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Effect of tea products on the *in vitro* enzymatic digestibility of starch

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ABSTRACT

The importance of postprandial hyperglycemia in the treatment of diabetes has been recognized recently. Tea products, such as tea polyphenols (TP), epigallocatechin gallate (EGCG), matcha, and instant tea, were chosen as constituents of tea-flour food, aimed at regulating the release of glucose from starchy foods in the postprandial period. Six starches were chosen for internal composition analysis and hydrolysis studies *in vitro*. Corn starch, wheat starch, and lily root flour appeared to have higher resistant starch content, slower digestion profiles, and lower kinetic constants, implying sustained release of glucose in the gastrointestinal tract. The effect of tea products on starch digestion was determined in order to get a desired formulation of dietary product for patients with hyperglycemia. Compared with macha and instant tea, TP and EGCG exerted greater inhibition of amylase and amyloglucosidase, especially for corn starch with 0.5% TP or 0.5% EGCG.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia resulting from an absolute or a relative insulin deficiency. Clinically, blood glucose values are often used to monitor the occurrence and progression of DM, and help DM patients adjust their diet and lifestyle (Welschen et al., 2005). According to the diagnostic criteria proposed by WHO and IDF, a diagnosis of DM may be made in patients with fasting blood glucose levels greater than 7.0 mM or postprandial blood glucose over 11.1 mM (WHO/IDF Consultation, 2006). Compared with fasting blood glucose, postprandial blood glucose has received more attention in recent years, not only because of the prolonged postprandial hyperglycemia seen in DM patients, but also because long-term hyperglycemia after meals may increase the risk of impaired glucose tolerance and chronic complications (Livesey, Taylor, Hulshof, & Howlett, 2008; Manzano & Williamson, 2010), such as cardiovascular and microvascular diseases. Accordingly, control of postprandial hyperglycemia is of particular importance for the prevention and treatment of DM.

DM patients have stringent dietary requirements, particularly in limiting starchy food intake. The monosaccharides produced from gastrointestinal starch digestion are the main source of postprandial blood glucose. The most significant enzymes in the digestive process of starch are α-amylase and α-glucosidase, respectively hydrolyzing starches into maltose or dextrin, and also yielding glucose, by breaking α-1,4 and α-1,6 glycosidic bonds (Hanhineva et al., 2010; Williamson, 2013). Based on the above mechanism, α-glucosidase inhibitors, such as acarbose, voglibose, and miglitol, have been developed and widely used in clinical practice as hypoglycemic drugs (Hwang et al., 2007). However, this type of medicine is a double-edged sword which can cause flatulence, diarrhea, nausea, and other side effects, resulting in poor compliance (Andayani, Ibrahim, & Asdie, 2010).

At present, the development of natural and effective hypoglycemic substances is a hot topic in the field of DM treatment. Tea polyphenols are tea extracts with various health benefits, including inhibition of amylase and glucosidase activities (Hara & Honda, 1990; He, Lv, & Yao, 2006; Kwon, Apostolidis, & Shetty, 2008; Qiu et al., 2017). Catechins account for 60–80% of tea polyphenols, and include epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and other components. Of the catechins, EGCG accounts for approximately 65%. Yilmazer-Musa et al. reported that EGCG had a marked inhibitory effect on α-amylase (1000 U/mg) and α-glucosidase (10 U/mg) with IC_{50} of 24 μg/ml and 0.3 μg/ml, respectively (Fei et al., 2014; Yilmazer-Musa, Griffith, Michels, Schneider, & Frei, 2012). By reducing the digestion of polysaccharides such as starch, tea polyphenols can result in hypoglycemic effects similar to those of glucosidase inhibitors, with greater patient comfort and safety.

The requirement for dietary control of postprandial hyperglycemia and the side effects of long-term medication appears more necessary now than ever. The ideal dietary agent, rather than being a drug substitute, would actually be a functional food, designed according to pathological characteristics. In theory, it should be suitable for long-term consumption with no toxicity, and should improve postprandial glucose and lower drug dependence. Many DM patients are advised to control their diet, thereby experiencing less satiety and dietary enjoyment.

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Advantages of dietary antihyperglycemic therapy include decreased hunger and the ability to eat starchy foods.

Our group therefore undertook research on special dietary within tea polyphenols as active ingredient for patients with hyperglycemia. Slowly digested starches were chosen to investigate sustained release of glucose in the gastrointestinal tract. To choose the desired starch, *in vitro* hydrolysis studies and internal composition analysis were performed. The influence of tea products on starch digestion was also studied, in order to optimize the formulation of a dietary product for patients with hyperglycemia.

