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Interaction between Amylose and Tea Polyphenols Modulates the Postprandial Glycemic Response to High-Amylose Maize Starch

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ABSTRACT: High-amylose maize starch (HAM) is a common source material to make resistant starch with its high content of amylose (>70%). In the current investigation, the self-assembly of amylose in the presence of bioactive tea polyphenols (TPLs) and resulting slow digestion property of starch were explored. The experimental results using a mouse model showed a slow digestion property can be achieved with an extended and moderate glycemic response to HAM starch cocooked with TPLs. Further studies using a dilute aqueous amylose solution (0.1%, w/v) revealed an increased hydrodynamic radius of amylose molecules, indicating that TPLs could bridge them together, leading to increased molecular sizes. On the other hand, the bound TPLs interrupted the normal process of amylose recrystallizaiton evidenced by a decreased viscosity and storage modulus (G') of HAM (5%) gel, a rough surface of the cross-section of HAM film, and decreased short-range orders examined by Fourier transform infrared spectral analysis. Single-step degradation curves in the thermal gravimetric profile demonstrated the existence of a self-assembled amylose−TPL complex, which is mainly formed through hydrogen bonding interaction according to the results of iodine binding and X-ray powder diffraction analysis. Collectively, the amylose−TPL complexation influences the normal self-assembling process of amylose, leading to a low-ordered crystalline structure, which is the basis for TPLs' function in modulating the digestion property of HAM starch to produce a slowly digestible starch material that is beneficial to postprandial glycemic control and related health effects.

KEYWORDS: *high-amylose maize starch, tea polyphenols, slowly digestible starch, postprandial glycemic response, self-assembly of amylose*

INTRODUCTION

Starch, as the product of photosynthesis occurring in cereal, tuber, and other food crops, is an important dietary carbohydrate comprising essentially linear amylose and highly branched amylopectin, and its digestibility is intimately associated with its nutritional properties, which are expressed by the percentages of rapidly digestible starch (RDS), slowly digestible starch (SDS, such as native cereal starch), and resistant starch (RS, such as high-amylose starch).[1] As the prevalence of glucose homeostasis related diseases is increasing, particularly type 2 diabetes, SDS and RS have been considered as healthy dietary carbohydrates that are beneficial to blood glucose control.[2,3] However, RDS, which is the major carbohydrate in refined starchy food ingredients, is detrimental to health due to resulting large fluctuations of postprandial glucose level and strong enhancement to oxidative stress,[4,5] which is one of the causative factors to many chronic diseases.[6] Thus, consumption of foods with high content of SDS and RS is a desirable choice for the prevention of and interference with these chronic diseases. With regard to the structural basis of RS and SDS, the content of amylose in the starch is significantly correlated with the content of RS, whereas the amylopectin is the structural basis for SDS.[7] Thus, high-amylose maize starch (HAM) is a natural resource of RS,[8] but regular waxy starch is not a good candidate for SDS because there are no specially structured amylopectin molecules in regular waxy starches.[9] This not only manifests the scarcity of SDS[10] and the fact that there is no commercialized SDS but also indicates the difficulty in producing SDS by only considering the starch itself. Innovative ways are needed to produce heat-stable SDS to facilitate research on SDS and applications of SDS-containing food products.

Tea polyphenols (TPLs), mainly including (−) epigallocatechin gallate (EGCG), (−) epigallocatechin (EGC), (−) epicatechin gallate (ECG), and (−) epicatechin (EC), are some of the most studied bioactive materials with a variety of biological functions.[11] With regard to their functions in regulating carbohydrate metabolism, TPLs, as antioxidant compounds, could not only ameliorate the oxidative stress elicited by postprandial hyperglycemia[12] that is characteristic of RDS[13] but also affect the activity of α-amylase[14] and other α-glycosidases[15] that are important for glucose liberation from starch or other glycemic carbohydrate materials. Thus, TPLs, as representatives of versatile phenolic compounds, are theoretically beneficial to glucose homeostasis.

Besides the above effects of TPLs on carbohydrate metabolism, another important aspect of TPLs' function, just like other phenolic compounds, is their effects on the physiochemical properties of starch. TPLs have been shown to inhibit starch retrogradation[16] and in vivo starch hydrolysis in a rat model study,[17] and hydrogen-bonding interactions during starch gelatinization were also observed.[18] Further study showed that amylose and the linear fragments of amylopectin are the major components interacting with the phenolic compounds of tannin[19] accompanying a decreased starch

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digestibility. Comparatively, we found no significant reduction of postprandial glycemic response to normal and waxy corn starch with 10% TPLs (based on weight of starch), but a significantly increased postprandial glycemic response to high amylose maize starch was observed,[20] which is contradictory to most literature papers. Apparently, although there have been reports on the interactions between phenolic compounds and starch as well as implications for starch digestion, there are still many controversies in this field due to the complicated system containing starch, phenolic compounds, and digestive enzymes, and more work is needed to clarify the details of their relationships and resulting implications to the nutritional properties of starches. According to our previous study,[20] in which a high glycemic response was produced by high-amylose maize starch in the presence of TPLs, we hypothesize that TPLs or other phenolic compounds' disruptive function on the normal process of amylose self-assembly to form ordered crystalline structure is the main determinant of the nutritional properties (proportion of RDS, SDS, and RS) of HAM starch when TPLs or other phenolic compounds are present. It was expected that novel strategies to produce heat-stable SDS could be realized through food component interactions.

