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Effect of steeping temperature on antioxidant and inhibitory activities of green tea extracts against α-amylase, α-glucosidase and intestinal glucose uptake

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ABSTRACT

The objective of the present study was to evaluate the effect of steeping temperature on the biological activities of green tea, including the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity, α-glucosidase and α-amylase inhibitory activities, and glucose uptake inhibitory activity in Caco-2 cells. Results showed that, with increasing extraction temperature, the polyphenol content increased, which contributed to enhance antioxidant activity and inhibitory effects on α-glucosidase and α-amylase. Green tea steeped at 100 °C showed the highest DPPH radical-scavenging activity and inhibitory effects on α-glucosidase and α-amylase activities with EC_{50} or IC_{50} values of 6.15 μg/mL, 0.09 mg/mL, and 6.31 mg/mL, respectively. However, the inhibitory potential on glucose uptake did not show an upward trend with increasing extraction temperature. Green tea steeped at 60 °C had significantly stronger glucose uptake inhibitory activity ($p < 0.05$). The integrated data suggested that steeping temperature should be considered when evaluating the biological activities of green tea.

1. Introduction

Diabetes is characterized by chronic hyperglycemia and has become a major health problem all over the world. A sustained high plasma glucose level is associated with an increased risk of cardiovascular disease, neuropathy, retinopathy, nephropathy, and other dysfunctions (Brownlee, 2001). The therapeutic approach for diabetes is to maintain the plasma glucose level within normal range, and the glucose uptake capacity has been reported to be increased in the small intestine of diabetic patients (Debnam & Unwin, 1996). Moreover, the mRNA and protein levels of sodium-dependent glucose transporter 1 (SGLT1), Na^+-K^+-ATPase, and glucose transporter 2 (GLUT2) were found to be over-expressed in streptozotocin-induced diabetic mice (Li et al., 2013). Carbohydrates are hydrolyzed into monosaccharides by digestive enzymes, and then absorbed through glucose transporters, mainly SGLT1 and GLUT2. Therefore, inhibiting digestive enzymes or reducing glucose uptake in small intestine is an effective therapeutic approach to delay the expeditious generation of blood glucose in diabetic patients after food uptake.

Diabetes is strongly associated with oxidative stress. Kumar and Rizvi (2015) found that plasma antioxidant potential showed a significant decrease (73%) in diabetic rats, and black tea extract supplementation improved this potential. Oxidative stress, which may weaken the antioxidative defense system and increase free radicals levels, is involved in the etiology of diabetes development and diabetic complications affecting the eyes, nerves, kidneys, and blood vessels (Baynes, 1991).

Tea, prepared from the buds and leaves of the plant *Camellia sinensis* (L.) O. Kuntze, is one of the most popular beverages worldwide. Due to differences in manufacturing, tea is generally classified into three types: non-fermented tea (green tea), partially fermented tea (oolong tea), and fully fermented tea (black tea). Green tea contains a large amount of polyphenol, which has been identified to have antioxidant activity and beneficial properties in diabetes (Almajano, Carbó, Jiménez, & Gordon, 2008; Kobayashi et al., 2000; Shimizu, Kobayashi, Suzuki, Satsu, & Miyamoto, 2000). Green tea is a traditional beverage in China and is usually brewed in hot water for 20–40 s, and this infusion is repeated seven times. In recent years, cold water steeping has become a popular to make tea, especially in Taiwan, due to the advantages of lower caffeine, lower bitterness, and stronger aroma in the infusions of tea leaves steeped in water at 25 °C for 2 h or at 4 °C water for over 4 h (Venditti et al., 2010). A comparative study of hot and cold tea infusions by Lin, Liu, and Mau (2008) revealed that hot

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water extracts were more effective in lipid peroxidation inhibition and showed greater reducing power, while cold water extracts had more effective scavenging ability for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals and chelating ability for ferrous ions. Therefore, temperature is one of the most crucial parameters affecting the bioactive compounds of tea infusions and their biological capacities. However, little information is available about the effect of steeping temperature on the bioactive compounds and biological capacities of green tea extracts steeped at different temperatures. The objective of the present study was to evaluate the effects of various steeping temperatures on the main components of green tea infusions. The relationship between those biological activities and steeping temperature was also investigated by analyzing the DPPH free radical-scavenging capacity, α-glucosidase and α-amylase inhibitory activities, and glucose uptake inhibitory activity in Caco-2. This research provides guidance on the scientific consumption of green tea for diabetic adults.

