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Effect of Green Tea Catechins on the Postprandial Glycemic Response to Starches Differing in Amylose Content

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ABSTRACT: The effect of tea polyphenols (TPLs), specifically tea catechins, on the postprandial glycemic response to cooked starches differing in amylose contents was investigated. The in vivo test using a mouse model showed a moderate reduction of the postprandial glycemic response to co-cooked normal (containing 27.8% amylose) or waxy corn starch with 10% TPLs (dry weight of starch), while an augmented glycemic response with a delayed blood glucose peak was observed when high amylose corn starch (HAC, containing 79.4% amylose) was used as the starch component. Enzyme kinetics results demonstrated that TPLs noncompetitively inhibit the digestion of waxy or normal corn starch, while the digestion rate of HAC starch was increased in the presence of TPLs, which supports the observed postprandial glycemic responses. Further studies using X-ray powder diffraction showed that the diffraction intensity (area under the diffraction curves) of normal and HAC starch was increased by 45% and 74%, respectively, whereas no change was observed for waxy corn starch. Consistently, dynamic laser light scattering studies using a solution of pure amylose showed an increased hydrodynamic radius of amylose molecules from ~54 nm to ~112 nm in the presence of TPLs. These experimental results indicate that there might exist an interaction between TPLs and amylose, which facilitates the association of amylose molecules to form a special nonordered structure that can produce a high and sustained postprandial glycemic response. Thus, a combination of tea polyphenols and specific starches could be used to manipulate postprandial glycemic response for glycemic control and optimal health.

KEYWORDS: Tea polyphenols, postprandial glycemic response, resistant starch, glycemic control

INTRODUCTION

As the prevalence of type 2 diabetes increases worldwide, glucose metabolism abnormity has become a hot research field. Glucose is not only the biological fuel preferred by the central nervous system but also a signal molecule regulating the expression of many genes related to glucose homeostasis and energy metabolism.[1] Thus, maintaining a normal level of serum glucose is essential to regular physiological processes and optimal health. Although there exists a complex regulatory system for glucose homeostasis, glucose release from glycemic carbohydrate digestion is the first critical point for glycemic control. Starch, as the main dietary carbohydrate from cereal and tubers, has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) to reflect its nutritional properties related to the rate and extent of glucose release and the corresponding postprandial glycemic response. It is known that a large postprandial glycemic fluctuation, which is characteristic of RDS, can produce excessive free radicals leading to oxidative stress[2] that is a risk factor for many chronic diseases.[3] SDS, which could provide a slow and prolonged glucose release, is the preferred carbohydrate for glycemic control and the prevention of glycemic fluctuation. The classification of starch fractions based on the in vitro Englyst method[4] facilitated the preparation and quantification of the nutritional qualities of dietary starches, and there have been many studies on[5−7] and related patents for[8,9] SDS. However, the fact that there is no commercially available SDS in the market suggests alternative ways from different perspectives are needed to achieve the physiological effects of SDS for glycemic control and the prevention of related diseases.[2]

Tea is one of the most popular beverages in the world, and green tea polyphenols, particularly the catechins including epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) have been proved to be capable of inhibiting the activity of α-amylase[10] and the absorption of glucose in the small intestine,[11,12] which might lead to a reduction of postprandial blood glucose level.[13,14] However, not all the studies on tea polyphenols showed positive results for glycemia control[15−17] and blood glucose-lowering effect,[18] indicating that other factors need to be considered to achieve a health benefit. Since glycemic carbohydrates are the major source of blood glucose, it is reasonable to take their nutritional properties into consideration in experimental designs or clinical trials. In the current investigation, a combination of tea catechins and starches with different amylose contents was used to study its effect on the postprandial glycemic response in order to better understand the antihyperglycemic effect of tea polyphenols[19] in the presence of other food components, which is important for their practical applications in functional foods.

MATERIALS AND METHODS

Normal corn starch, waxy corn starch, and high amylose corn starch were from National Starch and Chemical Company (Shanghai, China). Tea polyphenols (TPLs) were obtained from Lideshi Chemical Industry Co. Ltd. (Rizhao, China) with a total tea catechin content of ~99%, and its composition was analyzed using a common HPLC method.[20] α-Amylase (EC 3.2.1.1, type VI-B from porcine pancreas, 19.6 U/mg) and amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, 21100 U/g) were

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purchased from Sigma Chemical Co. (St. Louis, MO). The hexokinase (HK) kit for D-glucose assay was from Megazyme International Ireland Ltd. (Wicklow, Ireland).