MATERIALS AND METHODS

HAM starch and waxy maize starch were obtained from National Starch and Chemical Co. (Shanghai, China). TPLs with a total tea catechin content of ∼99% were from Lideshi Chemical Industry Co., Ltd. (Rizhao, China). α-Amylase (EC 3.2.1.1, type VI-B from porcine pancreas, 19.6 U/mg) and amyloglucosidase (AMG, EC 3.2.1.3, from *Rhizopus* mold, 21.1 U/mg) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The hexokinase (HK) kit for D-glucose assay was from Megazyme International Ireland Ltd. (Wicklow, Ireland).

Pure amylose was isolated using 1-butanol as the selective precipitating reagent,[21] and amylose solution was prepared by dissolving isolated amylose in a solvent of 90% dimethyl sulfoxide (DMSO) (20 mg/mL) incubated in a boiling water bath for 1 h with continuous stirring, and then ethanol with a final concentration of 80% was added to precipitate the amylose. After centrifugation for 3 min at 10000 rpm, the amylose was vacuum-dried and dissolved in hot distilled water (2.0 or 1.0 mg/mL) by heating at 90 °C for 20 min.

Starch Film Preparation. HAM starch (2.5 g) and TPLs (2.5 and 5% starch weight based) were mixed in 40 mL of distilled water and cooked at 135 °C for 2 h with continuous stirring. The starch film was prepared by pouring the gelatinized starch into a casting device that was leveled and preheated in a drying oven set at 60 °C. After drying for 5 h, the film was pulled and stored at a desiccate for further analysis.

Preparation Self-Assembled HAM−TPL Complex. HAM starch (3 g) and TPLs (2.5 and 10%, based on starch weight) were mixed in 60 mL of distilled water and cooked at 135 °C for 2 h with continuous stirring to completely gelatinize the HAM starch. The HAM−TPL complex was prepared by slowly cooling the cooked samples to room temperature and then freeze-dried after storage at 4 °C for 0 and 7 days. The prepared samples were bottled for further analysis.

High-Performance Liquid Chromatography (HPLC) Analysis of TPLs. An Agilent HPLC 1200 instrument coupled with a GraceSmart reverse-phase C18 column (4.6 × 250 mm, particle size = 5 μm) (Deerfield, IL, USA) was used to analyze TPL samples with a final concentration of 1 mg/mL (5 μL sample size). Two different solvents at a flow rate of 0.8 mL/min at 30 °C were used as the mobile phase. Solvent A was composed of acetonitrile, distilled water, and trifluoroacetic acid (TFA) in a ratio of 10:90:0.05, respectively, whereas a ratio of 30:70:0.05 was used as solvent B. Beginning with 100% solvent A, a linear gradient mobile phase (decreasing polarity) with solvent B was used until 20 min approaching 100% solvent B. After continuous running for 5 min, the mobile phase was returned to 100% solvent A until 30 min to finish an analysis cycle.

The total phenolic content was measured by a UV−vis spectrophotometric method by reading the absorbance at 295 nm.[22]

In Vitro Starch Hydrolysis. The effect of TPLs on starch digestion was studied according to the Englyst method[1] with minor modifications. Briefly, 500 mg of starch and TPLs (10% based on starch weight) were first cocooked at 135 °C in 20 mL of sodium acetate buffer (100 mM, 5 mM $CaCl_2$, pH 5.2) for 2 h with continuous stirring and then transferred to a 37 °C water bath before the addition of 5 mL of dual enzyme solution (α-amylase 3800 U/mL, AMG 13U/mL). The released glucose was measured with D-glucose-HK kit at 0, 20, 40, 60, and 120 min.

Thermogravimetric Analysis (TGA). A Mettler Toledo TGA/SDTA851^e thermogravimeter (Mettler Toledo Corp., Zurich, Switzerland) with STAR^e software (version 9.01) was used to analyze the thermal stability of the HAM−TPL complex prepared above. Samples (2.0 mg in each 70 μL alumina pan) were heated from 50 to 400 °C (10 °C/min) under a continuous nitrogen gas flow (20 mL/min). The thermal decomposition curve was recorded and analyzed.

X-ray Powder Diffraction. A Bruker D8-Advance diffractometer (Bruker AXS Corp., Nanjing, China) equipped with Cu Kα radiation at 40 kV and 40 mA was used to obtain the X-ray diffractograms of the HAM−TPL complexes by scanning from 3° to 40° 2θ at a rate of 0.02°/3 s.

Fourier Transform Infrared Spectral (FTIR) Analysis. A Nicolet Nexus 470 Fourier transform infrared spectrometer (Thermo Electron Corp.) equipped with a ATR cell and EZ Omnic software (version 7.0) was used to obtain the spectrograms of the HAM−TPL complexes. Accurately weighed samples and KBr (100 times the weight of each sample) were fully milled together to get the infrared information from 4000 to 600 cm^{-1} by accumulating 32 scans per spectra at a resolution of 4 cm^{-1}.

