Effect of Starch-Polyphenol Interactions on Starch Hydrolysis

by

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Phenolic compounds have attracted much attention due to numerous health benefits, including high antioxidant properties, reduced risk of cancer, and inhibition of digestive enzymes. Recent research has suggested that different phenolic compounds may interact with starch. The first objective was to investigate the effect of green or black tea extracts on hydrolysis of wheat, rice, corn, and potato starches. Cooking starches in the presence of either tea reduced their hydrolysis. Potato starch cooked with black tea was the most effective treatment. Observations suggested that hydrolysis may be affected by interactions and by impact on specific enzymes based on starch structure. The second objective was to determine if similar effect could be observed in product system. Addition of green tea extract to sponge cake significantly reduced in vitro starch digestibility, thus could reduce the expected glycemic index. In addition, significant increases in dietary fibre, resistant starch, and antioxidant properties were observed.
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Chapter 1: Introduction

Consumers are becoming more health conscious. As a result, more research is being carried out to develop healthier products by addition of different ingredients. Starch is a major carbohydrate in many food products, including white bread, white rice, cookies, cakes, and potatoes. These products are known to have a high glycemic index (GI). This means that upon consumption of such products, there is a large increase in blood glucose levels. Chronic consumption of high GI products can lead to ill health issues such as obesity and diabetes. Different strategies to address these health concerns have been investigated. For example, many products incorporate whole grains in an attempt to reduce their glycemic index. Another strategy to reduce the glycemic index of foods is the use of phenolic compounds to reduce starch digestibility. This was attributed to the inhibition of digestive enzymes. Research suggested that interactions between starch and phenolic compounds exist (Beta and Corke 2004; Zhu and others 2008, 2009; Wu and others 2009). Could it be that starch-phenolics interactions influence starch digestibility?

Therefore, this study investigated the effect of tea extracts on hydrolysis of starches from different botanical sources. Furthermore, the effect of tea extract was also tested in a product system and the nutritional qualities, including bioactive components, antioxidant capacity, starch digestibility and glycemic index were evaluated. Also, the effect of the green tea extract on textural attributes of the cake was investigated.
Chapter 2: Literature Review

Phenolic compounds and their health benefits

Phenolic compounds from a variety of sources

Polyphenols are a large class of natural or synthetic organic compounds comprised of phenol structural units. Many different phenolic compounds can be found in fruits and vegetables and, as a result, they are an integral part of human diet. While polyphenols have been extensively studied for their ability to bind macromolecules, recent interest in these compounds has increased because of their antioxidant properties. Despite the abundant amount of research on polyphenols (Bravo 1998; Hackman and others 2007), they continue to attract many researchers studying new sources of phenolic compounds and their antioxidant properties.

Consumption of products containing wholegrain cereals offers a variety of health benefits, such as increased fibre intake (Ragaee and others 2011). In addition, wholegrain cereals have also been studied for their antioxidant properties. This antioxidant activity is due to the presence of phenolic compounds. Phenolic acids represent the major group of phenolics found in grains and are comprised of three fractions: free, conjugated, and bound. Free phenolics are the smallest fraction and could account for as little as 1% of total phenolic acid content in wheat (Li and others 2008). Conjugated phenolic acids make a bigger contribution
(22%) to total phenolics (Li and others 2008). The remaining phenolics are bound phenolic acids, which have been reported to be the major fraction in grains (Liyana-Pathirana and Shahidi 2006; Li and others 2008) and in breads containing wholegrain flours (Ragaee and others 2011). Most common phenolic acids in grains are ferulic, vanillic, caffeic, syringic, and p-coumaric acids. Ferulic acid has been shown to be the dominant phenolic compound (Moore and others 2005; Mpofu and others 2006; Li and others 2008). Total phenolic content, phenolic acid composition, and antioxidant activity of grains may depend on a variety of factors including genotype and growing location (Mpofu and others 2006).

Similarly to cereals, legumes contain phenolic acids. No free phenolic acids were reported in the flours and hulls of ten legume species (Sosulski and Dabrowski 1984). The soluble ester fraction of most legumes contained trans-ferulic, trans-p-coumaric, and syringic acids. Trans-ferulic acid was the major phenolic acid in eight legume flours. For example, navy bean, lupine, lima bean, chickpea, and cowpea contained 4-9 mg/100 g of flour of trans-ferulic. Alkali treatment of the flour residue, however, did not release any additional phenols acids. In other words, the legume flours did not contain any bound phenolic acids (Sosulski and Dabrowski 1984). Hydrolysis of hull residues, on the other hand, yielded several phenolic acids including protocatechuic, syringic, and gallic acids (Sosulski and Dabrowski 1984). Lopez-Amoros and others (2006) also reported that beans, peas, and lentils contain phenolic acids, which included protocatechuic, p-hydroxybenzoic, vanillic, and trans-ferulic acids. P-Hydroxyphenylacetic acid was only detected in beans while p-hydroxybenzoic aldehyde was only detected in lentils. In addition, trans-p-coumaric acid was found in lentils and peas while
cis-p-coumaric was only present in peas (Lopez-Amoros and others 2006). Other phenolic compounds that were detected in lentils include flavonoids and tannins (Xu and Chang 2007).

Another great source of phenolic compounds is fruits. Fruits may contain a variety of phenolics including flavonoids, tannins, and phenolic acids (Naczk and Shahidi 2006). Raspberries, for example, were found to contain a number of phenolic acids including ferulic, gentisic, gallic, chlorogenic, ellagic, p-hydroxybenzoic, vanillic, caffeic, and p-coumaric acids. The major acids were ferulic and gentisic acids (Zhang and others 2010). In addition, raspberries contain anthocyanins. The total phenolic content was variety dependent and ranged from 41 to 99 mg of gallic acid equivalents/g dry weight (Zhang and others 2010). Lingonberry, which has been shown to possess anticancer properties, was reported to contain a large number of identifiable phenolics such as phenolic acids and anthocyanins (McDougall and others 2008). The berry extract was fractionated into bound and unbound fractions. The unbound fraction was rich in anthocyanins, including cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, and cyanidin-3-O-arabinoside. The bound tannin-rich fraction was found to contain proanthocyanidins comprised of catechin units or procyanidins. It was the bound fraction that retained the antiproliferative activity (McDougall and others 2008).

In addition to whole grain cereals and fruits, some recent work has been carried out to investigate new sources of phenolic compounds. For example, Rebey and others (2012) studied the polyphenol composition of cumin, which is a small herbaceous plant and a popular spice. The authors identified 19 phenolic compounds in cumin seeds. The major phenolic acid was p-coumaric acid, which ranged from 2.33 mg/g in Indian cumin to 4.83 mg/g in Tunisian cumin. Flavonoids ranged from 1.77 mg/g (Indian) to 2.88 mg/g (Tunisian) (Rebey and others 2012).
Seo and others (2012) analyzed phenolic content of *Lonicera japonica* Thunb., which is a species of honeysuckle found in eastern Asia. This plant is used as spring greens, for making wine or tea, and as a remedy to a number of diseases. Twenty five phenolic compounds were detected in the plant including 15 hydroxycinnamic acids and 10 flavonoids. The concentration of the phenolic acids was higher than that of flavonoids in every tissue. In fact, hydroxycinnamic acids accounted for more than 97% of total phenolics. The stem was found to contain the largest amount of phenolics while in the flower it was the lowest. Dicaffeoylquinic acid was found to be the dominant phenolic acid, while kaempferol 3-O-hexoside and kaempferol were the most abundant flavonoids. Interestingly, the antioxidant activity was highest in the leaf and lowest in the stem, indicating that higher amount of phenolics may not necessarily mean better antioxidant properties. The authors noted that the trend of antioxidant activities depended on flavonoid content as opposed to phenolic acids (Seo and others 2012).

Grape seed extract and grape pomace were reported to contain a number of different polyphenols including gallic acid, procyanidins B1 and B2, and a variety of catechins (Chamorro and others 2012). Concentrations of polyphenols could be affected by different enzymes during extractions. For example, treating grape seed extract with tannase resulted in a 41% increase of total phenolic content. This was accompanied by decrease in concentrations of epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), and epicatechin gallate (ECG). These observations suggested that the enzyme hydrolysed ester bonds from natural substrates enhancing the release of catechins. In addition, tannase was able to hydrolyse EGCG, GCG, and ECG producing gallic acid and epicatechin. The authors noted that the use of enzymes, such as tannase and
pectinase, can enhance the release of phenolic compounds in grape by-products, thus improving their antioxidant properties (Chamorro and others 2012).