Postprandial Glycemic Response Measurement. Nine-week-old male Kunmin (km) mice were purchased from Silaike Co. (Shanghai, China) and kept under an automatic light schedule of 07:00 a.m.−19:00 p.m. and a temperature maintained at 22 ± 3 °C. The mice were conditioned by feeding ad libitum with a laboratory diet (Silaike Co. Shanghai, China) and drinking water. Experiments were performed one week later after an overnight fasting (10 mice per experiment). Starch samples (10% w/v in distilled water) with and without TPLs were cooked (normal and waxy starch in boiling water bath, and high amylose starch at 120 °C) for 20 min to gelatinize the starches. After the samples were cooled to room temperature, the postprandial glycemic response was measured by feeding different test diets (TPLs, 100 mg/kg body weight [BW]; starch, 1 g/kg BW; 10% TPLs, based on starch, was used for starch + TPLs diet) administrated via gavages. Blood samples were taken from the lateral tail vein at 0, 15, 30, 45, 60, 90, and 120 min after gavages. The blood glucose concentration was measured using a glucose analyzer (Medisense, Abbott Park, IL) and expressed as the mean ± standard error (S.E.). All the procedures were approved by the Experimental Animal Review Committee at Jiangnan University of China.

In Vitro Starch Hydrolysis. The in vitro digestion of starch samples was carried out according a modified Englyst method.[4] Starch (200 mg) and/or TPLs (10% w/w, based on starch) in 8 mL sodium acetate buffer (100 mM, $CaCl_2$ 1 mM, pH 5.2) were first cooked (95 °C for normal and waxy corn starch, 120 °C for high amylose starch) for 20 min and placed in a 37 °C water bath. The enzyme solution (α-amylase and amyloglucosidase mixed in a proportion of 300 U/200 U/mL) was preincubated at 37 °C for 5 min. The reaction was started by adding the enzyme solution (2 mL) to the cooked starch samples, and an aliquot of 100 μL reaction mixtures was put into a plastic tube containing 0.9 mL of ethanol at 0, 20, 40, 60, and 120 min. The samples were vortexed and then centrifuged at 6,000 rpm for 10 min at room temperature. The glucose concentration was measured using a D-glucose-HK kit, and the rate of starch digestion was calculated by converting the amount of glucose into starch with a factor of 0.9. The concentration of total TPLs was measured spectrophotometrically according to their absorption at 295 nm, and the amylose content of the starches was measured by a colorimetric method based on the iodine−amylose complex.[21]

Enzyme Kinetics of Starch Digestion. The Michaelis−Menten kinetic model was employed to evaluate the effect of TPLs on starch hydrolysis under the reaction conditions of the Englyst test.[4] The amount of glucose liberated under different substrate concentrations (5−25 mg/mL) of cooked starch in the presence of TPLs (4 mg/mL) was used to measure the type of inhibition. Briefly, the reaction was carried out at 37 °C for 3 min in a sodium acetate buffer (100 mM, $CaCl_2$ 1.0 mM, pH 5.2) containing 3 U/mL α-amylase and 2 U/mL amyloglucosidase. A Lineweaver−Burk plot between 1/[substrate] (mg/mL) and 1/[V] (reaction rate, mmol/L/min per unit of α-amylase) was used to examine the action type of TPLs on starch hydrolysis.

X-ray Powder Diffraction. The samples of starch and starch + 10% TPLs were first cooked as described above, cooled to room temperature, and then freeze-dried for testing. A Bruker D8 advance speed X-ray diffractometer (Bruker AXS, Rheinfelden, Germany) equipped with a copper tube at 40 kV and 40 mA with Cu Kα radiation (λ = 0.154 nm) was employed to measure the degree of crystallinity of starch samples. The samples were mounted on a sample carrier with an amorphous background, and the diffractograms were obtained by scanning from 4° to 40° (2θ) at a rate of 4°/min. A software of MDI Jade 5.0 was used to analyze the diffractograms. Diffraction intensity that is the area under the diffraction curves was used for comparison.