Rheological Property Measurement. HAM starch (5%) with or without TPLs (10% based on dry weight of starch) was first cocooked at 135 °C for 2 h to completely gelatinize the starch (for waxy starch, it was cooked at 100 °C for 20 min), and then the apparent viscosity (Pa.s) of the samples was measured at 25 °C along the shear rate from 0.1 to 100 s^{-1} using an AR-G2 rheometer (TA Instruments-Waters LLC, Shanghai, China) with a 40 mm diameter steel plate gapped by 1 mm.

To measure the viscoelastic property of HAM starch (5%) affected by TPLs (10% based on starch), a strain sweep test on HAM starch (5%) paste was first performed from 0.1 to 100% strains at 1 Hz and 25 °C to identify the linear viscoelastic region. Then, the frequency sweep procedure for the samples of HAM and HAM + TPLs was run from 0.1 to 10 Hz in their corresponding linear strain range at 25 °C, and the storage modulus G' and loss modulus G'' were recorded to represent their viscoelastic properties.

Dynamic Laser Scattering Analysis (DLS). A commercial laser light scattering spectrometer (ALV/DLS/SLS-5022F, ALV Co., 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 (1.0 mg/mL) with different amounts of EGCG or EC (0.0, 1.0, 2.5, and 5.0%, amylose dry weight base) was mixed and 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 DLS measurements were obtained at 90°, and CONTIN FIT (ALV Co.) was performed to obtain the hydrodynamic radius distribution of amylose molecules.

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 7:00 a.m.−7:00 p.m. and a temperature 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 1 week later after an overnight fasting (10 mice per group). HAM starch samples (1.5 g in 150 mL of distilled water) with TPLs (control and

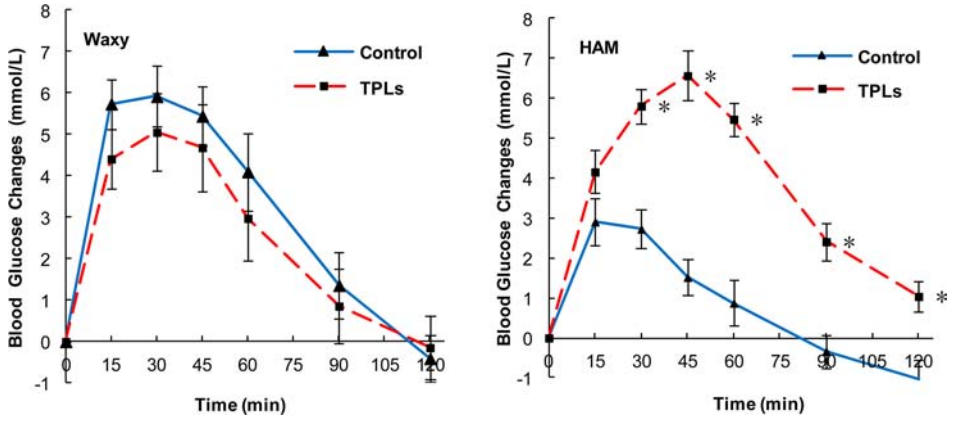

Figure 1. Postprandial glycemic response to waxy and HAM starches in the presence of 10% TPLs (reprinted from ref 20; copyright 2011 American Chemical Society).

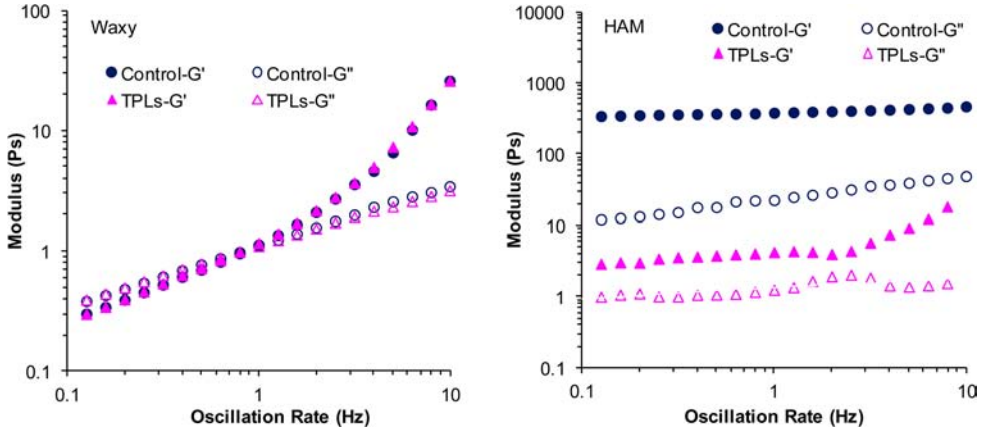

Figure 2. Viscoelastic properties of starch gels affected by TPLs. Waxy, waxy starch; HAM, high-amylose maize starch; G', storage modulus; G'', loss modulus.

50, 75, 100, and 150 mg TPLs) were put into a high-pressure reactor and then cooked at 135 °C for 120 min with continuous stirring. After the cooked samples had cooled to room temperature, TPLs were added to a total 10% (w/w) except for the control. The postprandial glycemic response was then measured by feeding different test diets (starch, 1 g/kg body weight (BW)] administered 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, USA) and expressed as the mean ± standard error (SE). All of the procedures were approved by the Experimental Animal Review Committee at Jiangnan University of China.