2. Materials and methods

2.1. Materials

Tea polyphenols (Total polyphenol content of 90%) and EGCG (95% purity) were obtained from Ebeikar Tea & Extracts Co., Ltd. (Hangzhou, China). Instant tea (from green tea) and matcha were made in our laboratory, respectively containing 23% and 14% tea polyphenols, and 8.4% and 4.5% EGCG. Corn starch, wheat starch, mung bean starch, lotus root starch, pueraria powder, yam flour, and lily root flour were purchased from Hangzhou local market. Porcine pancreatic α-amylase (CAT. No. S31302) and amyloglucosidase from *Aspergillus niger* (CAT. No. S10017) were supplied by Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). DNS, absolute ethanol, anhydrous sodium acetate, acetic acid, and other chemicals were obtained from Zhejiang Meidikang Trading Co., Ltd. (Hangzhou, China).

2.2. In vitro digestion of different starches

Eight starches, including corn starch, wheat starch, mung bean starch, lotus root starch, pueraria powder, yam flour, and lily root flour were used to determine digestibility *in vitro*. Several slowly digested starches were selected, based on their hydrolysis characteristics, for further study.

2.2.1. Measurement of free glucose (FG) and total starch (TS)

Starch samples (100 mg) and phosphate buffer (0.2 M, pH 5.2) were mixed uniformly and heated at 95 °C for 20 min. After the temperature was reduced to 37 °C, the gelatinized starch sample was centrifuged at 3000 rpm for 20 min. The DNS method was used to determine the glucose content by mixing supernatant with DNS reagent. The percentage of FG in the starch was calculated as follows.

$$\text{FG}\ (\%) = \frac{\text{Content of glucose in supernatant}}{\text{Weight of starch sample}} \times 100\%$$

Each starch sample (100 mg) was suspended in 5 ml distilled water and gelatinized at 95 °C for 20 min. When the temperature of the starch paste dropped to 37 °C, 6 ml 2 M KOH solution were added, followed by magnetic stirring at room temperature for 30 min. After mixing, 3 ml of sodium acetate buffer (0.4 M, pH 4.75) were added, and 2 M HCl or 0.5 M NaOH solution was used to adjust the pH value to 4.75. The mixture was incubated with amyloglucosidase (600 U) in a shaking water bath at 55 °C and 120 rpm for 45 min. At the end of the incubation, the reaction system was centrifuged at 3000 rpm for 20 min, and supernatant was taken to determine the TS content. The percentage of TS could be calculated by the following formula.

$$\text{TS}\ (\%) = \frac{(\text{Content of glucose in supernatant} - \text{FG}) \times 0.9}{\text{Weight of starch sample}} \times 100\%$$

2.2.2. Hydrolysis curves of different starches in vitro

Starch samples (100 mg) were gelatinized in 5 ml of phosphate buffer (0.2 M, pH 5.2) by heating at 95 °C for 20 min. The solution was then cooled to 37 °C in a water bath and equilibrated for 5 min. The enzyme suspension with 30 U α-amylase and 15 U amyloglucosidase was prepared with pH 5.2 phosphate buffer and added to the gelatinized starch samples, which were then shaken in a water bath at 37 °C at a speed of 150 rpm. At predetermined time points of 5, 10, 20, 30, 45, 60, 90, 120, and 180 min, 95% ethanol solution was added at a volume ratio of 3:1 to terminate digestion. Subsequently, the mixture was centrifuged at 3000 rpm for 20 min and aliquots of supernatant were taken to determine the glucose content by the DNS method. The hydrolysis rate was calculated by the following formula.

$$\text{Hydrolysis rate}\ (\%) = \frac{\text{Content of hydrolyzed glucose} \times 0.9}{\text{Weight of starch sample} \times \text{Percentage of TS}} \times 100\%$$

Hydrolysis curves were plotted of the reaction time and hydrolysis rate of different starches.

2.2.3. Determination of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) content

Referring to the relevant literature, the starches were classified as RDS, SDS and RS, according to the rate of hydrolysis in the gastrointestinal tract. RDS, SDS, and RS were the fractions digested within 20 min, between 20 and 120 min, and undigested after 120 min, respectively. Therefore, the RDS, SDS, and RS content could be calculated, based on the hydrolysis data in section 2.2.2 above, with the following equations.

$$\text{RDS}\ (\%) = \frac{(\text{Glucose}_{20min} - \text{FG}) \times 0.9}{\text{Weight of starch sample}} \times 100\%$$

$$\text{SDS}\ (\%) = \frac{(\text{Glucose}_{120min} - \text{Glucose}_{20min}) \times 0.9}{\text{Weight of starch sample}} \times 100\%$$

$$\text{RS}\ (\%) = \frac{\text{TS} - \text{Glucose}_{120\text{min}} \times 0.9}{\text{Weight of starch sample}} \times 100\%$$

$\text{Glucose}_{20\text{min}}$ and $\text{Glucose}_{120\text{min}}$ represented the glucose released within 20 min and 120 min, respectively.