Dynamic Laser Light Scattering. A commercial laser light scattering spectrometer (ALV/DLS/SLS-5022F, ALV Company, Langen, Germany) equipped with an ALV-5000/EPP multi-τ digital time correlator covering 125 ns−37 h in delay time and a He−Ne laser (Uniphase, output power ≈20 mW at λ = 632.8 nm) was used to measure the hydrodynamic radius of amylose molecules. An amylose solution (1 mg/mL) with different amounts of TPLs (0.0%, 2.0%, 8.0%, and 10.0%, amylose weight base) was used as the sample for analysis. Each sample solution was first passed through a 0.45 μm Millipore syringe filter into a dust-free cell. The dynamic light scattering measurements were obtained at 90°, and CONTIN FIT (ALV Company, Langen, Germany) was performed to obtain the hydrodynamic radius (expressed as a log scale) distribution of amylose molecules.

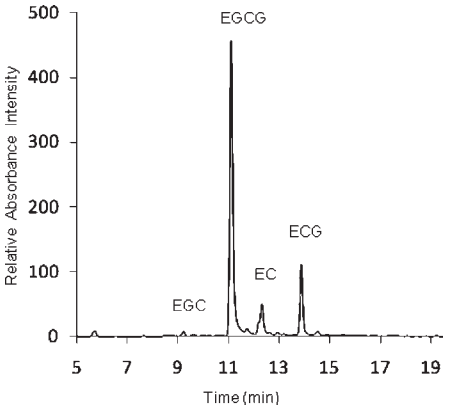

Figure 1. HPLC profile of tea polyphenols (TPLs) examined with a diode array detector at 280 nm.

Statistical Analysis. Experimental data of in vivo glycemic response were expressed as the mean ± standard error (S.E.), and standard deviation of the mean was used for other experimental results. Statistical analysis was carried out using the one-way analysis of variance (one-way ANOVA) in SPSS 11.5 for Windows software (SPSS) to examine the effect of TPLs on the postprandial glycemic response. A z-test was used to show the effect of TPLs on the proportion of RDS/SDS/RS for each starch sample. The statistical significance was accepted at $p < 0.05$.

RESULTS

Postprandial Glycemic Response to Starches in the Presence of TPLs. The in vivo test using a mouse model showed different postprandial glycemic response profiles among starch samples in the presence of TPLs, which is composed of EGCG (66.90%), EGC (5.88%), ECG (10.62%), and EC (15.70%) based on a liquid chromatography analysis (Figure 1). For normal and waxy corn starches, which are rapidly digestible after cooking,[6] the postprandial blood glucose concentration reached a peak value of 5.5−6.0 mmol/L above the baseline after gavage for 15 min. After the addition of TPLs (10%, based on starch dry weight), a similar glycemic response profile with a slightly reduced blood glucose concentration was shown (Figure 2B and C), and no significant difference of the area under the glycemic curves (AUC) was found compared with starch samples alone (Table 1). This result was not consistent with the literature reports on the antihyperglycemia function of tea catechins.[14,22] However, for HAC starch that is naturally a resistant starch[23] with a very low glycemic response, the addition of the TPLs significantly increased postprandial glycemia with a delayed blood glucose peak (Figure 2 D and Table 1). As shown in Table 1, the major difference among these starch samples is their amylose content, and the high amylose content in HAC starch is