Iodine Binding Analysis. Iodine solution (2% KI, 0.2% I_2) stored in a nonactinic bottle was used as the standard iodine solution. The amylose sample solutions (2 mg/mL) containing different contents of EGCG [0, 0.5, 1, 2, 6, and 10% (w/w of amylose)] was first prepared, and then the solution (200 μL) was diluted to 10 mL and then mixed with 20 μL of iodine solution. After reaction for 15 min at room temperature, absorbance at 680 nm was measured using a UV−vis spectrophotometer (model TU-1900, Peaking Puxi Inc., Beijing, China), and the absorbance was regarded as the relative iodine binding capacity of each amylose sample.

Scanning Electron Microscopy (SEM). To analyze the changes of the supramolecular structure of HAM starch affected by TPLs in the film, cross sections of dried films were used as samples for SEM analysis. The sample was first fixed by osmium tetroxide and sputter coated with platinum to a level of 250−500 nm. Scanning electron micrographs were then obtained with a Quanta 200 scanning electron microscope (FEI Co., Switzerland) under a vacuum of 13.33 Pa and an operating voltage of 20 kV.

Statistical Analysis. The data reported in all tables were the average of at least triplicate experimental results, and Statistical Package for the Social Science (SPSS, version 11.5) was used to analyze the results. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Amylose Is the Major Component Interacting with TPLs. High-amylose maize starch is a special type of starch containing ∼79% amylose, whereas waxy starch is almost purely composed of amylopectin with negligible amylose (0.28%). From the previous study, we (as illustrated in Figure 1 from our previous study) have shown that TPLs did not significantly reduce the postprandial glycemic response to waxy starch even though there had been literature report on decreased postprandial blood glucose levels due to the inhibitory effect of TPLs on starch hydrolysis enzymes.[17] However, for HAM starch, a dramatic increase of the glycemic response was observed in the presence of TPLs,[20] indicating starch−TPL interaction; especially amylose−TPL interaction is the molecular basis for the increased postprandial glycemic response. To further ensure the role of amylose, a rheological study was carried out, and distinct viscoelastic profiles were shown between waxy and HAM starches (Figure 2). For waxy starch, the addition of TPLs did not affect either the storage modulus (G') or the loss modulus (G''), whereas both the G' and G'' of HAM starch were significantly reduced by TPLs, which confirms that it is the amylose, and not the highly

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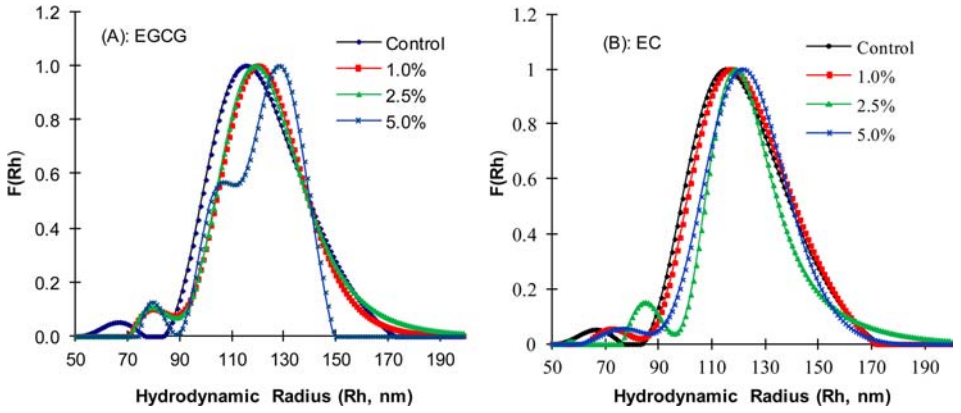

Figure 3. DLS analysis of the molecular size distribution of amylose under different concentrations of TPL monomers of EGCG and EC.

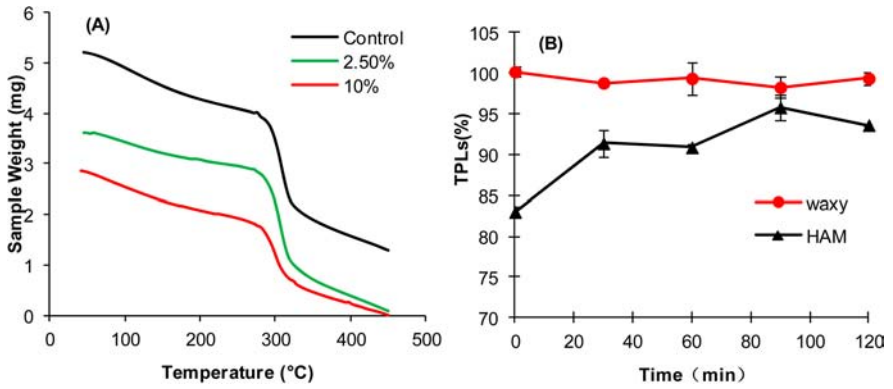

Figure 4. TGA analysis of amylose−TPL complex (A) and release of TPLs as the digestion of the complex made with 10% TPLs (B). The percentage represents the concentration of TPLs based on the dry weight of starch.

branched amylopectin, that interacts with TPLs, although HAM starch does have amylopectin contributing to the G' and G'' of HAM starch. Actually, this result is consistent with a literature report that amylose and possibly the linear fragment of amylopectin are the molecules interacting with phenolic compounds.[19]

Complexation between Amylose and TPLs. The postprandial glycemic response reflects the digestion property of HAM starch, and the viscoelstic profiles indicate the association properties among starch molecules. To understand how the presence of TPLs causes the increase of glycemic response and decrease of modulus of G' and G'', a deep understanding of how amylose and TPLs interact with each other is needed.