While health benefits of many different phenolic compounds have been well documented in terms of their antioxidant activity (Cai and others 2004; Fardet and others 2008; Hackman and others 2007; Choueiri and others 2012), researchers continue to investigate other potential medical uses of phenolics. For example, grape seed polyphenol extract comprised of proanthocyanidins has been shown to have a potential use in treating Alzheimer’s disease (Ksiezak-Reding and others 2012). Ulcerative colitis is a chronic disease of gastrointestinal tract that affects the large intestine and may be caused by an abnormal immune response. Apple polyphenols were used to successfully treat colonic damage that was induced in rats. Macroscopic injury was reduced by 60% while microscopic injury was lowered by 55% (D’Argenio and others 2012). Alcohol-induced liver damage in rats was successfully treated by polyphenols from *Ecklonia cava*, which is a brown alga produced in Korea. This suggested that phenolic compounds can be used to prevent ethanol-induced liver injury (Takahashi and others 2012). Many different plant polyphenols, including flavonoids and phenolic acids, have been shown to possess chemopreventive properties against liver cancer (Stagos and others 2012).

**Polyphenols from tea**

Different teas, such as green and black tea, contain different phenolic compounds because they are processed differently. Green tea is produced by steaming fresh leaves to prevent fermentation, which results in a dry and stable product (Khan and Mukhtar 2007).
Green tea polyphenols are comprised of catechins including catechin (C), epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), gallocatechin gallate (GCG), and epicatechin (EC). EGCG was reported to be a major catechin in green tea (He and others 2006; Lu and others 2010; Liu and others 2011). Structures of some of the major polyphenols are shown in Figure 2.1. Black tea production involves crushing of tea leaves, which allows polyphenol oxidase to catalyze polymerization of catechins. While the resulting polyphenol profile depends on the manufacturing method, some common polyphenols include catechins, theaflavins, thearubigins, theasinensins, oolongtheanins, and uncharacterized polymer-like oxidation products (Khan and Mukhtar 2007; Kusano and others 2008). Structures of theaflavins and thearubigens are shown in Figure 2.2.

Since tea is one of the most popular beverages, its health benefits have been well studied. Green tea polyphenols have been shown to possess strong antimutagenic properties. This may due to polyphenols affecting carcinogen metabolism, DNA adduct formation, interaction of ultimate carcinogen, and free radical scavenging (Wang and others 1989). However, tea polyphenols vary in their effectiveness. For example, among tea catechins, EGCG was the most effective at inhibiting growth of cancerous cells, followed by EGC and ECG. Theaflavins from black tea also exhibited strong inhibitory activity (Chung and others 1999). This antimutagenic effect of tea polyphenols was observed in humans when consumption of green tea was associated with a slightly decreased risk of breast cancer (Shrubsole and others 2009). In addition to reduced risk of cancer, phenolic compounds from tea can also reduce blood glucose levels. Matsumoto and others (1993) demonstrated that by feeding rats tea catechins prior to administration of soluble starch or sucrose. When rats consumed 60 or 80 mg
of catechin, the increase of glucose and insulin in the plasma was significantly suppressed. Since only slight suppression was observed for 40 mg of catechin, the authors concluded that the quantity of catechin was an important factor. Similar results were observed when sucrose was fed to rats. This ability of tea catechins to reduce blood glucose levels was attributed to inhibition of intestinal $\alpha$-amylase and brush border sucrase (Matsumoto and others 1993). In vitro tests have shown that the polyphenols in oolong tea were more effective against intestinal sucrase than human salivary $\alpha$-amylase (Nakahara and others 1994). Unlike the catechins in green tea, the oolong tea extract had no effect on blood glucose levels in rats loaded with soluble starch, which may be explained by the extract’s weak inhibitory properties against $\alpha$-amylase (Nakahara and others 1994). Interestingly, the beneficial effects of tea polyphenols on blood glucose levels could be enhanced by addition of soluble dietary fibre such as $\beta$-glucan. In fact, combination of tea polyphenols and $\beta$-glucan was more effective at improving glucose metabolism than polyphenols or fibre alone (Gao and others 2012). Additional potential health benefits of tea polyphenols include protection against hypertension (Negishi and others 2004) and reduction of total and LDL cholesterol (Davies and others 2003).

**Composition and properties of native starches**

Starch exists in a form of granules and can be found in cereal grains, roots, tubers, stems, and in legume seeds. Shapes and sizes of granules vary greatly depending on the source. For example, potato starch granules are large (5 – 100 µm) and oval in shape while corn starch granules are small (2 – 30 µm) and polygonal in shape (Swinkels 1985). Rice starch granules
range from 3 to 5 µm and are pentagonal or angular-shaped (Singh and others 2003). Wheat starch granules are round and lenticular in shape and possess a bimodal size distribution. Small granules range from 0.5 µm to 10 µm and large granules range from 10 µm to 45 µm in size (Swinkels 1985). Starch contains two kinds of glucose polymer: amylose and amylopectin. Amylose is essentially linear while amylopectin is highly branched. Contents of amylose and amylopectin depend on the source of starch. Rice starch contains less amylose (5 – 28%) than wheat (18 – 30%), potato (20 – 31%), or corn (22 – 33%) (Singh and others 2003). Degree of polymerisation (DP) of amylose also varies from one source of starch to another. For example, amylose from potato starch has higher DP (average 3000 glucose units) than wheat or corn (average 800 glucose units). DP of amylopectin, on the other hand, is about 2 million glucose units regardless of the botanical origin (Swinkels 1985).

In addition to the major polysaccharide components, starches also contain other compounds including proteins, lipids, and phosphorus. The protein can be found on the surface and in interior parts and its content ranges from 0.1 – 0.7% by weight. Lipids (free fatty acids and lysophospholipids) can also be found in starches, especially cereal starches, at concentrations of up to 1.5%. Starches contain phosphorus as phosphate monoesters and in phospholipids (Perez and Bertoft 2010). Potato starch contains the highest amount of phosphate monoester groups (Swinkels 1985; Singh and others 2003). Phosphate groups are found in the amylopectin, which contains one group per about 300 glucosyl residues (Swinkels 1985; Takeda and Hizukuri 1982). It was suggested that one third of the phosphate groups are present in the inner sections of the B-chains and two thirds are found in the A-chains and the
outer sections of the B-chains. In addition, no phosphate groups are present in the vicinity of branch points (Takeda and Hizukuri 1982).

Structure and composition of starch can influence its functional properties. Copeland and others (2008) noted that a better understanding of where amylose is located in a granule can help researchers relate structure to various properties such as swelling, gelatinization, and susceptibility to enzymatic attack. In addition, both amylose content and amylopectin architecture may influence thermal properties and gel formation (Copeland and others 2009). Amylose molecular size and amylopectin branch chain length were found to influence viscosity and gel properties of starches (Jane and Chen 1992). Amylose from normal corn (intermediate molecular size) had the greatest viscosity, followed by amylose from potato (large molecular size) and high-amylose corn (small molecular size). Amylopectin from rice (short branch chains) had the greatest viscosity, followed by amylopectin from high-amylose corn (long branch chains) and waxy corn (intermediate branch chains). In addition, a synergistic effect of interactions between amylose and amylopectin on viscosity was observed (Jane and Chen 1992). The greatest synergistic effect on viscosity was observed for the combination of long branch chain amylopectin and the intermediate molecular size amylose. Synergistic effects were also observed for gel formation upon storage at different conditions (Jane and Chen 1992). Minor starch components, such as phosphate groups, can also have an impact on starch properties (Noda and others 2007). Potato starch with high phosphorus content (1110 – 1244 ppm) had the highest swelling power, followed by medium-phosphorus potato starches (711 – 716 ppm) and low-phosphorus starches (308 – 395 ppm). Higher phosphorus content also resulted in increased peak viscosity and breakdown as tested by RVA (Noda and others 2007).
The high phosphorus content found in potato starch may explain why potato starch exhibits higher peak viscosity and swelling power than other starches such as corn and wheat starches (Swinkels 1985). The lower swelling power of corn and wheat starches may be partially explained by the presence of amylose-lipid complexes (Swinkels 1985).

As discussed above, there is a great diversity in composition and properties of starches from different botanical sources. Therefore, it is important to consider these factors when attempting to investigate the impact of polyphenols on starch hydrolysis.

Interactions between starch and phenolic compounds

Effect of phenolic compounds on pasting properties of starches

While there are few studies on interactions between phenolic compounds and starch, several studies investigated the effect of different phenolic compounds on pasting properties of starch. Addition of phytochemical extracts from pomegranate, green tea, and Chinese galls to wheat starch significantly increased peak viscosity. The extract from Chinese hawthorn had no major effect on peak viscosity. Peak time was also reduced by pomegranate and Chinese galls extracts. All extracts significantly increased breakdown. These different observations were attributed to different phenolic compounds present at different concentrations in the extracts. For example, Chinese galls contain high levels of gallotannins while green tea is rich in catechins. Phenolic compounds contain hydroxyl and carboxyl groups and can affect functional properties of starch by competing for water. In addition, all extracts reduced the pH of the
starch-water suspensions. This effect was attributed to the presence of phenolic acids, such as gallic and chlorogenic acids. pH was found to be positively correlated with final viscosity ($R^2=0.84$). All extracts significantly reduced the final viscosity with Chinese galls having the greatest effect. Besides pH, another reason for these results might be the interactions of phenolic compounds with hydrophobic regions of leached amylose and with amylopectin side chains through hydrogen bonds and van der Waals forces. The extracts also had an impact on textural properties of starch gels. All extracts reduced the hardness of the gels with Chinese galls having the greatest effect and pomegranate exhibiting the least effect. Adhesiveness was increased by the Chinese galls extract while other extracts had no effect. Other textural properties, such as springiness and cohesiveness, were hardly affected. The observed changes in the textural properties could be explained by the phenolic compounds interacting with amylose and changing the properties of the continuous phases, which could weaken the intermolecular interactions between amylose chains (Zhu and others 2009).