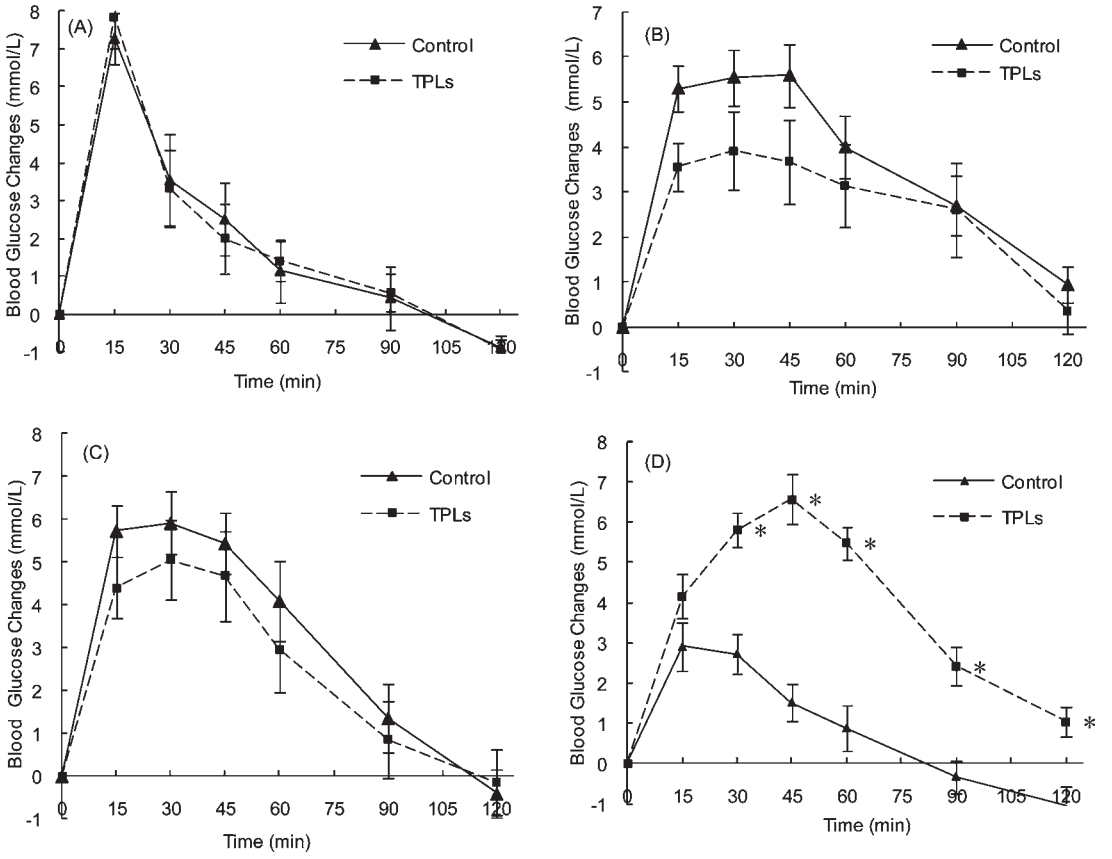

Figure 2. Postprandial glycemic response to starches affected by the addition of TPLs. The blood glucose level is the relative changes from its baseline before gavages. (A) glucose control, (B) normal corn starch, (C) waxy corn starch, and (D) high amylose corn starch. The statistically significant difference ($p < 0.05$, *) is labeled.

Table 1. Estimated Area under the Glycemic Response Curves (AUC) Affected by TPLs and the Amylose Content (Weight Percentage) of Starch Samples

sample	amylose content (%)	AUC[a] (0−120 min)	normalized AUC[b]
glucose	N/A	234.5	1.00
glucose + TPLs		241.2	1.03
NCS	27.8 ± 0.5	431.2	1.84
NCS + TPLs		322.0	1.37
waxy	0.4 ± 0.0	384.7	1.64
waxy + TPLs		302.6	1.29
HAC	79.4 ± 0.6	123.2	0.53
HAC + TPLs		459.3	1.95

[a] AUC = time × glucose concn (min·mM). [b] Based on the AUC of glucose.

likely the major reason for its increased glycemic response in the presence of TPLs. Regarding the control sample of glucose that reached a level of 7.5 mmol/L at the glycemic peak, an almost identical postprandial glycemic response profile (Figure 2A) and similar AUC (Table 1) were shown whether in the presence or absence of TPLs, indicating that TPLs have negligible effect on glucose absorption in the small intestine under the used dosage. Apparently, the postprandial glycemia-lowering effect of TPLs is dependent on starch structure, specifically the amylose content.