Amylose in a dilute aqueous solution (1.0 mg/mL) is generally believed to have a random coil conformation with irregular helical segments,[23] and its chain length independent critical concentration for amylose gelation is ∼1.0% (w/v).[24] Thus, the used amylose solution (0.1%, w/v) is far below the concentration required for amylose aggregation or gelation. An increased hydrodynamic radius of the amylose from DLS analysis (Figure 3) demonstrates that EGCG, as the major component of TPLs from HPLC analysis (2.7, 4.6, 73, and 19.6% for EGC, EC, EGCG, and ECG, respectively), might act as a bridge to link amylose molecules together, leading to an increased molecular size with a lower polydispersity (narrower distribution of their molecular size [for both EGCG and EC)] or cause the extension of amylose leading to an increased hydrodynamic radius. Two peaks in the presence of EGCG at a concentration of 5% suggest a possible nonuniformity for their interactions, which might result from the presence of linear and helical segments in aqueous amylose solution, and segments with different conformations might interact with TPLs differently. Additionally, different profiles in the presence of EGCG and EC, especially at a high concentration of 5%, suggest the molecular structure of TPLs also affects the manner of their interactions.

Thermogravimetric analysis showed single-step decomposition curves for each concentration of TPLs and decreased decomposition temperature throughout the increase of TPLs' concentration (Figure 4A), indicating an amylose−TPL complex was formed during their interactions. Additionally, the decomposition temperature was also shown to be increased during the storage time (Table 1), suggesting the stability of the amylose−TPL complex is not likely so high, which might be related to the nonuniformity of the amylose−TPL interaction observed from DLS or the dynamic complexation process to reach a thermodynamic equilibrium that is characteristic of

Table 1. Thermogravimetric Analysis Result of Amylose−TPL Complexes

	decomposition temperature[a] (°C)		
sample	day 0	day 1	day 7
HAM	312.2 ± 0.1	312.3 ± 0.1	312.1 ± 0.0
HAM + 2.5% TPLs	307.9 ± 0.2	308.0 ± 0.2	308.1 ± 0.1
HAM + 10% TPLs	298.3 ± 0.0a	300.5 ± 0.2b	302.4 ± 0.0c
TPLs		280.1 ± 0.2	

[a]Different letters represent significant difference at $p < 0.01$.

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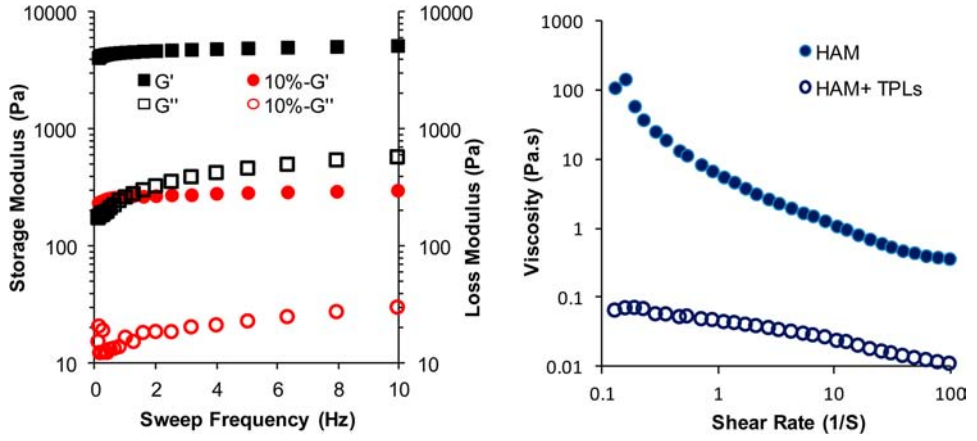

Figure 5. Rheological test of HAM starch in the presence of TPLs (10% w/w): (left) frequency sweep viscoelastic property; (right) shear rate dependent viscosity.

most structuralization processes of starch-based systems.[25] The formation of the self-assembled amylose−TPL complex was also supported by the release of TPLs during the digestion time (Figure 4B), whereas no change was found in waxy starch.

Disruptive Function of TPLs on Normal Process of Amylose Self-Assembly. TPLs play an important role in bringing amylose molecules together in a dilute aqueous solution where no association between amylose molecules would occur due to the low concentration of amylose, which is far below the gelation concentration of 1%. However, in the prepared complex, the TPLs could be regarded as penetrating into the matrix of amylose molecules to form complexes during the self-assembly of amylose molecules. No matter which process, the TPLs' binding to amylose is likely a certain event, and the effect of TPLs on the normal process of amylose self-assembly to form double helices and crystalline structures is the key to explaining the increased postprandial glycemic response (Figure 1) and decreased modulus of amylose gel (Figure 2).