2.3. Effect of tea products on digestibility of starch in vitro

Several slowly digested starches were selected to investigate the influences of tea products on digestibility. The starch samples (100 mg) were weighed and suspended in 5 ml phosphate buffer (0.2 M, pH 5.2). Tea polyphenol, EGCG, matcha, or instant tea solution were then added at mass percentages of 0.25%, 0.5%, and 1%, depending on the starch. The control group was prepared by replacing the tea product solution with an equivalent volume of phosphate buffer. The mixtures were then heated at 95 °C for approximately 20 min, and allowed to cool to 37 °C in a thermostatic water bath. After mixing with enzyme solution (α-amylase 30 U, amyloglucosidase 15 U), the reaction system was shaken at 150 rpm in the 37 °C environment for 3 h. Every hour, the enzymes were inactivated in a subset of samples by adding 95% ethanol solution at three times the sample volume. After centrifugation at 3000 rpm for 20 min, the supernatant glucose content was determined and used to calculate the digestive inhibition rate according to the equation below.

$$\text{Inhibitionrate}\ (\%) = \frac{\text{Glucose}_{\text{control}} - \text{Glucose}_{\text{sample}}}{\text{Glucose}_{\text{control}}} \times 100\%$$

$\text{Glucose}_{\text{control}}$ and $\text{Glucose}_{\text{sample}}$ represented glucose hydrolyzed from pure starch and starch-tea mixture, respectively.

2.4. Statistical analysis

All samples were prepared and analyzed in triplicate. Data were analyzed by using SPSS (version 17.0) with variance analysis (ANOVA). A P value < 0.05 was considered statistically significant.

Table 1
Analysis of starch composition.

Starches	FG%	TS%	RDS%	SDS%	RS%
Corn starch	–	88.9 ± 0.98	10.2 ± 1.20	27.2 ± 1.97[a]	51.5 ± 2.56[a]
Wheat starch	0.93 ± 0.05	93.4 ± 1.48	10.8 ± 2.00	25.9 ± 2.83[a]	56.7 ± 1.03[a]
Yam flour	4.44 ± 0.27	64.8 ± 4.94	10.5 ± 0.99	26.2 ± 2.44[a]	28.1 ± 1.89[c]
Pueraria powder	4.05 ± 0.17	72.6 ± 4.30	12.6 ± 0.20	26.5 ± 1.72[a]	33.5 ± 1.68[c]
Lily root flour	–	96.4 ± 1.84	11.9 ± 1.47	31.9 ± 1.52[ab]	52.6 ± 2.39[a]
Mung bean starch	–	88.8 ± 1.87	11.2 ± 0.32	34.6 ± 3.91[b]	43.0 ± 3.94[b]

The symbol "–" indicates that the glucose content was too low to be detected.
[a,b,c]Different letters in the same column indicate significant differences between mean values ($P < 0.05$).

3. Results and discussion

3.1. In vitro digestion of different starches

3.1.1. Measurement of FG, TS, and starch fractions

The FG and TS percentage data of different starches are shown in Table 1. As shown, yam flour and pueraria powder contained more free glucose, approximately 4%, which was almost four times as much as wheat starch. Meanwhile, the FG levels of corn starch, lily root flour, and mung bean starch were below the detection limit. By contrast, the TS contents of yam flour and pueraria powder were low, while the other four starches contained more TS, over 88%. The differences in FG and TS contents of these six starches were associated with many factors, including types, origins, growth conditions.

Analysis of variance indicated that there were no significant differences among the RDS% values of the six starches. Compared with other starches, the mung bean starch had a larger proportion of SDS (approximately 35%), while its RS fraction was much lower than those of corn starch, wheat starch, and lily root flour ($P < 0.05$). The higher RS content implied slow digestion in the stomach and intestine, and that most of the starch would be degraded in the colon by microorganisms. The lower FG and higher RS ratios identified corn starch, wheat starch, and lily root flour as promising dietary products component for patients with hyperglycemia.

3.1.2. Hydrolysis characteristics of different starches

Hydrolysis–time curves of the different starches were plotted as presented in Fig. 1. The yam flour and pueraria powder exhibited fast digestion during the whole experiment. For mung bean starch, the hydrolysis rate remained relatively constant for 45 min, before rapidly increasing by 10% from 45 min to 60 min. From the hourly data of increased hydrolysis rate, the release rate of glucose from all three starches gradually decreased. As shown in Fig. 1, the hydrolysis rates of corn starch, wheat starch, and lily root flour were significantly lower than those of the other three starches at 60 and 90 min ($P < 0.05$). At 120 min, the corn and wheat starches appeared to have the lowest digestion percentage ($P < 0.05$). At the end of experiment, the least-hydrolyzed starches were wheat starch and lily root flour ($P < 0.05$).