Enzyme Kinetics of Starch Digestion in the Presence of TPLs. Although it is well known that TPLs inhibit the activity of α-amylase for carbohydrate digestion, most of the literature reports[10,24] were studied under the optimum condition for enzyme activity. In this study, the reaction condition of the Englyst test[4] containing amyloglucosidase and porcine pancreatic α-amylase at a pH of 5.2, which is used to classify the starch factions with different digestibilities, was used to examine the inhibitory effect of TPLs on enzyme activity when starch was used as the substrate. A decreased reaction velocity without affecting the enzyme's affinity for substrate demonstrates that TPLs could noncompetitively inhibit (Figure 3) the digestion of normal and waxy corn starches. Although there are two types of enzymes in the reaction, TPLs did not show significant effect on amyloglucosidase (data not shown); thus, this noncompetitive inhibition indicates that α-amylase is the main component affected by TPLs through their noncompetitive binding to α-amylase (not the catalytic sites). Although TPLs inhibit the digestion of both normal and waxy corn starches, the degree of inhibition (estimated by V_{max}) is 50% and 60% with an enzyme affinity (K_m) of 6.25 and 4.54 mg/mL for normal and waxy corn starch, respectively, which suggests that waxy corn starch, with a negligible amylose content, is more susceptible to enzyme digestion than normal corn starch, and TPLs' inhibition effect is also more efficient for waxy corn starch.

When enzyme kinetics was studied using HAC starch as the substrate, a significant increase of the digestion rate without affecting the enzyme's affinity (Figure 4A) was shown in the

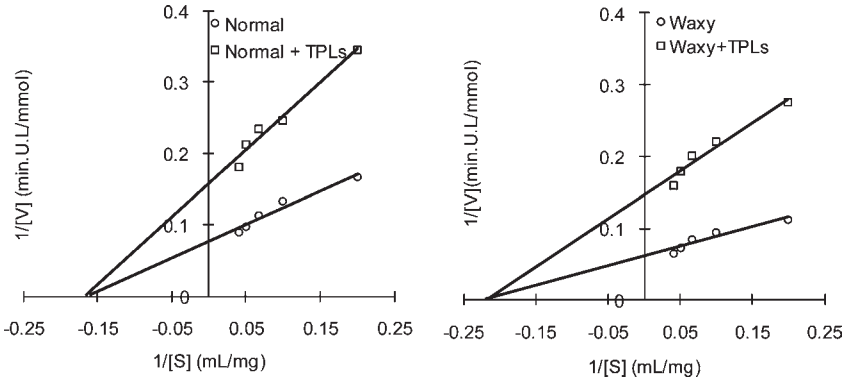

Figure 3. Effect of TPLs on the enzyme kinetics of digesting normal and waxy corn starches.

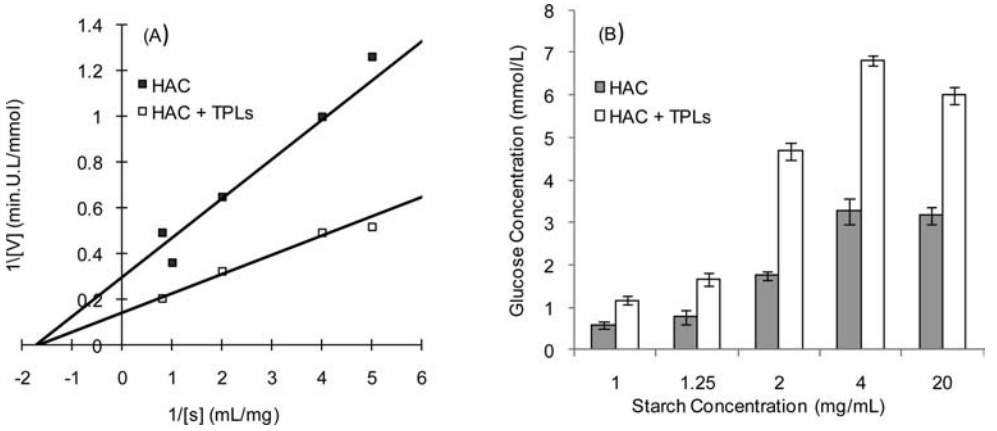

Figure 4. Effect of TPLs on the enzyme kinetics of HAC starch digestion. (A) Low substrate concentration for enzyme kinetics; (B) liberated glucose concentration in 3 min under different substrate concentrations of HAC starch.

presence of TPLs, which suggests that TPLs might act as enzyme activators to accelerate the digestion of HAC starch (V_{max} increased by 115%). When the substrate concentration was increased above the linear range (0.2−1.25 mg/mL) for the enzyme kinetics study, a similar effect was also observed (Figure 4B). Thus, although the samples of HAC and HAC + TPLs were the most difficult to be digested (lowest velocity with the same substrate concentration compared to those of normal and waxy starches) among the tested samples, an opposite effect of TPLs on HAC starch digestion, compared to that of normal and waxy corn starch, was discovered, indicating the complexity of the enzymatic reaction in the presence of TPLs.