When investigating the effect of pure phenolic compounds on pasting properties of wheat starch, 21 out of 25 compounds were reported to increase peak viscosity (Zhu and others 2008). All 12 phenolic acids studied also increased peak viscosity. The low negative correlation between pH and peak viscosity ($R^2=0.27$) indicated that other factors must be influencing the pasting properties of wheat starch. One of these factors was believed to be the structural differences between phenolic compounds. For example, four hydroxybenzoic acids increased peak viscosity to different extent. Gallic acid caused the highest increase while 3-hydroxybenzoic had the least effect. These phenolic acids have different numbers of hydroxyl groups at different positions, which may explain their different influences on peak viscosity.
Syringic acid resulted in a higher increase in peak viscosity than vanillic acid, which was attributed to syringic acid having two methoxy groups while vanillic has one. These two acids increased peak viscosity to a higher extent than other hydroxybenzoic acids that have no methoxy groups, which suggested that these groups can have a significant influence on the peak viscosity. While all hydroxycinnamic acids also increased the peak viscosity, the influence of the hydroxyl and methoxy groups was found to be different from that in hydroxybenzoic acids. For example, trans-cinnamic acid without any hydroxyl groups on the aromatic ring resulted in a higher increase than those with hydroxyl groups (Zhu and others 2008). The authors also reported that different flavonoids had different effects on peak viscosity of wheat starch. Quercetin caused the highest increase while catechin caused the highest decrease. These different effects were mainly attributed to structural differences between different types of flavonoids, such as flavonols (quercetin) and flavan-3-ols (catechin). Hot paste viscosity and final viscosity were also influenced by phenolic compounds. Most of the compounds reduced both of those properties with phenolic acids having the greatest effect. While pH was deemed to be a dominant factor influencing hot paste and final viscosities, structural differences (e.g., types of head groups and functional groups) were also important. For example, 3-hydroxybenzoic and 4-hydroxybenzoic acids had significantly different effects on these properties (Zhu and others 2008).

Beta and Corke (2004) stated that the interactions between small phenolic compounds, such as ferulic acid and catechin, and starch can be explained by inclusion complexes (clathrates). Similarly to lipids, these phenolic compounds form inclusion complexes with amylose molecules, which led to a reduction of swelling power of maize and sorghum starches.
Ferulic acid and catechin also reduced hot paste and final viscosities. These reductions were hypothesized to be due to formation of starch-phenol complexes that impeded the reassociation of starch molecules. While catechin was shown to associate with amylose (Deshpande and Salunke, 1982), it did not affect the pasting properties as much as ferulic acid did.

**Effect of tea polyphenols on starch retrogradation**

Addition of purified green tea polyphenols may have a reducing effect on starch retrogradation (Wu and others 2009). Rice starch containing 10%, 14%, or 20% tea polyphenols did not exhibit retrogradation endotherm on the DSC after 10 days of storage. The authors suggested that hydroxyl groups of tea polyphenols interact with OH groups on starch molecules, thus reducing the reassociation of starch polymers during retrogradation. This effect may also depend on the strength of the hydrogen bonds between starch and the polyphenols (Wu and others 2009). Green tea polyphenols can reduce retrogradation of rice starch regardless of its amylose content (Xiao and others 2011). In fact, enthalpy of retrogradation of starches containing 10% or 15% polyphenols was only detected after 20 days of storage. The degree of retrogradation was greatly reduced as well. For example, addition of tea phenolics to high amylose rice starch at a concentration of 15% reduced the degree of retrogradation from 79% (control) to 11.7% (Xiao and others 2011).
The above results, which show that phenolic compounds can influence starch properties, provide evidence for starch-phenolics interactions. Therefore, different phenolic compounds can be added to starchy foods to improve their nutritional and quality attributes.

**Effect of polyphenols on starch digestibility**

Starch is a major dietary carbohydrate and, as a result, is a source of energy. However, starchy diets can lead to a higher glycemic response which, in turn, can lead to different chronic diseases including diabetes, cardiovascular diseases, and obesity (Dickinson and Brand-Miller 2005). Therefore, an increased intake of low-glycemic foods has been recommended (FAO 1998). One strategy to reduce the glycemic index of food products could be the use of phenolic compounds to reduce starch digestibility. Starch breakdown involves a number of enzymes including salivary and pancreatic α-amylases and intestinal α-glucosidases. As a result, it is important to consider the effect of phenolics on each enzyme.

**Inhibition of salivary α-amylase**

An early study by Thompson and Yoon (1984) was interested in investigating the effect of tannic acid, catechin, and phytic acid on starch digestibility by human salivary α-amylase. After pre-incubation of the enzyme with polyphenols, tannic acid reduced starch digestibility by 13% (after 5 h of digestion) while catechin had no effect. Phytic acid was much more effective as it lowered starch breakdown by 60% at 5 h. However, there was no synergistic effect on
starch breakdown when both tannic acid and phytic acid were added to the enzyme (Thompson and Yoon 1984).

Tannins are high molecular weight polyphenols that are found in plants and plant-based foods. Kinetics of inhibition of human salivary α-amylase by tannins were studied by Kandra and others (2004). The researchers noted that the inhibition was of the mixed non-competitive type. From their kinetic data, the authors concluded that tannin can bind to the active site or to the secondary site of the enzyme. Since tannin appeared to be as effective an inhibitor as acarbose, it was suggested that tannins could be used for the prevention of dental caries (Kandra and others 2004). Similar findings were reported by Zajacz and others (2007) who studied inhibition of salivary α-amylase by tannin isolated from a gall nut of Aleppo oak. When amylose was used as a substrate, a mixed type of inhibition was reported suggesting that Aleppo tannin can interact with amylose substrate. The type of inhibition depended on the concentration of inhibitor. At low concentrations of tannin, the inhibition was competitive-like, and at high concentrations it was non-competitive-like. Competitive inhibition involved galloylated glucose binding to the active site of salivary α-amylase and interacting with aromatic or subsite residues of the enzyme. In case of non-competitive inhibition, the tannin can interact with the secondary site of the enzyme or with the substrate (Zajacz and others 2007).

McDougall and others (2005) compared the efficacy of phenolic extracts from different sources against human salivary α-amylase. The authors found the strawberry and raspberry extracts to be most effective inhibitors followed by blueberry, blackcurrant, and red cabbage. The authors attributed this inhibitory effect to the presence of tannins. In fact, their results
suggested that α-amylase inhibitors may be soluble, hydrolysable tannins. These tannins included a mixture of ellagitannins and ellagic acid as determined by LC-MS.

**Inhibition of pancreatic α-amylase**

Preliminary experiments by Deshpande and Salunkhe (1982) suggested that tannic acid and catechin could reduce breakdown of different starches by pancreatic α-amylase. This inhibition was attributed to associations between starches and the phenolic compounds. Fish and others (1991), however, found that pre-incubation of potato starch with tannic acid did not affect starch breakdown. Pre-incubating α-amylase with tannic acid, on the other hand, resulted in a significant reduction of its activity, which was attributed to polyphenol-enzyme interactions.

Porcine pancreatic α-amylase has been shown to be effectively inhibited by phenolic compounds from different sources including strawberry, raspberry, blueberry, blackcurrant, and red cabbage. The order of inhibition was the same as that for human salivary α-amylase but with less effectiveness. Tannins were again suspected to be responsible for this inhibitory activity (McDougall and others 2005). Nine different Bangladeshi fruits were also found to possess α-amylase inhibitory properties by Hossain and others (2008) but the responsible phenolic compounds were not identified. The authors did suggest that inhibition was non-competitive.

Green tea polyphenols also possess enzyme inhibitory properties. Out of different digestive enzymes (α-amylase, pepsin, trypsin, and lipase) α-amylase was inhibited most
effectively (He and others 2007). Since molecular weight is an important factor when it comes to macromolecular interactions, α-amylase’s highest molecular weight may have made it most susceptible to inhibition. Tea polyphenols contain hydroxyl and galloyl groups which can form hydrogen bonds with polar groups of enzymes. The amount and type of these polar groups can affect the formation and stability of hydrogen bonds between phenolic compounds and enzymes. In addition, polyphenols could interact with enzymes through hydrophobic associations. Such interactions between digestive enzymes and tea polyphenols could enable tea phenolic compounds to act as an antinutritional factor (He and others 2007). Similar results were also obtained by Kusano and others (2008), who reported that polyphenols from black tea could reduce activity of α-amylase and lipase. Unlike green tea polyphenols, which mainly consist of monomeric catechins, black tea contains many different phenolic compounds that are produced during the fermentation process. In addition to known polyphenols, such theaflavin and theasinensin, authors detected polymer-like oxidation products that played an important role in enzyme inhibition. These findings are in agreement with Koh and others (2010) who reported that black tea slowed down the digestion of rice noodles by pancreatin.