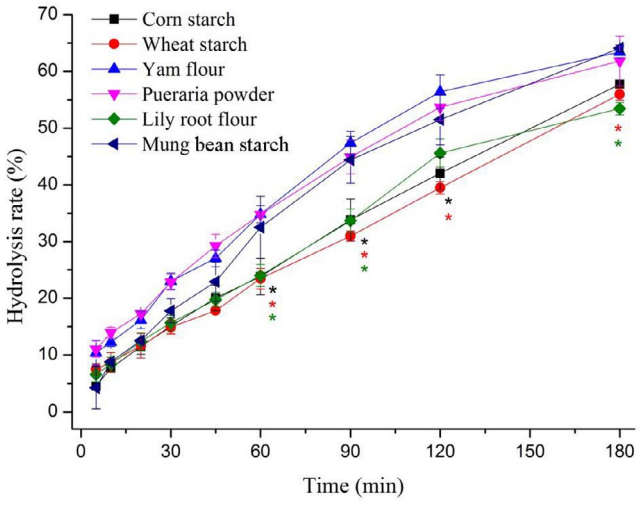

Fig. 1. *In vitro* hydrolysis profiles of starches. The symbols "*" "⁎" "⁎" respectively indicate the significant differences in hydrolysis rates of corn starch, wheat starch and lily root flour compared with those of the other three starches.

In order to further understand the dynamic, nonlinear process of starch hydrolysis, a first-order kinetic equation was used to fit the digestion profiles (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). The following equation was used:

$$C = C_\infty \times (1 - e^{-kt})$$

where C represents the starch hydrolysis rate (in%), C_∞ is the percentage of equilibrium after 180 min (in%), and k is the kinetic constant implying the reaction speed of hydrolysis (in min^{-1}). The detailed parameters and fitting curves are presented in Table 2 and Fig. 2, respectively.

Consistent with the results of hydrolysis profiles, the k values of corn starch, wheat starch, and lily root flour were lower than those of the other starches. The k values of yam flour and pueraria powder were higher, 0.013 min^{-1} and 0.022 min^{-1}, respectively, which may explain the fast digestion profiles in Fig. 1. It has been reported that the hydrolysis degree of kudzu starch is greater than that of sweet potato starch (Guo, Hu, Zhou, Li, & Du, 2016), which is similar to the present result. There was some disagreement between the C_∞ values in Table 2 and RS percentages shown in Table 1. We hypothesized that the fitting property of C_∞ led to the discrepancy. The starch digestibility was affected by various factors, such as origin, structural features, granule size, amylose/amylopectin ratio (Svihus, Uhlen, & Harstad, 2005), which might be a possible explanation for different hydrolysis rates among those starches. Generally, A-type (most cereals and tapioca) starches are more easily hydrolyzed by α-amylase than are B-type (amylomaize and potato) starches. However, *in vitro* enzymatic digestibility of starch is determined mainly by enzyme binding to starch rather than the ordered structures of starch (Wang, Wang, Liu, Wang, & Copeland, 2017). Based on the hydrolysis rate and k values, corn starch, wheat starch and lily root flour were considered to be slowly digested materials and were used to conduct the next experiment.

Table 2
Kinetic equation parameters for hydrolysis of different starches.

Starches	C_∞/%	$k \times 10^3/min^{-1}$	R^2
Corn starch	75.0 ± 6.56	8.06 ± 1.42	0.9925
Wheat starch	102 ± 24.84	4.28 ± 1.30	0.9659
Yam flour	69.4 ± 4.39	13.1 ± 1.88	0.9767
Pueraria powder	49.0 ± 4.64	21.5 ± 3.49	0.9609
Lily root flour	69.3 ± 7.42	8.13 ± 1.54	0.9801
Mung bean starch	78.8 ± 3.69	9.30 ± 0.90	0.9956