HAM starch gel is a viscoelstic material. The rheological test of the HAM starch (5%) gel (Figure 5, right) showed a shear-thinning behavior that is probably because of the alignment of amylose chains with shearing. The addition of TPLs dramatically decreased the viscosity, but in the meantime, the degree of shear-thinning was lessened. When the viscoelstic property was tested using a frequency sweep testing (Figure 5, left), a low phase angle ($G' > G''$) indicates the amylose gel is more rigid and elastic. The addition of TPLs decreased both the G' and G'', but did not change the inherent rigid property of the amylose gel with the same trend of $G' > G''$. As the rheological behavior of amylose gel is related to the motions of single amylose chains (more mobile) connecting the entangled network junctions (more rigid) formed by interchain double helices,[24] the addition of TPLs might decrease the entanglement and formation of amylose double-helix junction zones, which would lead to a decreased viscosity or friction and storage modulus G' representing the solid-like behavior of amylose gel. On the other hand, the TPLs can also bridge the mobile amylose chains to form some loosely connected complex, so the shear-thinning was lessened, and the loss modulus of G'' representing the liquid-like behavior of amylose gel was also decreased.

FTIR is normally used to detect the function group of molecules based on signals from specific wavelengths. With regard to starch structure analysis, the FTIR spectrum has been used to describe the short-range order such as chain conformation and helices[26] based on the ratio of absorbance at specific wavelengths in the region of 800−1200 cm^{-1}. Three main vibration modes with maximum absorbance at 995, 1022, and 1047 cm^{-1} have been used to describe the starch structure, and the absorbance at 1022 cm^{-1} has been correlated with vibration mode in the amorphous region of starch, whereas the bands at 995 and 1047 cm^{-1} are correlated with the degree of order.[27] In the current investigation, the ratio of absorbance at 995 cm^{-1} to that at 1022 cm^{-1} was used to characterize the structural orderness of HAM starch affected by TPLs (Figure 6). Clearly, the addition of TPLs showed a concentration-dependent decrease of the short-range order of HAM starch with a lower ratio of absorbance at 995 to that at 1022 cm^{-1}. This result further supports the above view that TPLs interfere with the normal process of amylose self-assembly to form ordered structure of double helices and crystalline domains.

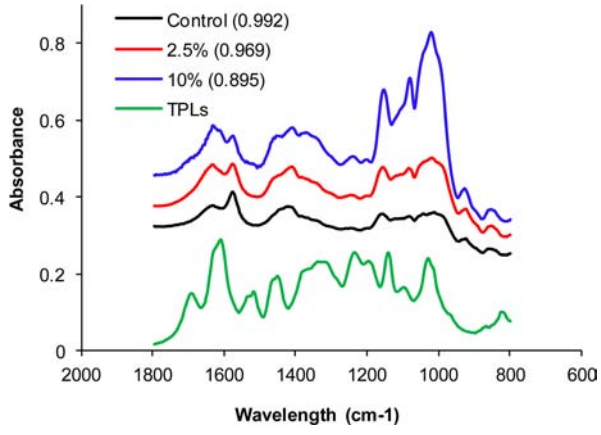

Figure 6. FTIR analysis of the amylose−TPL complex. The number is the absorbance ratio of 995/1022 cm^{-1}.

To view the disruptive function of TPLs on the microstructures formed through amylose self-assembly during gelation, an amylose film was produced in the presence of 2.5% TPLs as no film can be formed at higher concentration of TPLs. The cross section of film showed a distinct rough surface compared to the control (Figure 7), which demonstrates that the basic microstructural component during amylose gelation was changed by the addition of TPLs showing a rod-like

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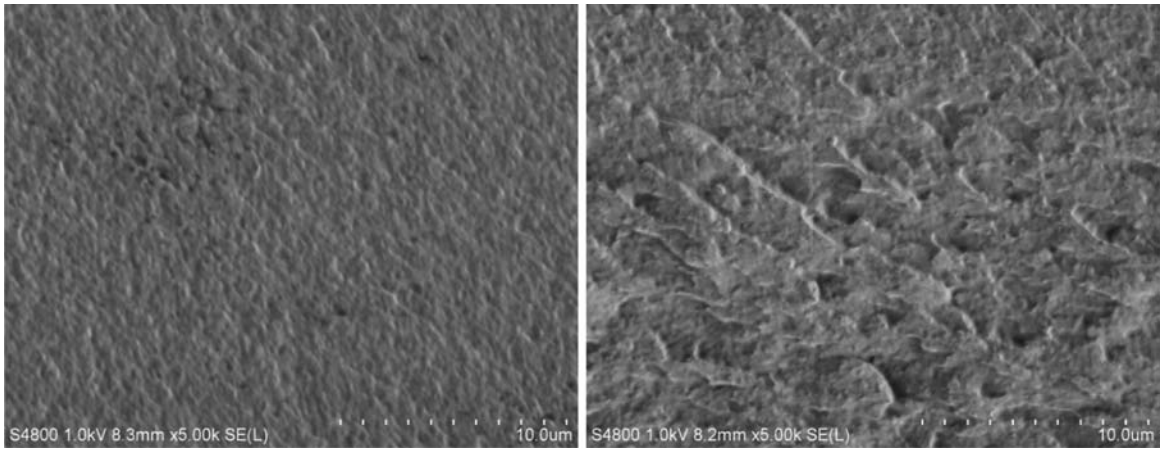

Figure 7. SEM observation of the cross section of amylose films: (left) amylose control; (right) amylose + 2.5% TPLs.