Phenolic acids, such as cinnamic acids, were reported to have no effect on α-amylase activity (Adisakwattana and others 2009).

**Inhibition of α-glucosidase**

McDougall and others (2005) reported that α-glucosidase (from acetone powder from rat intestine) activity was inhibited by different polyphenol extracts and the most effective
extracts were blueberry and blackcurrant followed by strawberry, raspberry, and red cabbage. This inhibitory effect was attributed to the anthocyanin contents of the extracts. This was different for $\alpha$-amylase, which was inhibited by the tannins present in the same extracts (McDougall and others 2005).

Unlike in the case of $\alpha$-amylase, phenolic acids appear to have inhibitory properties against rat intestinal sucrases. Tannic acid has been found to be the most effective phenolic compound at reducing the enzyme’s activity (78% reduction) compared to other phenolic acids such as ferulic (30% reduction), gallic (26% reduction) or caffeic (18% reduction) acids (Welsch and others 1989). However, tannic acid was only effective in the native form. When oxidized, it lost its ability to inhibit sucrase possibly due to becoming sterically inaccessible or reducing its number of reactive sites. In contrast, catechol and epicatechin were effective under oxidizing and unoxidizing conditions (Welsch and others 1989). Chauhan and others (2007) have also demonstrated the ability of tannic acid to inhibit rat sucrase. Similar inhibition was also observed for sucrase obtained from mice intestine. Interestingly, rabbit sucrase appeared to be relatively resistant to inhibition by tannic acid. The type of inhibition was reported to be competitive and pH dependant. However, the pH dependency was different for different animals. For example, rat intestinal sucrase was strongly inhibited (78-91%) in the acidic pH range and slightly inhibited (14%) at pH 8.5. For the rabbit sucrase, however, the inhibition pattern was the opposite of that observed in the rat (Chauhan and others 2007).

Adisakwattana and others (2009) investigated the effect of eleven cinnamic acids on the activity of rat intestinal $\alpha$-glucosidase. $\alpha$-Glucosidase activity was measured by maltase and sucrase assays. Cinnamic acid was a weak inhibitor of intestinal sucrase and maltase. However,
the inhibition was increased when a hydroxyl group was added to cinnamic acid. The most effective inhibitors were caffeic, ferulic, and isoferulic acids. These compounds were more effective against sucrase than maltase. When investigating inhibition kinetics, the authors found that all three compounds exhibited a mixed competitive and non-competitive type of inhibition against maltase. Combining these three compounds with acarbose produced an additive inhibition of sucrase but not of maltase (Adisakwattana and others 2009).

Zhang and others (2010) studied the inhibitory properties of different raspberry extracts against yeast α-glucosidase. All extracts were effective at inhibiting the enzyme but the efficacy varied depending on the cultivar. Fractionation of the extracts by an LH-20 column yielded three fractions: phenolic acids, anthocyanins and flavanols, and tannins. The first two fractions were effective inhibitors while tannins had a minimal effect on α-glucosidase.

In addition to phenolic acids, polyphenols from different teas were reported to inhibit rat intestinal α-glucosidase (Koh and others 2010). Black tea was the most effective inhibitor, followed by oolong tea, and then by green tea which was a relatively weak inhibitor. The stronger inhibitory property of black tea was attributed to its theaflavin content. However, theaflavins alone did not account for the whole activity of the tea because it contains other polyphenols which can contribute to the inhibition. Oolong and green teas were less effective because their main polyphenols are catechins, which were shown to be weak inhibitors (Koh and others 2010). Tea polyphenols (theaflavins and catechins) were found to be more effective at inhibiting maltase than sucrase (from rat acetone powder) (Matsui and others 2007). The inhibitory activity of theaflavins and catechins depended on their structure. For example, esterified catechins (ECG and EGCG) were 15 – 20 times more effective against maltase than
nonesterified ones (EC and EGC). The inhibitory activity of theaflavin was influenced by the presence of a free hydroxyl group at R2 position and esterification of theaflavin with a mono-galloyl group (Figure 2.2) (Matsui and others 2007).

The above studies indicate that starch digestive enzymes, including salivary and pancreatic α-amylases and α-glucosidases, can be inhibited by phenolic compounds. However, there are many factors that influence the inhibition. For example, differences in structures of enzymes make them susceptible to different phenolic compounds: α-amylases can be inhibited by polyphenols such as tannins while α-glucosidases tend to be inhibited by smaller phenolic compounds such as phenolic acids. Interestingly, tea polyphenols appear to inhibit both pancreatic α-amylase and intestinal α-glucosidase. In addition, the source of the enzymes is also a factor that can influence the phenolics’ inhibitory behaviour. Majority of the described research attributes the inhibitory properties of the phenolics to protein-phenolic interactions. However, it has been suggested that starch can also interact with different phenolic compounds as discussed in the previous section. This suggests that starch-phenolics interactions may also play a role in starch digestibility, which requires further investigation.

**Fortification of different baked products with green tea extract**

As consumers are becoming more health aware, there is an increasing demand for healthier and more nutritious products. As a result, addition of different healthy ingredients to baked goods is being investigated. A number of reports have investigated fortification of different products with green tea extracts (GTE) as described below.
Stability of tea catechins in baked products

Green tea contains a variety of catechins and their stability during bread making was studied by Wang and Zhou (2004). Their results revealed that epicatechin (EC), epicatechin gallate (ECG), and catechin gallate (CG) were more stable than epigallocatechin gallate (EGCG), epigallocatechin (EGC), and gallocatechin gallate (GCG). CG proved to be most stable (94% - 100% retention) while EGC was least stable (63% - 68% retention). Approximately 16% of total tea catechins were lost during the bread making. The losses could be caused by a variety of factors including oxidation, isomerization/epimerization, and degradation. In addition, the interactions between the phenolic compounds and wheat proteins could also reduce the amount of free catechins and contribute to their apparent loss (Wang and Zhou 2004). Wang and others (2008) investigated the reaction kinetics of the degradation and epimerization of tea catechins during bread baking. Degradation of catechins in the crumb was found to follow first-order reaction. The retentions of phenolic compounds in the crumb were similar under three baking temperatures (200, 215, and 240 °C) possibly due to small variations in the temperature and moisture profiles. Catechins underwent more degradation in the crumb than in the crust probably because the crust had a much lower moisture content which reduced the mobility and collisions of molecules. Evidence for epimerization of tea catechins in the crumb was reported as EGCG was observed to decrease with increase in baking time resulting in an increase of its epimer GCG. Similar trend was observed in the crust but the stability of catechins was highly dependent on baking temperature. Similarly to degradation, epimerization reactions followed first order kinetics. By developing mathematical models, stability of catechins under different baking conditions can be predicted (Wang and others 2008).
Catechin concentrations in biscuits was measured at intervals throughout the baking process by Sharma and Zhou (2011). As baking progressed, the concentrations of EGCG and ECG decreased. The amount of catechins lost during baking increased proportionally with the increase in GTE amount in the dough. In fact, a very strong linear relationship was observed between the initial GTE concentration in the dough and the loss of EGCG ($R^2=0.999$) and ECG ($R^2=0.977$). The loss of catechins in biscuits was much greater than in bread. For example, addition of 150 mg of GTE per 100 g of flour resulted in EGCG retention of 21% while in bread it was 83%. This indicated that stability of catechins could not be extrapolated by using other models. In addition to baking conditions and level of GTE addition, product matrix also plays a major role in tea polyphenol stability.

**Effect of green tea extract on quality of baked products**

Quality of bread can be affected by addition of green tea extract (GTE) (Wang and others 2006). GTE was found to have a negative effect on bread volume. Two different extracts were used and the one with higher catechin content resulted in a larger volume reduction (approx. 17% reduction at a level of 1.5 g/kg of flour). GTEs also had a negative impact on volume of bread made by a frozen dough process. Since bread volume depends on proper development of gluten network, the authors speculated that addition of tea catechins resulted in excessive SH groups and insufficient SS bonds. This would lead to a bread with a smaller volume and harder texture. Firmness is another important bread quality attribute, which significantly increased in the presence of GTEs. While frozen storage also increased firmness, no synergistic effect between GTEs and frozen storage was reported. GTEs continued to exhibit a negative effect on firmness throughout storage at ambient temperature. The researchers noted
that reduction in volume alone may not explain increase in firmness. Another possible mechanism may involve the activity of amylases in wheat flour being reduced by tea polyphenols. This, in turn, would reduce the amount of maltose produced, which is utilized by yeast. Reduced yeast activity and increased starch content of breads with GTEs could result in lower volume and increased starch retrogradation, respectively. Thus, GTE can have a detrimental effect on bread quality by interacting with gluten proteins, inhibiting amylase activity, and/or reducing yeast activity (Wang and others 2006). Similar results were observed by Wang and others (2007) who also noted that bread density increased and volume decreased when GTE was added. In fact, increasing GTE concentration was found to increase bread hardness. In addition, stickiness increased as well. Addition of GTE also affected the taste profile of bread. For example, bread containing 5 g/kg of GTE was found to be significantly less sweet and more astringent by both trained and untrained panelists. Tea catechins are likely to be responsible for these changes in taste profile, especially epigallocatechin gallate, which exhibited the lowest threshold concentration for astringency (Wang and others 2007).