In Vitro Starch Digestion. In order to elucidate the mechanism of the in vivo glycemic results, an in vitro digestion of starch according to the method of Englyst was also conducted. The digestion results (Table 2) showed that TPLs did not significantly affect the enzyme digestion of normal and waxy corn starches, while a significant increase of SDS and a decrease of RS were observed for HAC starch. Apparently, TPLs transform the RS portion of HAC starch into the digestible fractions, specifically the SDS fraction, which led to a high and sustained postprandial glycemic response to HAC starch in the presence of TPLs.

TPLs' Concentration in Co-Cooked Starch Suspension and X-ray Diffraction. TPLs exhibited their inhibitory effect on normal and waxy corn starch digestion, but for HAC starch, TPLs promoted its digestion. This contradictory result suggests that there might exist an interaction between TPLs and amylose.

Table 2. Effect of TPLs on Starch Fractionation (Percentages) Measured by the *Englyst* Method[a]

sample	RDS (%)	SDS (%)	RS (%)
NCS[b]	69.8 ± 2.9	17.4 ± 2.8	12.8 ± 1.0
NCS + TPLs	73.4 ± 1.4	9.2 ± 1.5	17.4 ± 0.7
waxy	79.1 ± 0.4	6.3 ± 0.5	14.6 ± 0.6
waxy + TPLs	71.4 ± 2.1	10.2 ± 1.5	18.4 ± 1.2
HAC[b]	24.1 ± 0.4	30.7 ± 0.1	45.2 ± 0.5
HAC + TPLs	31.3 ± 2.1	47.3 ± 1.4	21.5 ± 3.1

[a] For the HAC sample, the addition of TPLs significantly ($p < 0.05$) increased and decreased the proportion of SDS and RS, respectively. For NCS and Waxy starches, TPLs had no significant effect on starch fractionation. [b] NCS: normal corn starch. [c] HAC: high amylose corn starch.

By measuring the concentration of total TPLs in the supernatant of co-cooked samples of starches and TPLs, waxy corn starch with negligible content of amylose showed the highest concentration of TPLs (Figure 5), and the lowest content of TPLs was detected from the supernatant of high amylose corn starch. This result clearly demonstrates that high amylose corn starch, most likely amylose, can bind TPLs, and this binding is possibly related to its high susceptibility to enzyme digestion.

X-ray diffraction is a common method used to examine the semicrystalline structure of starches. The experimental results of

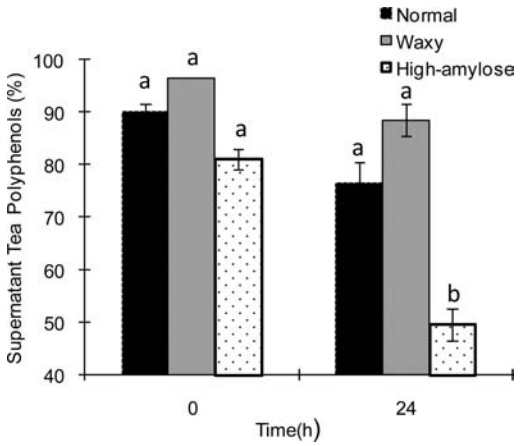

Figure 5. TPL percentage in the supernatant after gelatinization for 0 and 24 h. Different letters represent the statistical difference at $p < 0.05$.

the freeze-dried cooked samples (Figure 6) showed that the diffraction intensity (area under the diffraction curves) was increased by 45% and 74% for normal and HAC starches, respectively, while the diffraction pattern of freeze-dried cooked waxy starch was independent of the presence of TPLs. This result also showed that amylose is the major player interacting with TPLs, and this interaction or binding might make amylose molecules easier to associate with each other to form a special structure with a high content of SDS (Table 2). However, no clear peaks in the diffractograms of all the samples indicate that no ordered long-range crystalline structure was formed under the current experimental conditions, and the difference in the diffraction intensity area might only reflect the difference in shorter range structures, such as scattered short double helices or single helical structures formed through amylose association with the help of TPLs.