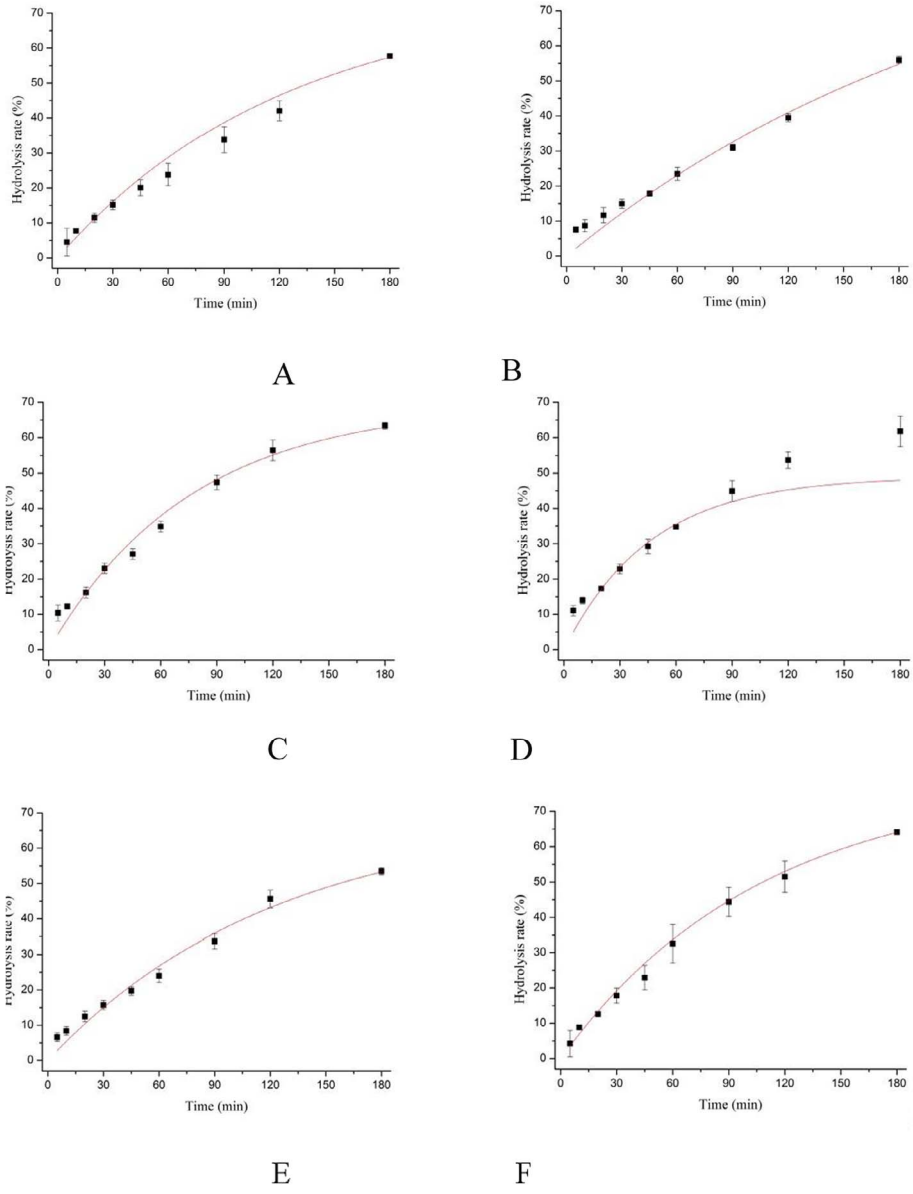

Fig. 2. Kinetic model of starch hydrolysis. A: Corn starch; B: Wheat starch; C: Yam flour; D: Pueraria powder; E: Lily root flour; F: Mung bean starch.

3.2. *Effect of tea products on digestibility of starch in vitro*

The effects of TP, EGCG, matcha, and instant tea on starch digestion were investigated by *in vitro* enzymatic hydrolysis tests. The inhibition rates at different intervals, with three additions of tea products, are listed in Tables 3. As shown, tea products either inhibited or promoted the digestibility of starch. After 1 h, 0.5% TP and 0.5% EGCG, for example, were capable of reducing the hydrolysis rate of corn starch by 20.6% and 25.1%, respectively. However, 0.5% matcha accelerated the starch digestion process by 3.75% after 1 h. This implied a complex interaction between inhibitors and the mixed enzyme system. As previously reported in the literature, the inhibitory effect of tea extract on the enzymes was related to the hydrogen bonds formed by hydroxyl groups of TP and catalytic residues of enzyme (Lo Piparo et al., 2008; Sun, Warren, Netzel, & Gidley, 2016). Another possible mechanism would be stabilization of active site interactions through a conjugated π-system associated with the unsaturated C-ring in the structure of TP (Miao et al., 2013; Sun et al., 2016; Xiao, Ni, Kai, & Chen, 2013). According to our data, interactions between the active ingredients in tea and digestive enzymes could also increase the hydrolysis rate of starch, contrary to the above inhibition theory. This experimental phenomenon warrants further study to aid the development of dietary products for patients with hyperglycemia. Similarly, it is reported that tea polyphenols noncompetitively inhibited the digestion of waxy or normal corn starch, but increased the digestion rate of high amylose corn starch due to a possible interaction between tea polyphenols and amylose (Liu, Wang, Peng, & Zhang, 2011). Meanwhile, the enzyme activation effect of tea products might be explained by the other components, such as caffeine and ions, enhancing α-amylase activity and counteracting the inhibition effect of tea products (Yang & Kong, 2016).

Another interesting finding was the change in inhibition rate after 3 h with one particular tea product at a certain addition dosage. Taking corn starch as an example, inhibition was gradually decreased by 0.5% TP, while 1% TP would enhance the inhibitory effect within the first 2 h, before exerting a promoting effect of approximately 4.1%. The inhibition of wheat starch digestion with 0.5% EGCG showed an increasing trend. When mixing matcha or instant tea solution with other starch samples, inhibition rates rose after an initial decline in many cases. For example, the inhibition rates of corn starch digestion with 0.25% matcha were 3.07% at 1 h, −13.4% at 2 h, and finally, 0.25% at 3 h. The various reaction courses could be correlated with the dynamic balance between substrates, inhibitors and enzymes. A series of studies

Table 3
Inhibition rates of TP, EGCG, matcha and instant tea on starches (%, mean ± SD, n = 3).