Lu and others (2010) reported similar effects of green tea powder on textural attributes of sponge cake. Increasing the level of green tea resulted in reduced cake volume, which was attributed to an increasing amount of cellulose. Hardness increased as well, which was related to reduced volume. In addition, green tea powder resulted in lower cohesiveness, adhesiveness, springiness, and resilience, while gumminess and chewiness increased. Despite these changes, sensory liking results suggested that replacing of up to 20% of flour with green tea powder was acceptable (Lu and others 2010).
Besides textural properties, lipid oxidation is another factor that can influence food product quality. The effect of GTE on lipid stability in biscuits was investigated by Mildner-Szkudlarz and others (2009). Tea polyphenols are good antioxidants and, as a result, reduced the oxidation of fatty acids by inhibiting the decomposition of monounsaturated and polyunsaturated fatty acids. In addition, biscuits containing 1% GTE exhibited lower hydroperoxides formation (by 47% to 73%), which was more effective than synthetic antioxidant BHA (16% to 60% inhibition). Because of these beneficial effects, biscuits containing the natural antioxidants were rated higher in terms of overall acceptance than those containing BHA (Mildner-Szkudlarz and others 2009).

**Health benefits**

Despite the popularity of green tea and its well-known healthy attributes, not much research is available on fortification of baked products with green tea in terms of nutritional benefits. A recent study by Lu and others (2010) added green tea powder to sponge cakes and studied their composition and antioxidant properties. Total dietary fibre was significantly increased by all replacement levels (10%, 20%, and 30%). Replacement level of 30% (GT30) increased fibre content from 0.65% (control) to 2.51%. GT30 also significantly increased ash and protein contents. Addition of green tea powder greatly improved antioxidant properties of the cakes, which was attributed to the presence of different catechins. Since tea can be consumed in much bigger quantities than other antioxidants, such as BHA, it can prove to be a useful food additive that can improve nutritional quality of different food products.
As discussed above, green tea can be incorporated into different food products. Since green tea can be added in different forms, such as tea powder or polyphenol-rich extracts, more research is needed to investigate the healthy attributes of GTE fortified foods. In addition, green tea has a potential to reduce starch digestibility. Due to the increasing demand of low glycemic index food products, it is important to study the effect of GTE addition on starch digestibility and glycemic index of food products.
Figures

Figure 2.1: Major green tea polyphenols (Khan and Mukhtar 2007).

Figure 2.2: Structures of theaflavins and thearubigens (Khan and Mukhtar 2007).
Chapter 3: Mechanism of Hydrolysis of Native and Cooked Starches from Different Botanical Sources in the Presence of Tea Extracts

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Short version of the title: Effect of green and black tea on starch
Abstract

A series of experiments were conducted to highlight the mechanism of inhibition of hydrolysis and differences in hydrolysis among starches from different sources in the presence of green or black tea extract. The first experiment showed that black tea extract was more effective at reducing final viscosity for all starches. The second experiment showed that black tea was more effective at inhibiting starch hydrolysis compared to green tea when starch, tea extract, and pancreatin were added at the beginning of pasting. The third experiment, when starches were pre-treated with tea extracts, showed that both treatments reduced starch hydrolysis. Analysis of supernatant free phenolic content and of soluble dextrins showed that amylglucosidase activity was affected, with exceptions for potato starch. These observations suggest that starch hydrolysis is affected by interactions and also by the impact on specific enzymes based on starch structure.

Keywords: starch, green tea extract, black tea extract, phenolic compounds, hydrolysis, digestibility, mechanism
**Introduction**

Green and black teas are popular beverages that have high phenolic content. Tea polyphenols are well known for their health benefits including reduced risk of breast cancer, attenuated blood pressure, reduced total and LDL cholesterol, and anticarcinogenic and antimitagenic properties (Wang and others 1989; Ahmad and Mukhtar 1999; Davies and others 2003; Negishi and others 2004; Shrubsole and others 2009). In addition, catechins in black tea were reported to be bioavailable because only a small fraction of ingested catechins were excreted (Warden and others 2001).

Tea polyphenols are flavonoids such as catechins and theaflavins. The profile of flavonoids present in green or black tea varies due to the different processing methods used in their production. Black tea production involves fermentation, which results in enzymatic oxidation of polyphenols. This process produces catechin polymers, such as theaflavins and thearubigins and many uncharacterized polyphenols. These polyphenols have an ability to inhibit digestive enzymes. Green tea production, on the other hand, involves heating which inactivates enzymes. As a result, green tea polyphenols are not oxidized and consist of monomeric catechins, such as epicatechins, epigallocatechins, and their galloyl esters (Kusano and others 2008; Koh and others 2010).

Starch is a major dietary carbohydrate and, as a result, is a source of energy. However, starchy diets can lead to a higher glycemic response which, in turn, can lead to different chronic diseases including diabetes, cardiovascular diseases, and obesity (Dickinson and Brand-Miller 2005). Therefore, an increased intake of low-glycemic foods has been recommended (FAO
A number of studies investigated enzymatic hydrolysis of different starches in cooked and native forms (Zhang and others 2006; Goldstein and others 2010; Dona and others 2010). Cooked starches tend to be hydrolysed more rapidly than native starches and thus it is attributed to higher glycemic index. One strategy to reduce the glycemic index of food products could be the use of phenolic compounds to reduce starch digestibility. Many studies (McDougall and others 2005; Qiang and others 2007; Hossain and others 2008; Kusano and others 2008; Zhang and others 2010; Koh and others 2010) have investigated the effect of different phenolic compounds on starch digestibility but the results were inconsistent. For example, contradictory results have been reported for the efficacy of raspberry extracts as \( \alpha \)-amylase inhibitor (McDougall and others 2005; Zhang and others 2010). Reduction in starch digestibility was attributed to the ability of phenolic compounds to inhibit digestive enzymes.

Reducing starch digestibility is not the only aspect that has been studied. Researchers have also investigated the effect of phenolics on pasting properties of starches (Beta and Corke 2004; Zhu and others 2008, 2009) and on starch retrogradation (Wu and others 2009). While these findings suggested interaction between starch polymers and phenolic compounds, the researchers studying digestion implied enzyme inhibition and not interactions as a mechanism of hydrolysis inhibition. More recently, a possible interaction was suggested between amylose and green tea polyphenols, which influenced starch digestion (Liu and others 2011).

Starches from different botanical sources differ in several aspects including their morphology and structure that consequently have an impact on their functional properties. Recent research has shown that these architectural differences, such as the organization of polymers within the granule, affect properties like granule swelling and polymer leaching (Dhillon and others 2011).
Therefore, it is important to consider these factors as well in attempting to investigate the impact of tea polyphenols on starch digestion.

This study shows the impact of green or black tea extracts on starch hydrolysis and suggests that the mechanism of inhibition is related to interactions between phenolic compounds and starch and also highlights differences in hydrolysis between starches from different botanical sources.

**Materials and Methods**

**Materials**

Wheat (34% amylose), corn (22% amylose), potato (22% amylose), and rice (9% amylose) starches were obtained from MGP Ingredients (Atchison, KS, USA), National Starch (Bridgewater, NJ, USA), Penford Food Ingredients Company (Centennial, CO, USA), and Sigma (St. Louis, MO, USA), respectively. Green and black teas were obtained from a local market (Guelph, ON). Pancreatin (P1625) and ferulic acid (128708) were purchased from Sigma. Amyloglucosidase (E-AMGDF100) and glucose oxidase/peroxidase (GOPOD) reagent (K-GLUC) were purchased from Megazyme (Bray, Ireland). All other reagents were obtained from Fisher Scientific (Ottawa, ON, Canada).

**Preparation of tea extracts and determination of their total phenolic content**

Tea extracts were prepared by exposing 12 g of tea leaves to 200 mL distilled water at 95 °C for 30 min. Total phenolic content (TPC) of tea was determined by using the Folin-Ciocalteau assay (Beta and others 2005). Briefly, 1 mL of tea extract was diluted to 100 mL with distilled water
and 0.1 mL of it was added to 0.75 mL of 10-fold diluted Folin-Ciocalteau reagent. After allowing the mixture to equilibrate for 5 min, 0.75 mL of sodium carbonate solution (60 g/L) was added. After incubation for 90 min the absorbance measured at 725 nm. Ferulic acid was used as a standard (regression equation $y=0.0049x-0.0406$ and $R^2$ of 0.99) and the results were expressed as ferulic acid equivalents.