Dynamic Laser Scattering (DLC) Measurement. Since the high amylose corn starch has the highest amylose content, pure amylose was used to further the understanding of the binding between amylose and TPLs at a molecular level. An increase of hydrodynamic radius of amylose molecules from ~54 to ~112 nm was discovered from the DLC measurements (Figure 7) in the presence of TPLs (2−10% based on amylose). This result directly demonstrated a possible interaction between amylose and TPLs, and it was consistent with the X-ray diffraction results.

DISCUSSION

Postprandial Glycemic Response to Different Corn Starches. Tea catechins, as bioactive phytochemicals, have been reported to have a variety of biological functions such as hypolipidemic activity, antidiabetic, antitumor, and anticardiovascular disease properties.[25] They also affect carbohydrate metabolism through different mechanisms including inhibiting the enzyme activity and glucose transporter in the small intestine, suppressing hepatic glucose production, increasing insulin sensitivity, and modulating intracellular signaling pathways related to glucose homeostasis.[24] TPLs' attenuation of postprandial glycemic response has been mainly attributed to their inhibitory activity on α-glucosidase[26] and the sodium-dependent glucose transporter of SGLT1 for glucose absorption.[27] Compared to the current study, the inhibitory activity of TPLs on α-amylase is

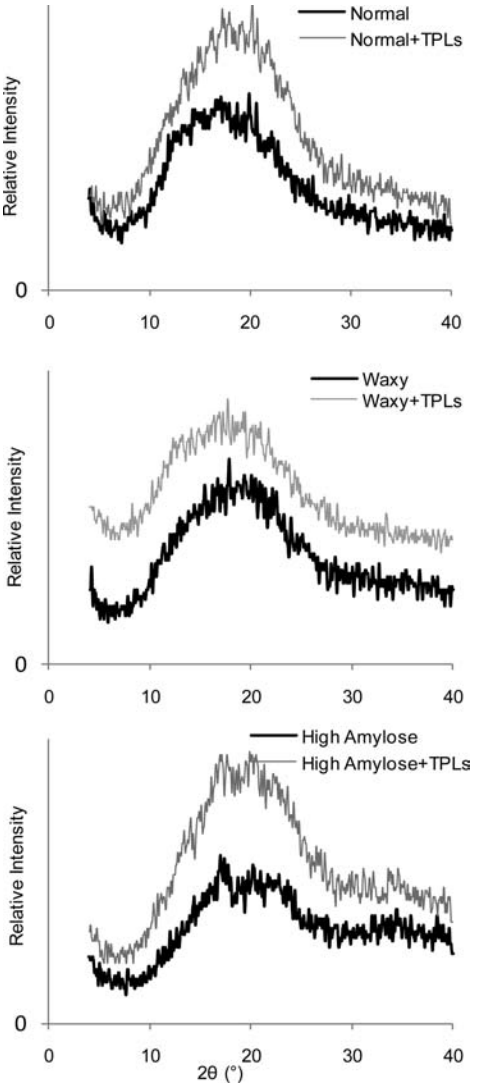

Figure 6. X-ray diffraction pattern of starch samples affected by TPLs. The diffraction intensity is referred to the area under the diffraction curves.

consistent with the literature report when normal and waxy corn starches are used as the carbohydrate component. However, the inhibition activity on the SGLT1 for glucose absorption is not supported by the current study as no difference was found between glycemic response profiles from glucose and glucose + TPLs (Figure 2A and Table 1), and the reason might be that the dosage used in this study is not high enough to demonstrate the effect.