Starches	Time (h)	Addition of TP			Addition of EGCG			Addition of matcha			Addition of instant tea		
		0.25%	0.5%	1%	0.25%	0.5%	1%	0.25%	0.5%	1%	0.25%	0.5%	1%
Corn starch	1	12.1 ± 3.76	20.6 ± 1.17	11.8 ± 1.96	14.8 ± 2.64	25.1 ± 3.75	1.88 ± 4.48	3.07 ± 2.59	−3.75 ± 2.85	−4.78 ± 1.35	4.67 ± 1.95	5.64 ± 4.13	−0.56 ± 2.06
	2	8.49 ± 3.93	12.2 ± 0.22	13.9 ± 1.25	6.66 ± 2.08	14.0 ± 1.73	5.00 ± 1.87	−13.4 ± 4.40	−0.25 ± 2.12	−3.11 ± 1.36	−10.4 ± 1.84	−4.34 ± 1.02	−0.45 ± 5.39
	3	5.21 ± 1.40	6.80 ± 2.81	−4.10 ± 2.16	5.17 ± 0.23	11.7 ± 2.57	−3.39 ± 6.01	0.25 ± 2.89	−12.55 ± 3.68	−7.68 ± 1.57	3.48 ± 1.39	−1.73 ± 1.03	1.23 ± 1.34
Wheat starch	1	−3.78 ± 2.91	−2.21 ± 0.85	−4.54 ± 4.91	3.59 ± 2.66	0.86 ± 1.98	6.29 ± 1.25	−2.51 ± 3.99	−0.32 ± 2.03	1.71 ± 2.86	−2.94 ± 1.36	−9.27 ± 1.11	−4.64 ± 2.87
	2	−5.29 ± 6.65	0.22 ± 1.45	−22.5 ± 1.72	−2.94 ± 1.84	1.40 ± 0.25	6.60 ± 0.66	−4.51 ± 2.07	−1.8 ± 0.67	−2.46 ± 2.05	−3.17 ± 1.80	−4.20 ± 2.60	−1.31 ± 3.06
	3	6.60 ± 2.48	−19.50 ± 4.28	−17.6 ± 0.12	0.26 ± 2.42	3.66 ± 2.21	2.04 ± 2.19	2.31 ± 0.94	−0.5 ± 2.46	−0.2 ± 0.64	3.09 ± 1.90	1.31 ± 4.66	1.24 ± 1.25
Lily root flour	1	−2.51 ± 1.67	1.69 ± 7.85	1.82 ± 8.83	1.33 ± 3.24	4.93 ± 2.69	0.68 ± 0.55	0.18 ± 0.42	2.77 ± 1.12	4.26 ± 1.7	0.68 ± 2.78	−2.74 ± 2.83	2.14 ± 3.31
	2	7.10 ± 1.32	3.67 ± 2.31	8.90 ± 1.26	4.99 ± 1.99	1.11 ± 3.43	−2.90 ± 6.16	0.42 ± 1.77	−4.95 ± 2.26	−5.94 ± 2.09	−6.23 ± 4.26	−3.02 ± 5.04	−1.77 ± 2.78
	3	5.05 ± 1.97	1.17 ± 5.18	1.68 ± 7.28	4.44 ± 2.05	−3.47 ± 5.34	−2.03 ± 3.68	−4.73 ± 3.14	0.53 ± 2.11	0.34 ± 0.65	−5.78 ± 2.36	1.44 ± 1.14	3.89 ± 1.54

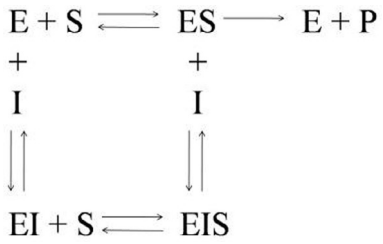

Fig. 3. Sketch map of non-competitive inhibition. E: Enzyme; S: Substrate; P: Product; I: Inhibitor.

had reported that the inhibition of digestive enzymes by active tea compounds was of a non-competitive type (Miao, Jiang, Jiang, Zhang, & Li, 2015). Forester, Gu, and Lambert (2012) observed a 34% non-competitive inhibition of pancreatic amylase by EGCG.

A sketch map of non-competitive inhibition is shown in Fig. 3, where a reaction mechanism between inhibitor, substrate, and enzyme exists. It can be seen that the combination of enzyme and inhibitor would not influence the binding of substrate to enzyme, while the substrate would not obstruct the enzyme and inhibitor. In other words, the amount of either substrate or inhibitor has less effect on the combination of enzyme with the other. This characteristic might explain why there was little correlation between the dose of inhibitors and inhibition rates obtained in our experiment. Furthermore, the binding interaction was reversible; however, the release of glucose was irreversible. It was hypothesized that the variations in inhibition rates after 3 h were related to the reversible nature of binding. At different response times, it is possible that minute changes in binding force or spatial arrangement led to a change of reaction direction, thus giving varying inhibitory or promoting results.