Pasting properties

A Rapid ViscoAnalyzer (model RVA-4; Newport Scientific, Warriewood, Australia) was used to study the effect of tea extracts on the pasting properties of starches. The RVA profile used was as follows: sample was kept at 37 °C for 5 min, heated to 95 °C at 10 °C/min, held at 95 °C for 5 min, and cooled to 37 °C at 10 °C/min. Samples for the RVA were prepared as follows: 3 g starch was weighed into a canister and a 25-g mixture of sodium acetate buffer (pH 5.2) and a tea extract (100 mg ferulic acid equivalents) was added to the starch.

Hydrolysis of starches in RVA

The above RVA profile was also used to study the effect of tea extracts on the hydrolysis of different starches. Samples were prepared as follows: 3 g starch was weighed into a canister followed by addition of a 25-g mixture of sodium acetate buffer (pH 5.2), 20 mg ferulic acid equivalent of tea extract and 0.1 mL of pancreatin (150 mg/mL). The RVA profile was then run and the inhibition of hydrolysis in RVA was calculated using peak viscosities.

Hydrolysis of starches cooked in the presence of tea extracts
Starches were heated using the above RVA profile in the presence of tea extracts (20 mg ferulic acid equivalents). The cooked samples were then freeze-dried. Hydrolysis of cooked starch was performed using the Englyst enzyme mixture (Englyst and others 1992), but at lower enzyme concentration. Sample (1.5 g) and 50 μL of enzyme mixture were used in order to maintain the same sample-to-enzyme ratio as that used in the RVA experiments stated previously. The hydrolysis was performed at 37 °C in a sodium acetate buffer (pH 5.2) for 120 min. Supernatant (0.1 mL) was sampled every 20 min into 0.8 mL of 80% ethanol and stored at -20 °C. Glucose in the supernatant was determined using the GOPOD reagent. Briefly, the supernatants were centrifuged at 1500g for 3 min. Then 200 μL of supernatant was mixed with 1.5 mL of GOPOD reagent and incubated at 50 °C for 20 min. Absorbance was measured at 510 nm. Soluble carbohydrate content of the centrifuged supernatant was determined by the phenol sulfuric acid method. Briefly, 0.1 mL of the supernatant was diluted to 1 mL with distilled water. Then 0.1 mL of this was mixed with 0.4 mL water followed by addition of 0.5 mL of phenol solution (5%) and 2.5 mL of concentrated sulfuric acid. The mixture was incubated at room temperature for 20 min and absorbance measured at 490 nm.

Statistical analysis

All analyses were performed at least in duplicate. Differences between means were compared by Tukey’s test using Statistical Package for Social Sciences (SPSS) software (version 16, SPSS, Inc, Chicago, IL, USA).

Results

Effect of polyphenols on pasting properties of different starches
The pasting properties of starches with different tea extracts are shown in Figure 3.1. Green and black tea extracts had different effects on the pasting profiles and the effects were also different for starches from different botanical sources. Black tea addition resulted in a lower final viscosity in all 4 starches and the reduction ranged from 39% for wheat starch to 19% for rice starch. Green tea addition, however, reduced the final viscosity to a smaller degree. In potato, the reduction was minimal while in other starches it ranged from 18% (wheat starch) to 12% (rice starch). Both extracts also reduced the trough viscosity (reduction ranged from 35% for rice starch with black tea to 2% for potato starch with green tea) and, as a result, increased the breakdown viscosities for most starches. While peak viscosity (PV) was affected as well, the effects were not significant. Since black tea polyphenols are larger and more complex than those in green tea, this suggests that the size and structure of the phenolic compounds are very important factors influencing the interactions between phenolics and starches and their resulting pasting properties.

Effect of polyphenols on hydrolysis of native starch by pancreatin

Table 3.1 summarizes the PV of all treatments. In the presence of pancreatin enzymes, PV of wheat starch without tea extracts was reduced by 88%. Potato starch was affected similarly with a 95% reduction in PV. PVs of corn and rice starches were reduced to a lesser extent by 21 and 41%, respectively.

Green tea extract did not affect starch hydrolysis by pancreatin. Black tea extract, on the other hand, was very effective at inhibiting hydrolysis by pancreatin. For example, the addition of black tea extract to wheat starch resulted in high PV (3362 cP) compared to 421 cP when no
extract was added, thus exhibiting a 91.5% inhibition of hydrolysis. The PVs of potato and corn starches were 6678 cP and 3296 cP, respectively, compared to 323 and 2591 cP in the absence of the extract resulting in a 91.5% and 100% inhibition of hydrolysis, respectively. Interestingly, the black tea extract was less effective for the rice starch with only 73.2% inhibition.

Hydrolysis of different starches cooked in the presence of green or black tea

For this part of the study, the experimental conditions (the TPC of tea extracts and the ratio of starch to enzyme) were the same as those used above. These experiments investigated the effect of enzymes on starches cooked in the presence of tea extracts. The changes in hydrolysis kinetics for the different starches are shown in Figure 3.2. Both green and black tea extracts reduced the hydrolysis kinetics of starch. However, the differences in extent of hydrolysis between green and black tea were only significant for potato starch (Figure 3.2B). For potato starch at 20 min, both extracts reduced the hydrolysis by 54% (from 0.97% to 0.45%). However, the reduction with black tea remained the same until 120 min of hydrolysis, while the reduction with green tea at 60 and 120 min were 23% (from 3.71% to 2.85%) and 14% (from 6.34% to 5.46%), respectively. Furthermore, the extracts were the least effective on rice starch. These observations suggest that botanical source of starch and phenolic extract also contribute to the differences observed.

Discussion

A number of recent studies have focused on the impact of phenolic compounds on starch digestion, both in native and in cooked starches. However, it is difficult to compare and contrast the inferences because of the different experimental conditions used by different researchers.
These include substrate environment (buffer or water), sources of starches, phenolic compounds, and enzymes, ratio of starch to enzymes to phenolics, and other experimental conditions. In general, the changes in the pasting properties observed in the presence of phenolics are attributed either to complexation of phenolics with starch or changes in intrinsic pH brought about by the phenolic acids (Beta and Corke 2004; Zhu and others 2008, 2009; Wu and others 2009). However, the impact on digestibility has been generally attributed to the inhibition of enzymes by the phenolic compounds (McDougall and others 2005; He and others 2007; Hossain and others 2008; Kusano and others 2008; Zhang and others 2010; Koh and others 2010).

The first experiment in this study also showed changes in pasting properties in the presence of tea extracts. However, there were significant differences between the effects of green tea extract vs. black tea extract. Green tea extracts have been reported to contain mostly low-molecular-weight catechins, while black tea extracts contain polymerized catechins (Kusano and others 2008; Koh and others 2010). Thus it is likely that the polymerized catechins in black tea interacted with starch during pasting resulting in the differences, while green tea catechins were not as effective in interacting with starch during pasting. It is important to note that in these experiments using buffers the effect of pH was eliminated; therefore, the results are likely only due to potential interactions.

Starch was significantly hydrolyzed by pancreatin during the pasting treatment (Table 3.1), as expected. Furthermore, the extent of hydrolysis was lower for starches with higher gelatinization temperature, such as rice and corn compared to wheat and potato, again as
expected. When this experiment was conducted in the presence of tea extracts, black tea was significantly more effective at preventing hydrolysis compared to green tea. Again some differences were observed between the starches, with black tea being less effective on rice starch compared to the other 3 starches. These observations suggested 2 possibilities: a) black tea extract was more effective at interacting with starch and reducing the number of binding sites for the enzyme or b) black tea extract was a better enzyme inhibitor than green tea extract. It also presented an alternate hypothesis, based on the above 2 experiments, that starch interaction with black tea polyphenols occurred during the heating phase, while the interaction with green tea happened during the cooling phase when the starch gel network is set.

The next experiment investigated the digestibility of starches pre-cooked with the 2 tea extracts. These results showed that both black and green tea extracts were effective at reducing starch hydrolysis. However, this was different from the observation in the previous experiment where only black tea was effective in inhibiting starch hydrolysis. This raised the question of the mechanism of inhibition: are the interactions of phenolics with starch resulting in reduced accessibility to the enzyme or are the 2 enzymes (α-amylase and amyloglucosidase) inhibited?

Free phenolic content (FPC) in the supernatant was measured during starch hydrolysis. FPC did not change throughout the hydrolysis duration (Table 3.2). However, there was a clear trend between the black tea FPC and the reduction in starch hydrolysis. For example, rice starch had the highest FPC values for the black tea and had the lowest reduction in hydrolysis. Conversely,
potato starch cooked with the black tea had the lowest FPC and also had the most reduction in hydrolysis. Therefore, the more black tea polyphenols bound by a starch, the more resistant it was to hydrolysis. This trend, however, was not as clear for the starches cooked with green tea. While rice starch bound the least green tea phenolics (more supernatant FPC) and it exhibited the least reduction in starch hydrolysis, corn, wheat, and potato starches had similar supernatant FPC values; but the reduction in potato starch hydrolysis was not as high as seen for wheat or corn starches. These observations suggest that the structural differences between the starches might play a role in the extent of interaction and/or hydrolysis. These observations also suggest that starch-phenol/polyphenol interactions influence hydrolysis by probably hindering the enzymes. However, the question of which enzyme, α-amylase or amylglucosidase, might be affected by the tea extracts is still open.