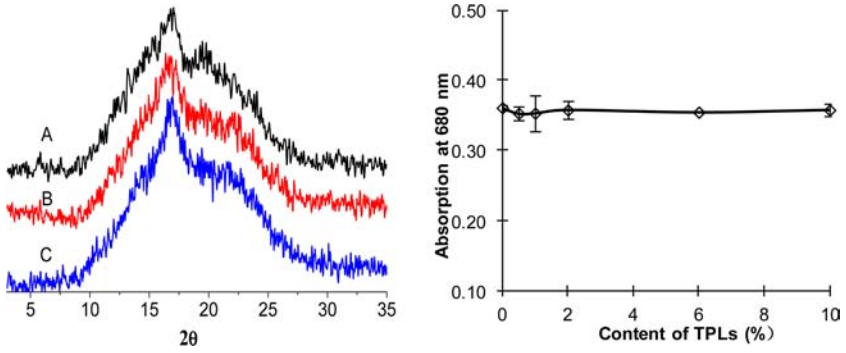

Figure 8. X-ray powder diffraction patterns (left) and iodine binding properties (right) of amylose−TPL complex: (A) control; (B) 2.5% TPLs; (C) 10% TPLs.

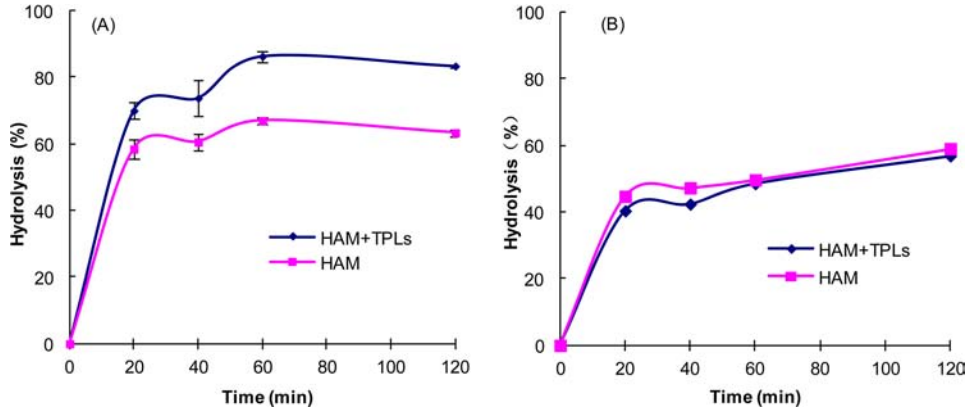

Figure 9. Digestion of HAM starch after retrogradation at 4 °C: (A) fresh cooked samples; (B) after retrogradation for 5 days.

building block and some empty spaces compared to the uniform and smooth surface of the control.

Collectively, all of the above experimental results on the concentrated amylose system showed a disruptive function of TPLs on the normal process of amylose self-assembly to form double helices and ordered structures.

Noncovalent Interactions for Amylose−TPL Complexation. A common interaction between linear amylose with a helical structure is the inclusion complex formed with guest molecules such as free fatty acids and monoglycerides.[28] For TPLs, there has been a report of an inclusion complex formed between (−)-epicatechin and β-cyclodextrin (β-CD)[29] or other catechins of ECG, EGC, and EGCG,[30] where the A and C rings of EGCG and ECG were included in the cavity of β-CD and the B and B′ rings were left outside. Because of the similarity between β-CD and the helical structure of amylose, it is possible for TPLs to form an inclusion complex with amylose. However, the X-ray powder diffraction pattern of the freeze-dried HAM starch after retrogradation for 7 days at 4 °C (Figure 8, left) showed a pronounced peak at 2θ of 17° and a shoulder at 23° for TPL-containing samples, which are more likely peaks of B-type crystal structure. A weak peak at 19.9° may indicate V-type crystal structure, but it also appears in the HAM starch control, so it might be formed with the indigenous lipids in HAM starch during recrystallizaiton.[31] Thus, the addition of TPLs to HAM starch did not show the

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characteristic structure of V-type crystalline structure peaked at 2θ of 7.5°, 13°, and 19.9°.[31] Furthermore, the iodine binding analysis (Figure 8, right) in a dilute TPL-containing amylose solution, where an increased molecular size was shown from DLS analysis, did not show changes of the iodine binding, indicating the amount of TPLs does not affect the available interior space of amylose helices. In other words, TPLs did not likely bind amylose through hydrophobic interactions in a form of inclusion complex,[32] and if there is an inclusion complex, it is very weak and does not affect the X-ray powder diffraction result.