The third concern about our results was the effect of active ingredient amount in the tea products on inhibition rate. The purity of TP used in the experiment was 90.0%, with 40.3% EGCG, 6.6% ECG, and 1.2% caffeine. The purity of the EGCG used was 95.0%. The instant tea and matcha, produced in our laboratory, respectively contained 22.7% and 14.0% tea polyphenols, 4.8% and 2.3% caffeine, 2.3% and 1% ECG, and 8.4% and 4.5% EGCG. It was reported that EGCG strongly inhibited α-glucosidase (Yilmazer-Musa et al., 2012). Similarly, Sun et al. (2016) observed the greater inhibition of α-amylase by the galloylated catechins, EGCG and ECG. Thus, compared with pure TP and EGCG, matcha and instant tea mostly exhibited promotion or weak inhibition of starch digestion. The lower catechin content in matcha and instant tea depressed the inhibitory action, and other complex components, such as ions and caffeine, also greatly influenced the hydrolysis efficiency. Several studies demonstrated that Cl^- (Aghajari, Feller, Gerday, & Haser, 2002), Co^{2+}, Ca^{2+} (Saboury, 2002), Ba^{2+}, and caffeine in tea products could enhance α-amylase activity (Kashani-Amin, Yaghmaei, Larijani, & Ebrahim-Habibi, 2013; Usha & Hemalatha, 2011). Yang and Kong (2016) reported that tea extracts and purified TP might exhibit different effect due to the presence of other components, and speculated that the caffeine and ions in tea extracts promoted the α-amylase activity, but retarded the mild inhibitory effect of polyphenols. The higher caffeine content in matcha and instant tea greatly increased the starch digestion rate.

According to the data in Table 3, the inhibition rate of TP and EGCG on corn starch digestion was greater than that of wheat starch and lily root flour. For corn starch, the inhibitory effects of 0.5% TP and 0.5% EGCG were superior to those of the corresponding 0.25% and 1% concentrations. Variance tests indicated that the inhibition rate of the 0.5% EGCG sample at 1 h was significantly higher than those of other corn starch samples ($P < 0.05$), with the exception of the 0.5% TP preparation. Consequently, corn starch, with 0.5% TP or 0.5% EGCG, was selected as the basic ingredient in our special dietary product for diabetes mellitus. Due to their slow digestion rate, these two formulations are expected to be further

developed into a truly special dietary product to reduce the postprandial blood glucose of diabetes patients.

4. Conclusion

Special dietary, or functional food, can be beneficial for improving postprandial glucose levels in diabetic patients, while also satisfying their demands for delicious food. In this article, tea products as glucose regulators were added to starchy foods, aimed at gaining a special dietary product for diabetes patients. Based on our data, corn starch, wheat starch, and lily root flour had lower FG, higher RS ratios, and lower hydrolysis rates than the other starches, showing that they underwent slow digestion. Compared with matcha and instant tea, TP and EGCG exerted greater inhibitory effects on amylase and amyloglucosidase, which suggested their potential as natural active ingredients for glycemic control. The inhibitory activity might be associated with hydrogen bonds between the hydroxyl groups of TP and the catalytic residues of enzyme and formation of a conjugated π-system that stabilized the interaction with the active site. Corn starch with 0.5% TP or 0.5% EGCG was the optimized preparation identified by this study. Further work is underway to apply these findings to real products that can improve postprandial glucose *in vivo*.

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Conflict of interest statement:

The authors do not have any possible conflicts of interest.

References

Aghajari, N., Feller, G., Gerday, C., & Haser, R. (2002). Structural basis of α-amylase activation by chloride. *Protein Science, 11*(6), 1435–1441.

Andayani, T. M., Ibrahim, M. I. M., & Asdie, A. H. (2010). The safety of triple therapy with oral antidiabetics versus insulin in type 2 diabetes. *Asian Journal of Pharmaceutical and Clinical Research, 3*(3), 201–203.

Fei, Q., Gao, Y., Zhang, X., Sun, Y., Hu, B., Zhou, L., ... Zeng, X. (2014). Effects of Oolong tea polyphenols, EGCG, and EGCG3″Me on pancreatic α-amylase activity *in vitro*. *Journal of Agriculture and Food Chemistry, 62*(39), 9507–9514.

Forester, S. C., Gu, Y., & Lambert, J. D. (2012). Inhibition of starch digestion by the green tea polyphenol, (-)-epigallocatechin-3-gallate. *Molecular Nutrition and Food Research, 56*(11), 1647–1654.

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437.

Guo, L., Hu, J., Zhou, X., Li, X., & Du, X. (2016). *In vitro* digestibility of kudzu starch by using α-amylase and glucoamylase. *Starch/Stärke, 68*, 140–150.

Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., & Poutanen, K. (2010). Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences, 11*(4), 1365–1402.

Hara, Y., & Honda, M. (1990). The inhibition of α-amylase by tea polyphenols. *Agricultural and Biological Chemistry, 54*(8), 1939–1945.