The glucose release measured thus far is a result of 2 enzymes: pancreatic α-amylase and amylglucosidase. Therefore, to parse out the effect of the phenolic compounds on the 2 enzymes, the supernatants that were analyzed for glucose were also analyzed for soluble starch content by the phenol-sulfuric acid method. α-Amylase breakdown products result in soluble dextrins. If α-amylase is affected by the tea extracts, the amount of soluble dextrins in the supernatants should be affected as well. Data in Figure 3.3 suggest that α-amylase was not affected by either green or black tea extract when treated with corn, wheat, or rice starch. However, α-amylase activity appeared to be significantly affected by black tea extract when treated with potato starch. This leads to 2 observations: a) The reduction in glucose release shown in Figure 3.2 for corn, wheat, and rice starch is likely due to a reduced activity of amylglucosidase and not reduced activity of α-amylase (Figure 3.3); and b) treating black tea
with potato starch resulted in the decreased activity of both enzymes. The second observation suggests that the polyphenolic compounds interact with the polymers in potato starch; it is well recognized that potato starch has longer external and internal chains compared to the other starches. Therefore, it might be considered that this would make potato starch more susceptible to \( \alpha \)-amylase activity. However, the contrary data observed here suggest that the interaction of the longer chains with phenolics likely reduces the number of active sites available for the \( \alpha \)-amylase, which in turn reduces the substrate available for amyloglucosidase activity.

**Conclusion**

The results suggested that starch-phenolic compound interactions were responsible for reducing starch hydrolysis by impacting specific enzymes depending on the starch structure. Further research is underway to investigate and characterize the nature of interactions between starches and phenolic compounds.
Figures and Tables

Figure 3.1. Effect of green or black tea extracts on pasting properties of A) wheat B) potato C) corn, and D) rice starches.
Figure 3.2. Hydrolysis of A) wheat B) potato C) corn, and D) rice starches cooked in the presence of green or black tea extract. Glucose measured by glucose oxidase/peroxidase kit and reported on the basis of starch content. The ratio of starch to Englyst enzyme mixture was 1.5 g to 50 μL. Reductions in hydrolysis (%) are shown at 20, 60, and 120 min for green (first value) and black tea (second value), respectively: % hydrolysis reduction = ((value for control – value for treatment)/Control) * 100.
Figure 3.3. Hydrolysis of A) wheat B) potato C) corn, and D) rice starches cooked in the presence of green or black tea extract measured by the sulfuric acid/phenol total carbohydrate method.
Table 3.1. Peak viscosities, measured by using the Rapid ViscoAnalyzer, of different starches with pancreatin and green or black tea

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<th>Starch</th>
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<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Wheat</td>
<td>3635±45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potato</td>
<td>6729±16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corn</td>
<td>3261±59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice</td>
<td>3500±4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means with different letters in each row are significantly different (p<0.05).
Table 3.2. Free phenolic content during hydrolysis of starches

<table>
<thead>
<tr>
<th>Starch</th>
<th>Green tea FPC (µg)</th>
<th>Black tea FPC (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>3576±140&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2665±102&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Potato</td>
<td>3686±63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1646±158&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Corn</td>
<td>3728±86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2238±104&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice</td>
<td>4980±195&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4040±187&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-d</sup> Means with different letters in each column are significantly different (p<0.05).
Chapter 4: Effect of Green Tea Fortification on Nutritional Attributes of Sponge Cake

(Submitted to Journal of Food Science)

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Short version of the title: Effect of green tea on sponge cake
Abstract

The current study investigated the nutritional aspects of sponge cake fortified with polyphenol-rich green tea extract (GT) using three different levels of reconstituted GT - 4% (GT4), 6% (GT6), or 9% (GT9). Textural attributes (hardness, gumminess, and chewiness) of the cakes were evaluated. All levels of GT increased the insoluble dietary fiber content of the cakes by approximately 70% while no change was observed in the soluble dietary fiber content. Resistant starch content increased significantly with GT9 exhibiting the highest increase (22%). All fortified cakes were significantly rich in polyphenols compared to the control, with the majority of phenolics present in the free form. The high phenolic content of the fortified cakes also resulted in higher antioxidant activity as compared with the control. A significant reduction in both rapidly and slowly digestible starch fractions in GT6 and GT9 suggests reduced starch digestibility. However, fortification of the cake with GTE significantly affected their textural attributes as observed by a significant increase in the hardness, gumminess and chewiness as compared with the control.

Keywords: sponge cake, green tea extract, starch digestibility, expected glycemic index, bioactive components
Introduction

Green tea is a popular beverage that is high in phenolic content. Tea polyphenols are well known for their health benefits including reduced risk of breast cancer, attenuated blood pressure, reduced total and LDL cholesterol, and anticarcinogenic and antimutagenic properties (Wang and others 1989; Ahmad and Mukhtar 1999; Davies and others 2003; Negishi and others 2004; Shrubsole and others 2009). In addition, tea catechins are bioavailable because only a small fraction of ingested catechins were excreted (Warden and others 2001).

Tea polyphenols are flavonoids such as catechins and theaflavins. The profile of flavonoids present in tea varies based on processing methods used in tea production. Unlike fermentation, involved in the production of black tea, green tea production involves heating which inactivates enzymes. As a result, green tea polyphenols are not oxidized and consist of monomeric catechins, such as epicatechins, epigallocatechins, and their galloyl esters (Kusano and others 2008; Koh and others 2010).

As consumers are becoming more health aware, there is an increasing demand for healthier and more nutritious products. As a result, addition of different healthy ingredients such as whole grains or soluble and insoluble fibers (Ragaee and others 2011; Sullivan and others 2012; Ktenioudaki and Gallagher 2012) to baked goods is a subject of interest in the research community. A number of reports have also investigated fortification of different products with green tea extracts (GTE) (Wang and Zhou 2004; Wang and others 2006; Wang and others 2007; Wang and others 2008; Sharma and Zhou 2011). Wang and Zhou (2004) studied the stability of green tea polyphenols during bread baking. They reported that catechins
were relatively stable with 84% retention and polyphenols that were lost was attributed to the combined effect of oxidation, isomerization/epimerization, and degradation of catechins. Wang and others (2008) developed mathematical models for stability of catechins during bread baking and found that the degradation and epimerization reactions followed first-order kinetics. Sharma and Zhou (2011) investigated the stability of catechins in biscuits and reported that their concentration decreased with baking time. However, the authors found that the stability increased when the initial concentration of catechins in the dough was increased.

Some studies also investigated the effect of GTE on the quality of baked products. For example, tea extracts had a negative effect on volume of breads prepared by either frozen on unfrozen dough process. In addition, GTE resulted in firmer crumb structure (Wang and others 2006). Increasing levels of GTE in bread resulted in darker colour and reduced sweetness, while hardness, stickiness, and astringency increased (Wang and others 2007). Green tea polyphenols were also reported to reduce oxidation of fatty acids in biscuits. However, the polyphenols did not improve lipid stability. Despite that, following storage, biscuits containing GTE received a higher overall acceptance score than those containing a synthetic antioxidant (Mildner-Szkudlarz and others 2009). In addition to quality, the effect of green tea powder on nutritional quality of sponge cakes has also been investigated (Lu and others 2010). However, the effect of such fortification on starch digestibility and glycemic index of baked products has not been investigated as yet. Since health issues, such as diabetes and obesity, are on the rise, studying these aspects is also imperative.

The aim of the current study was to evaluate the nutritional attributes, including the amount of bioactive components, antioxidant capacity, starch digestibility and estimated
glycemic index of sponge cakes fortified with a polyphenol-rich green tea extract. Furthermore, the effect of the green tea extract on textural attributes of the cake was also investigated.

**Materials and Methods**

**Materials**

Cake flour was provided by Mondelez mill (Mondelez, ON, Canada). Green tea extract was obtained from Tata Tea Extractions Inc., (Plant city, FL, USA). Pancreatin (P1625), invertase (I-4504), and ferulic acid (128708) were purchased from Sigma Chemical (St. Louis, MO, USA). Amyloglucosidase (E-AMGDF100), glucose oxidase/peroxidase (GOPOD) reagent (K-GLUC), total starch kit, and resistant starch kit were purchased from Megazyme (Bray, Ireland). All other reagents were obtained from Fisher Scientific (Ottawa, ON, Canada).

