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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.