2. Material and methods

2.1. Materials

Green tea (*Camellia sinensis* (L.) O. Kuntze) was purchased from Changchen Tea Factory (Enshi, Hubei, China). α-Glucosidase (EC 3.2.1.20, from *Saccharomyces cerevisiae*, G0660), α-amylase (EC 3.2.1.1, from hog pancreas, 10080), DPPH (D9132), and *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG, N1377) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Caffeine, gallic acid (GA), and catechin standard ((−)-epicatechin (EC), (+)-catechin (C), (−)-epigallocatechin (EGC), (−)-gallocatechin (GC), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), (−)-epigallocatechin gallate (EGCG), and (−)-gallocatechin gallate (GCG)) were purchased from Shanghai Yuanye Biological Technology Company Limited (Shanghai, China). Stock cultures of Caco-2 cells (the TC7 clone-human adenocarcinoma) were obtained from Dr. Paul Sharp in King's College London (London, UK). Dulbecco's modified eagle medium (DMEM), fetal bovine serum, non-essential amino acids, penicillin/streptomycin, and trypsin were purchased from HyClone Laboratories Inc. (Logan, Utah, USA); plasmocin and biodegradable scintillation solution from InvivoGen (San Diego, USA) and National Diagnostics (Atlanta, USA), respectively; and D-[^{14}C] glucose from Perkin Elmer (Boston, USA). All other reagents and solvents were purchased from China National Pharmaceutical Group Corporation (Beijing, China) and were of analytical grade. Purified water (18.2 MΩ) was prepared using a Millipore Mill-Q Ultrapure Water System (Billerica, MA, USA).

2.2. Preparation of tea extracts

Green tea (50 g) was cut into thin slices and extracted with 1 L water at varying temperatures (20, 40, 60, 80, and 100 °C) for 7 min. The mixture was then centrifuged at 4,000 rpm for 5 min, followed by collecting the supernatant and extracting the residue once more under the same conditions. The two supernatants were pooled and concentrated using a the vacuum rotary evaporator (RE-52AA, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) at 55 °C under reduced pressure and then freeze-dried (Coolsafe 110-4, Labogene ScanVac, Lynge, Denmark).

2.3. Characterization of the green tea extracts

Total polyphenol content in green tea extracts was determined according to the Folin-Ciocalteu method (Ranilla, Kwon, Apostolidis, & Shetty, 2010). The amino acids content was assessed according to the China National Standard (B/T 8314–2002, GAQSIQ, P. R. China, 2002). Water soluble carbohydrates were measured by the anthrone-sulfuric acid method (Morris, 1948). The total flavonoids and polysaccharides were measured as previously reported (Zhong, 1989). Caffeine, GA, and catechins were identified and quantified by high performance liquid chromatography (HPLC, Agilent 1100VL, Agilent Technologies Inc., CA, USA) as described in our previous work (Liu, Yu, Zhu, Zhang, & Chen, 2016).

2.4. DPPH radical scavenging assay

The DPPH free radical scavenging activity of each sample was determined as previously reported (Kondo, Tsuda, Muto, & Ueda, 2002). Briefly, 1.0 mL of various concentrations of samples was mixed with 1.0 mL of DPPH (0.15 mmol/L) radical solution dissolved in methanol. After standing at room temperature in the dark for 30 min, the absorbance was measured at 516 nm using a spectrophotometer (722N, Shanghai Jinghua Science & Technology Instruments Co., Ltd., Shanghai, China), and the DPPH radical scavenging activity (%) was calculated according to the following equation:

$$\text{Scavenging activity } (\%) = (1 - \text{OD}_{\text{sample}}/\text{OD}_{\text{control}}) \times 100$$

where $OD_{control}$ is the absorbance of DPPH standard solution; OD_{sample} is the value for added sample concentration.

2.5. Inhibitory assay of α-glucosidase

α-Glucosidase inhibitory activity was determined as previously reported (Zhu et al., 2014). Briefly, mixtures of 100 μL of enzyme solution (1 unit/mL) and 50 μL of the sample were incubated in a 96-well plate at 25 °C for 10 min, followed by the addition of 50 μL of *p*NPG (2.5 mmol/L) to each well, and incubation at 25 °C for 5 min. At the end of the reaction, the absorbance was determined at 405 nm using a microplate reader (ELX-800, Bio-Tek Instruments Inc., Winooski, USA). The enzyme, sample, and *p*NPG were dissolved in phosphate-buffered saline (PBS, pH 6.8). The α-glucosidase inhibitory activity was expressed as the IC_{50} according to the percentage inhibition and calculated by the following equation:

$$\text{Inhibition } (\%) = (1 - \text{OD}_{\text{sample}}/\text{OD}_{\text{control}}) \times 100$$

where OD_{sample} is the absorbance value of enzyme + sample + *p*NPG; $OD_{control}$ is the absorbance value of enzyme + PBS + *p*NPG.

2.6. Inhibitory assay of α-amylase

The pancreatic α-amylase activity was assayed with an iodine-starch kit as previously reported with slight modifications (Cheng et al., 2015). Briefly, mixtures of 50 μL of enzyme solution (0.01 mg/mL) and 50 μL of the sample in PBS (pH 7.0) were incubated at 37 °C for 5 min, followed by the addition of 1.0 mL of starch substrate (0.4 g/L). After 15 min incubation at 37 °C, 1.0 mL of iodine diluent (0.01 mol/L) was added to terminate the reaction. Finally, the reaction mixture was supplemented with 5 mL of distilled water, and the absorbance was measured at 660 nm using a 722N spectrophotometer. The inhibition of α-amylase activity was calculated by the following equation:

$$\text{Inhibition } (\%) = (1 - \text{OD}_{\text{sample}}/\text{OD}_{\text{control}}) \times 100$$

where OD_{sample} is the absorbance value of enzyme + sample + substrate; $OD_{control}$ is the absorbance value of enzyme + PBS + substrate.