He, Q., Lv, Y., & Yao, K. (2006). Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. *Food Chemistry, 101*(3), 1178–1182.

Hwang, J. Y., Zhang, J., Kang, M. J., Lee, S. K., Kim, H. A., Kim, J. J., & Kim, J. I. (2007). Hypoglycemic and hypolipidemic effects of *Saururus chinensis* Baill in streptozotocin-induced diabetic rats. *Nutrition Research and Practice, 2*, 100–104.

Kashani-Amin, E., Yaghmaei, P., Larijani, B., & Ebrahim-Habibi, A. (2013). Xanthine derivatives as activators of alpha-amylase: Hypothesis on a link with the hyperglycemia induced by caffeine. *Obesity Research and Clinical Practice, 7*(6), 487.

Kwon, Y. I., Apostolidis, E., & Shetty, K. (2008). Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. *Journal of Food Biochemistry, 32*(1), 15–31.

Liu, J., Wang, M., Peng, S., & Zhang, G. (2011). Effect of green tea catechins on the postprandial glycemic response to starches differing in amylose content. *Journal of Agriculture and Food Chemistry, 59*, 4582–4588.

Livesey, G., Taylor, R., Hulshof, T., & Howlett, J. (2008). Glycemic response and health – A systematic review and meta-analysis: Relations between dietary glycemic properties and health outcomes. *American Journal of Clinical Nutrition, 87*(1), 258S–268S.

Lo Piparo, E., Scheib, H., Frei, N., Williamson, G., Grigorov, M., & Chou, C. J. (2008). Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-amylase. *Journal of Medicinal Chemistry, 51*(12), 3555–3561.

Manzano, S., & Williamson, G. (2010). Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. *Molecular Nutrition and Food Research, 54*(12), 1773–1780.

Miao, M., Jiang, H., Jiang, B., Li, Y., Cui, S. W., & Jin, Z. (2013). Elucidation of structural difference in theaflavins for modulation of starch digestion. *Journal of Functional Foods, 5*(4), 2024–2029.

Miao, M., Jiang, B., Jiang, H., Zhang, T., & Li, X. (2015). Interaction mechanism between green tea extract and human α-amylase for reducing starch digestion. *Food Chemistry, 186*, 20–25.

Qiu, S., Yadav, M. P., Zhu, Q., Chen, H., Liu, Y., & Yin, L. (2017). The addition of corn fiber gum improves the long-term stability and retrogradation properties of corn starch. *Journal of Cereal Science, 76*, 92–98.

Saboury, A. A. (2002). Stability, activity and binding properties study of α-amylase upon interaction with Ca2+ and Co2+. *Biologia – Section Cellular and Molecular Biology, 57*(SUPPL. 11), 221–228.

Sun, L., Warren, F. J., Netzel, G., & Gidley, M. J. (2016). 3 or 3′-Galloyl substitution plays an important role in association of catechins and theaflavins with porcine pancreatic α-amylase: The kinetics of inhibition of α-amylase by tea polyphenols. *Journal of Functional Foods, 26*, 144–156.

Svihus, B., Uhlen, A. K., & Harstad, O. M. (2005). Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Animal Feed Science and Technology, 122*, 303–320.

Usha, B., & Hemalatha, K. P. J. (2011). Purification of α-amylase from germinating little millets (Panicum sumatrense). *Journal of Pharmacy Research, 5*, 1370.

Wang, S., Wang, S., Liu, L., Wang, S., & Copeland, L. (2017). Structural orders of wheat starch do not determine the *in vitro* enzymatic digestibility. *Journal of Agriculture and Food Chemistry, 65*, 1697–1706.

Welschen, L. M., Bloemendal, E., Nijpels, G., Dekker, J. M., Heine, R. J., Stalman, W. A., & Bouter, L. M. (2005). Self-monitoring of blood glucose in patients with type 2 diabetes mellitus who are not using insulin. *The Cochrane Database of Systematic Reviews* (2) CD005060.

WHO/IDF Consultation (2006). *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Report of a WHO/International Diabetes Federation Consultation.* Geneva, Switzerland: World Health Organization Document Production Services.

Williamson, G. (2013). Possible effects of dietary polyphenols on sugar absorption and digestion. *Molecular Nutrition and Food Research, 57*(1), 48–57.

Xiao, J., Ni, X., Kai, G., & Chen, X. (2013). A review on structure-activity relationship of dietary polyphenols inhibiting α-amylase. *Critical Reviews in Food Science and Nutrition, 53*(5), 497–506.

Yang, X., & Kong, F. (2016). Effects of tea polyphenols and different teas on pancreatic α-amylase activity *in vitro*. *LWT-Food Science and Technology, 66*, 232–238.

Yilmazer-Musa, M., Griffith, A. M., Michels, A. J., Schneider, E., & Frei, B. (2012). Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. *Journal of Agriculture and Food Chemistry, 60*(36), 8924–8929.