As for the postprandial glycemic response to HAC starch, TPLs induced a high level of glycemic response (Figure 2D and Table 1), and the enzyme kinetic studies (Figure 4) showed that TPLs did not have any inhibitory activity on HAC digestion but promoted the hydrolysis of HAC starch. Since the HAC starch has the highest amylose content, it is likely that the increased HAC starch digestion by TPLs is due to a possible interaction between TPLs and amylose. Actually, TPLs have been reported to have the effect of inhibiting starch retrogradation[28] and directly activating the activity of cytochrome P450 1A1,[29] which indirectly supports our findings. However, this increase of starch digestion is contradictory to the common knowledge of TPLs

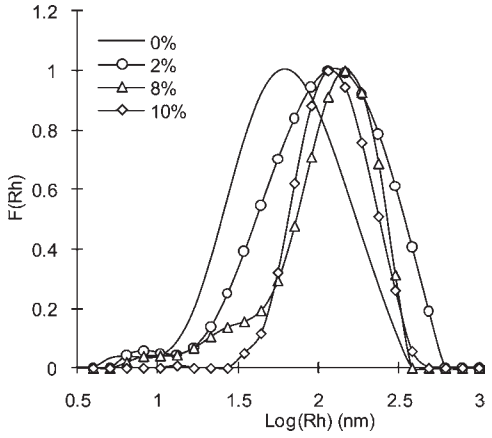

Figure 7. Distribution of hydrodynamic radius (Rh, nm) of amylose molecules affected by TPLs (2, 8, and 10%, amylose weight base). The Rh of amylose is ~54 nm and 112 nm in the presence of TPLs.

acting as an inhibitor of carbohydrate digestion enzymes from many literature reports,[10,26] and the reason for this inconsistency is probably the elimination of possible interactions between TPLs and substrates that are simple molecules (such as maltodextrin) in most studies.

Interaction between TPLs and Amylose. The acceleration of HAC starch digestion by TPLs is somehow a surprising finding, and in order to explain its mechanism, other studies were carried out. The X-ray diffraction result and the concentration of free TPLs after starch gelatinization clearly showed that amylose is the major player interacting with TPLs in the system. Furthermore, an increased diffraction intensity and hydrodynamic radius of amylose molecules in the presence of TPLs suggests that TPLs could, in some way, interact with amylose to form a special structure leading to an increased postprandial glycemic response. It is well known that amylose is an essentially linear molecule of glucose linked through α-1,4 glycosidic linkages and that it can form an inclusion complex with hydrophobic molecules such as free fatty acids[30] through its helical structure. TPLs might also form an inclusion complex with amylose through their hydrophobic moiety just like the inclusion complex formed between (+)-catechin/(−)-epicatechin and β-cyclodextrins[31] that is structurally similar to one turn of amylose helices. If this type of inclusion complex could be formed between amylose and TPLs, free TPLs molecules in the system would be decreased, and its availability to enzyme binding would be decreased. Additionally, the formed structure is also more susceptible to enzyme digestion[32] than the resistant long-range double helices formed from amylose molecules. Thus, it is possible that amylose and TPLs did form some nonordered inclusion complex as X-ray diffraction did not show a clear V-type crystalline structure (peaks at 8, 13.5, and 20°) that is the hallmark of a typical complex formed between amylose and other linear hydrophobic molecules.

The in vitro digestion results (Table 2) showed that the SDS content of HAC starch is significantly increased in the presence of TPLs and that the RS content is significantly decreased. From the studies of SDS, it is known that some nonordered crystalline structures formed during starch retrogradation is required for the starch to be slowly digestible.[33] From the structure of HAC starch, amylose retrogradation is very fast after it is gelatinized, and once it is retrograded, it becomes resistant to digestion (as shown in the current study). Thus, some methods are needed to prevent the quick retrogradation of amylose molecules to form resistant starch. A literature report[34] showed that a quick cooling of sterilized HAC starch can form slowly digestible starch due to a quick stabilization of its nonordered crystalline structure. Similarly, amylose retrogradation in the presence of TPLs might also form a nonordered crystalline structure with a slow digestion property. Thus, a nonordered structure formed through interaction with TPLs is likely the basis for TPLs' accelerated digestion of HAC starch and the increased and prolonged postprandial glycemic response.

HAC starch is naturally a resistant starch, but the addition of TPLs promoted its digestion. Although we hypothesize that a possible interaction between amylose and TPLs is the basis for an increased digestion of high amylose starch, the detailed mechanism including a deep analysis of the dose−response relationship still needs further studies in order to quantitatively manipulate the postprandial glycemic response and to understand the actual roles played by food matrix and functional components in health foods.

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