No inclusion complexation between HAM starch and TPLs was supported by the time-dependent digestion behaviors of the complex (Figure 9). Freshly cooked HAM starch and TPLs showed increased extent of digestion, but after retrogradation for 5 days at 4 °C, the digestibility decreased significantly (from ~85 to 50%) due to the recrystallizaiton of amylose to form crystalline structures that are resistant to digestion; in the meantime, a similar degree of digestion for both the TPL-containing sample and the control demonstrated that TPLs did not have any effect on the digestion. Apparently, the self-assembled amylose−TPLs complex may not have a thermodynamically stable structure, which has been suggested previously (Table 1), but the disruptive function of TPLs on amylose association becomes weaker and weaker during the process of retrogradation. Because the melting enthalpy of the inclusion complex has been used to measure the amylose content,[33] indicating a relatively stable structure of an inclusion complex that could not induce significant changes of starch hydrolysis during the storage time, the time-dependent digestion behavior in this study implies that no strong inclusion complex was formed between HAM starch and TPLs.

The hydrophobic interaction is the predominant noncovalent force for inclusion complex formation,[32] and because no inclusion complex was detected, the hydrophobic interaction cannot be the major force involved in the amylose−TPL complexation. Both the outer surface of amylose helices and TPLs have abundant hydroxyl groups, so the hydrogen bonding might be responsible for their interactions. Evidently, there has been a report that TPLs can interact with rice starch through hydrogen bonds.[18] The TPLs might be sandwiched between amylose molecules through H-bonds at the beginning of interaction (Figure 10) so as to interfere with the direct

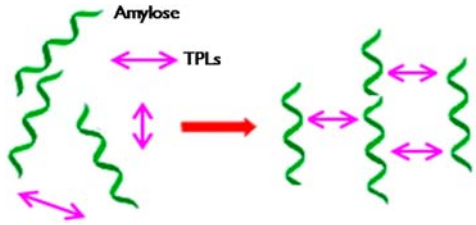

Figure 10. Schematic representation of amylose−TPL complexation through hydrogen bonding.

association of amylose chains, although amylose molecules can be pulled together by TPLs; however, in the long run, TPLs might be expulsed to the outside of amylose by the driving force of amylose recrystallizaiton (TPLs are still bound to amylose, data not shown) to reach a thermodynamic equilibrium, which is likely similar to syneresis of starch gels with an expulsion of liquid.[34] Thus, after a long time period of retrogradation, starch digestion might be solely determined by the retrograded HAM starch, and TPLs' effect on HAM starch digestion, if it even exists, would become negligible.

Health Implications. Although the hydrogen bonding induced complexation between amylose and TPLs is relativly unstable for a long time, their complexation did change the digestion property of HAM starch (Figure 1) by interrupting the normal process of retrogradation. If relatively greater association among amylose molecules is allowed (using less TPLs), a slow digestion property might be achieved. Indeed, the postprandial glycemic response to cooked TPLs-containing HAM starch showed that a slow digestion property of HAM starch has been achieved (Figure 11) by changing the amount

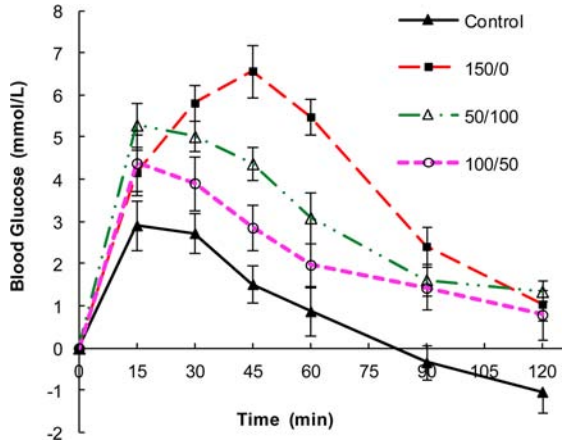

Figure 11. Postprandial glycemic response to HAM starch cocooked with different combinations of TPLs (the first number, mg). The second number means the amount TPLs added after the cooked sample was cooled to 37 °C. The total amount of TPLs is 10% of the starch (1.5 g).

of cocooked TPLs, such as the combination group of 100/50, which means for a total HAM starch of 1.5 g, 100 mg of TPLs was cocooked, and 50 mg was added after the cooked samples were cooled to 37 °C. Thus, adding different amounts of TPLs in different ways could produce a heat-stable slowly digestible starch. More importantly, the experimental result of the postprandial glycemic response demonstrates that the digestibility of HAM starch can be modulated by TPLs, which is the novel finding from the present study.

The current investigation, to our knowledge, is the first comprehensive study focusing on the interactions between neutral carbohydrate of amylose and tea polyphenols that act as a representative of bioactive phenolic compounds widely present in food products. The produced heat-stable SDS through amylose−TPL complexation can simultaneously bring the health benefits of SDS and bioactive components. Additionally, this study also adds new knowledge to the research field of the TPLs' function in carbohydrate metabolism: TPLs can act as a regulator to the digestibility of high-amylose starches through hydrogen bond-mediated amylose−TPL complexation. Although the self-assembled amylose−TPL complex is not thermodynamically so stable, the achieved extended and moderate postprandial glycemic response warrants further investigations to stabilize the structure of the amylose−TPL complex for its slow digestion property.[9] Additionally, it is also noted that the dosage used in the current study is too high for human consumption (according to an average of 3 cups of tea per day containing

240−320 mg of TPLs), but the experimental result does provide new insight into the production of heat-stable SDS with practical applications by using other compounds with properties similar to those of TPLs.

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Notes

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