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Green tea catechins reduced the glycaemic potential of bread: An *in vitro* digestibility study

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ABSTRACT

Green tea catechins are potent inhibitors of enzymes for carbohydrate digestion. However, the potential of developing low glycaemic index bakery food using green tea extract has not been investigated. Results of this study showed that addition of green tea extract (GTE) at 0.45%, 1%, and 2% concentration levels significantly reduced the glycaemic potential of baked and steamed bread. The average retention levels of catechins in the baked and steamed bread were 75.3–89.5% and 81.4–99.3%, respectively. Bread fortified with 2% GTE showed a significantly lower level of glucose release during the first 90 min of pancreatic digestion as well as a lower content of rapidly digested starch (RDS) content. A significantly negative correlation was found between the catechin retention level and the RDS content of bread. The potential of transforming bread into a low GI food using GTE fortification was proven to be promising.

1. Introduction

Baked bread has a history dating back to the Neolithic era and is currently the staple food of Europe, European-derived cultures such as the Americas, Middle East and North Africa. Steamed bread, a type of bread that originates from China, is made from fermented wheat flour dough and processed by steaming. Steamed bread is getting popular in Hong Kong, Taiwan and countries of Southeast Asia. Studies have shown that with the appropriate formulation and processing conditions, bread of acceptable qualities can be produced with the fortification of phytochemical based ingredients such as green tea extract (GTE) (Ananingsih, Gao, & Zhou, 2012; Wang, Zhou, & Isabelle, 2007).

GTE is derived from dried green tea leaves and contains mainly natural tea polyphenol antioxidants. One major component of the polyphenol antioxidants is known as tea catechins. Due to its increasingly evident health benefits such as being anti-oxidative and anti-mutagenic which contribute to lowered risk of chronic diseases, green tea and GTE enriched food are highly sought after among people who pursue healthier lifestyles (Basu & Lucas, 2007). The effects of GTE fortification on the physical and sensory characteristics of baked and steamed bread have been studied previously (Lee, 2012; Wang et al., 2007).

Studies conducted by Liu, Wang, Peng, and Zhang (2011), Yilmazer-Musa, Griffith, Michels, Schneider, and Frei (2012), Koh, Wong, Loo, Kasapis, and Huang (2010), showed that tea polyphenols such as tea catechins inhibited the activity of α-amylase and α-glucosidase which are two key enzymes for starch digestion in human. Another study showed that among 4 types of digestive enzymes (α-amylase, pepsin, trypsin and lipase) investigated, tea polyphenols inhibited the activity of α-amylase the most (He, Lv, & Yao, 2007). These findings suggested the potential role of tea catechins in influencing the digestibility of starch in GTE-fortified bread which has not yet been addressed in literature. The retarded starch digestion is favorable in developing low glycaemic index (GI) food (Robyt, 2009). The consumption of low GI foods results in relatively small fluctuations in blood glucose level (Englyst & Hudson, 1996) which can reduce the risk of developing type 2 diabetes mellitus over a prolonged period of time (Simmons, Unwin, & Griffin, 2010). Hence, fortification with green tea catechins might be able to reduce the glycaemic potential of bread.

Although it has been argued that food values intended as guidelines for glycaemic control must be validated based on clinical

Abbreviations: GTE, green tea extract; ECG, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; CG, catechin-3-gallate; GCG, (−)-gallocatechin-3-gallate; RDS, rapidly digested starch; SDS, slowly digested starch.

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trials, *in vivo* digestibility analysis has been reported to be intrinsically poor in precision even in well-controlled experiments (Monro, Mishra, & Venn, 2010). This is largely due to inherent variability in human blood glucose responses (Monro & Mishra, 2010). Therefore, many authors have used *in vitro* digestibility analysis to study the digestion of carbohydrate food (Chung, Liu, Peter Pauls, Fan, & Yada, 2008; Monro & Mishra, 2010; Monro et al., 2010; Woolnough, Monro, Brennan, & Bird, 2008). *In vitro* digestibility model described by Monro and Mishra (2010) has been used to determine important starch fractions such as rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS) in foods. Since the *in vitro* approach was demonstrated to be a viable, rapid and cost effective alternative to *in vivo* studies for the preliminary screening of the GI of foods (Woolnough et al., 2008), it can be used to investigate the effects of GTE fortification on the digestibility of bread which has yet to be reported in the literature.

In this study, the digestibility of baked and steamed bread containing various concentrations of GTE was investigated using an *in vitro* digestibility test. The amount of catechins in the bread after baking and steaming was quantified using high performance liquid chromatograph (HPLC) analysis (Wang & Zhou, 2004; Wang, Zhou, & Jiang, 2008). Correlations between the amount of green tea catechins retained and the digestibility of bread were analyzed and compared between baked and steamed bread. Results of this study would provide insights on the nutritional benefits of green tea catechins on the glycaemic potential of bread.

2. Materials and methods

2.1. Materials

Baked bread flour (13.1% protein, Prima, Singapore), steamed bread flour (7.9% protein, Gim Hin Lee, Singapore), shortening (Phoon Huat, Singapore), instant dry yeast (*Saccharomyces cerevisiae*) (Algict Bruggeman N.V., Belgium), fine salt and sugar were purchased from local supermarket. Green tea extract (GTE) was procured from Pure Herbal Remedies Pte Ltd. (Singapore), which was made from green tea (*Camellia sinensis*) leaves harvested in Guangxi, China. The GTE was specified by the manufacturer to contain total polyphenols (⩾95%), total catechins (⩾65%) and total (−)-epigallocatechin gallate (EGCG) (⩾30%) as a quality marker of GTE.

Four catechin standards (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (ECG), (−)-gallocatechin-3-gallate (GCG), and catechin-3-gallate (CG) were purchased from Sigma–Aldrich (St Louis, MO, USA). Pancreatin (P7545; 8X USP specifications; amylase activity 200 units/mg), pepsin (800–2500 units/mg), α-amylase (⩾10 units/mg solid) and amyloglucosidase (⩾300 units/mL) used in the *in vitro* digestion study were purchased from Sigma–Aldrich (St Louis, MO, USA). Amyloglucosidase (E-AMGDF; 3260 units/mL) used in the reducing sugar assay was purchased from Megazyme International (Wicklow, Ireland). Formic acid (ACS grade) was purchased from Merck (Germany). Methanol (HPLC grade) and absolute ethanol (HPLC grade) were purchased from Tedia Company Inc (USA). Chemicals used for 3,5-dinitrosalicylic acid (DNS) analysis were described in previous work (Ranawana & Henry, 2013).

2.2. Preparation of GTE fortified baked and steamed bread

Baked and steamed bread fortified with 0% (control), 0.45%, 1% and 2% GTE were prepared using a no-time bread-making process described by Wang and Zhou (2004), Ananingsih et al. (2012) with slight modifications, respectively. The ingredients of baked bread included 1 kg flour, 620 mL water, 40 g sugar, 30 g shortening, 20 g salt and 10 g dry instant yeast. The formulation of steamed bread was 1 kg flour, 550 mL water, 10 g of shortening, dry instant yeast, salt and sugar for each. GTE powder was added at levels of 0, 4.5, 10 and 20 g per 1000 g of flour for both types of bread. All ingredients were slowly mixed (44 rpm) for 1 min followed by an intense mixing (100 rpm) of 7 min or a mild mixing (66 rpm) of 5 min for baked and steamed bread, respectively. After mixing, the dough was rested for 10 min at 22 °C and then divided into spherical shape using an automated moulder (DR. ROBOT2, Daub Bakery Machinery B.V., Goirle, Netherlands). The dough pieces (57 ± 2 g each for baked bread and 53 ± 2 g each for steamed bread) were then proofed at 40 °C and 85% relative humidity for 75 and 45 min for baked and steamed bread, respectively. Finally, the dough pieces were baked at 185 °C for 15 min or steamed for 20 min. After cooling in room temperature for 1 h, crumb sample was taken from the center of the bread. The crust of baked bread was taken as the layer of 1.5–2 mm thickness from the surface while the skin of steamed bread was peeled off from the surface by hand. Samples of crumb and crust/skin were used for *in vitro* digestibility and HPLC analysis.

2.3. HPLC analysis of tea catechins

To determine the retention level of tea catechins in bread after baking or steaming, bread samples were lyophilized, ground and sieved (diameter 250 μm, Mesh 60) to ensure particle size consistency. Ground samples were weighed accurately (0.5 g) into a 50 mL centrifuge tube and homogenized with 50 mL of solvent mixture (70% methanol, 29.7% distilled water and 0.3% formic acid, pH∼3.4) using vortex. Next, the centrifuge tubes were shaken mechanically in a water bath maintained at 70 °C for 45 min. The supernatant was collected using vacuum filtration (Vacuubrand MDIC, Germany). In order to determine the amount of tea catechins in bread digesta, solvent mixtures were prepared by mixing 14.85 mL of digesta aliquot with 35 mL of methanol and 0.15 mL of formic acid. The percentage composition of this mixture was similar to the extraction solvent mixture used for the analysis of ground bread samples since the main bulk of the digesta was made up of distilled water. A syringe filter of 0.45 μm was used to transfer 3 mL of the supernatant or mixture into HPLC vials for analysis. The HPLC method used for catechin quantification was described previously (Wang & Zhou, 2004).

2.4. In vitro digestibility study

The protocol of *in vitro* digestion study was adapted from the study of Ranawana and Henry (2013). Bread skin/crust and crumb samples were cut into pieces (0.5 cm × 0.5 cm) and weighed (2.5 ± 0.1 g) into biopsy pots containing 30 mL distilled water and a magnetic stirring rod that continuous stirring at 130 rpm. The biopsy pots were placed in an aluminum block seated a circulating water bath (Model GD-120, Grant instruments, Shepreth, UK). The temperature of the biopsy pots was kept at 37 °C. *In vitro* digestion consisted of simulated oral, gastric and pancreatic digestion phases. The oral phase was initiated by adding 0.1 mL of 10% α-amylase solution dissolved in distilled water. After 1 min, 0.8 mL of 1 M aqueous HCl was added to stop oral digestion. Oral phase samples were collected by transferring 0.5 mL aliquot samples into tubes containing 2 mL ethanol. The gastric phase of digestion was initiated by adding in 1 mL of a 10% pepsin solution dissolved in 0.05 M HCl. After 30 min, the gastric digestion phase was halted by adding 2 mL of 1 M sodium bicarbonate and 5 mL of 0.2 M Na maleate buffer, pH 6. Gastric phase samples were collected by transferring 0.5 mL aliquot samples into tubes containing 2 mL ethanol. Final digesta volume for each pot was then topped up to 55 mL with distilled water. The pancreatic phase of digestion was