**Preparation of the tea extract and total phenolic content**

Green tea extract was prepared by dissolving 30 g of tea powder in 100 ml of water at 95 °C for 10 min. Total phenolic content (TPC) of tea was determined by using the Folin-Ciocalteau assay (Beta and others 2005). Briefly, 1 mL of tea extract was diluted to 100 mL with distilled water and 0.1 mL of it was added to 0.75 mL of 10-fold diluted Folin-Ciocalteau reagent. After allowing the mixture to equilibrate for 5 min, 0.75 mL of sodium carbonate solution (60 g/L) was added. The mixture was incubated for 90 min, and its absorbance was measured at 725 nm. Ferulic acid was used as a standard (regression equation y=0.0049x-0.0406 and R² of 0.99) and the results were expressed as ferulic acid equivalents.

**Preparation of sponge cakes**
Cakes were prepared according to Lu et al. (2010). Ingredients are listed in Table 4.1. Whole eggs and egg yolk were poured into a bowl and mixed. Sucrose and sodium chloride were added and the mixture was heated to 40 °C using a hot water bath. The mixture was then mixed using Kitchen Aid mixer with a whisk attachment at speed 8 for 3 min. Mixing speed was reduced to 2 and cake flour was gradually added into the bowl. The bowl was then removed from the mixer and the remaining ingredients were added and mixed manually with a plastic scraper until smooth. Since the reconstituted tea extract contained water, the amount of additional water added was reduced to maintain batter viscosity. Four hundred grams of batter was poured into a cake pan and baked at 160 °C for 40 min. Cakes were cooled for 1 hour prior to analysis.

Composition of cakes

Before chemical analysis, all cakes were oven dried at 45 °C overnight, ground, and passed through an 850 mm sieve. Moisture content was determined using Ohaus Halogen Moisture Analyzer (Ohaus Corporation, USA). Insoluble and soluble fiber contents were determined by AACC Method 32-07. Protein content was analysed using a nitrogen analyser (FP-528 Leco Instrument Ltd., Mississauga, ON. Canada). Ash content was determined by AACC Method 08-01. Total starch and resistant starch contents were determined by Megazyme Assays Kits.

Determination of phenolic content and antioxidant activity
Phenolic content of cakes was determined according to Ragaee and others (2011) with slight modifications. Briefly, 0.5 g of sample was weighed into a centrifuge tube and 5 ml of 80% methanol was added. The mixture was shaken in a platform shaker for 30 min and then centrifuged at 16800 g for 5 min. The supernatant was removed and extraction was repeated once more. The phenolics extracted this way were labeled as free phenolics. Fifteen mL of hexane was added to the pellet and shaken for 5 min, centrifuged, and the supernatant was discarded. The residue was digested with 5 mL of 2 M NaOH for 1 h under nitrogen using platform shaker. The phenolics released by NaOH treatment were labeled as bound phenolics.

The phenolic content of the methanol (free phenolics) and NaOH (bound phenolics) fractions was determined using the Folin-Ciocalteau method. Briefly, the extracts were mixed with the Folin-Ciocalteau reagent and neutralised with sodium carbonate (60 g/L). The mixture was incubated in the dark for 90 min and its absorbance was measured at 725 nm. For bound phenolics, absorbance values for control cake were used to correct for any interference from other reducing compounds. Results were expressed as ferulic acid equivalents.

Antioxidant activity of sponge cakes was determined according to Ragaee and others (2011). Briefly, 50 μL of the methanol fraction was mixed with 950 μL of 80% MeOH. Then 200 μL of fresh 0.1 mMol DPPH (2,2-diphenyl-1-picrylhydrazyl radical) solution was added and the mixture was incubated in the dark for 30 min. The absorbance was measured at 517 nm and the percent DPPH scavenging was calculated by \( 1 - (\text{absorbance of sample}/\text{absorbance of control}) \) x 100.

Starch digestibility
Starch digestibility was performed according to Englyst et al. (1992). This method involves controlled hydrolysis of a sample with a mixture of pancreatin, amyloglucosidase, and invertase. Glucose content was measured every 20 minutes using a glucose oxidase kit from Megazyme (K-GLUC). Furthermore, the amount of glucose released from starch alone was determined by excluding invertase from the enzyme mixture to eliminate the contribution of sucrose to glucose release.

Expected glycemic index

Expected glycemic index was calculated according to Goni and others (1997) and Granfeldt and others (1992).

Texture analysis

Texture profile analysis of cakes was performed using a TAXTplus texture analyser (Stable Micro Systems, Surrey, UK). Cakes were cut into cubes of 25 mm thickness and 30 mm height. A 5 g force was used to compress the samples to a depth of 10 mm at a test speed of 1.7 mm/s using a double cycle. Hardness, springiness, cohesiveness, gumminess, chewiness, and resilience were measured. All measurements were performed in quadruplicates.

Statistical analysis

All experiments were performed in duplicates except texture analysis, which was performed in quadruplicates. Duncan’s test was used to determine significant differences between means at the level of p < 0.05.

Results and Discussion

Bioactive components and antioxidant activity
Addition of different levels of tea extract to the cake formulation resulted in a significant, but similar, increase of total dietary fiber (TDF) (Table 4.2) - from 2.4% (control) to 3.3%, 3.1%, and 3.2% for cakes containing 4%, 6%, and 9% GT, respectively (approx. 30% increase). Lu and others (2010) reported an increase of TDF by 280% in their study with cakes, although it is not stated if the soluble or insoluble fraction changed. The green tea powder in that study contained 32.5% total fiber, while the extract used in the current study contained only 8.6% soluble fiber and negligible amounts of insoluble fiber. Results in this study showed that the increase in TDF was due to a higher content of insoluble fiber, which increased from 1.3% for control to approximately 2% (approx. 70% increase) for the fortified cakes, while the soluble fiber content remained unchanged.

Addition of green tea extract to sponge cakes significantly increased their resistant starch content (RS) (Table 4.2). GT9 cake had the highest RS content of 1.1%, which was 22% higher than control. Currently there are no other studies, to the best of our knowledge, reporting the effect of tea extracts on RS content. It is possible that green tea polyphenols interacted with starch polymers resulting in higher RS content (Guzar and others 2012). This increase in RS may have also contributed to higher insoluble fiber content as has been suggested by other researchers (Vasanthan and others 2002; Menga and others 2009). Since different levels of green tea resulted in similar RS values, this may explain why addition of tea also resulted in similar insoluble fiber contents.

As expected, addition of green tea extract to sponge cakes greatly increased their phenolic contents (Table 4.2). Free phenolic content ranged from 3631 μg/g to 8078 μg/g,
which corresponds to 2940 to 6665% increase compared to the control. The free phenolic acid content of the fortified cake is similar to that of wheat bran (Yu and others 2003; Zhou and Yu 2004; Liyana-Pathirana and Shahidi 2006). The fortified cakes were also found to contain bound phenolics and their concentration ranged from 525 μg/g to 974 μg/g. This bound phenolic fraction may have formed due to polyphenol-protein interactions (Siebert and others 1996; Charlton and others 2002; Frazier and others 2003; Papadopoulou and others 2005) and/or polyphenol-starch interactions (Zhu and others 2008, 2009; Liu and others 2011; Guzar and others 2012). Fortified cakes also exhibited high antioxidant activity (Table 4.2), which positively correlated with their free phenolic content ($r^2 = 0.984$). This improved antioxidant activity is due to the presence of catechins found in green tea. These results are in agreement with Lu et al. (2010). In addition to imparting numerous health benefits associated with antioxidants, these phenolic compounds can also reduce oxidation of fatty acids and formation of hydroperoxides in products (Mildner-Szkudlarz and others 2009). The above results indicate that addition of green tea extracts to food products is an excellent way to deliver phenolic compounds and improve antioxidant activity of the final product.

Starch digestibility

Addition of 6% or 9% of tea extract to sponge cake significantly affected starch digestibility as indicated by significant change in all three starch fractions indicating lower starch digestibility (Figure 4.1A-C) compared to the control. GT9 was the most effective treatment that reduced starch digestibility by 22.9% at 20 min, 17.8% at 60 min, and 16.6% at 120 min. Addition of 4% of tea, however, had no effect on starch digestibility. At fortification
level of 6% or 9% of tea extract, a significant reduction in both rapidly digestible starch (starch digested after 20 min) and slowly digestible starch (starch digested after 120 min) was observed. As a result, residual starch (starch not digested after 120 min) was increased. There are two possible reasons for this effect of polyphenols on starch digestibility. The first explanation may be inhibition of digestive enzymes by free phenolics (McDougall and others 2005; He and others 2007; Hossain and others 2008; Kusano and others 2008; Zhang and others 2010; Koh and others 2010). However, this may not be the case because GT9 cake had a considerably higher free phenolic content than GT6, but both had similar effect on digestibility. The second possibility was recently explored by our group and attributed to polyphenol-starch interactions (Guzar and others 2012). The authors investigated effect of cooking of different starches in the presence of green or black tea on starch hydrolysis. Both green and black tea extracts were effective at reducing starch hydrolysis. In addition, free phenolic content (FPC) in the supernatant was measured during starch hydrolysis and a trend was observed: the lower the FPC, the higher reduction in hydrolysis. In other words, starches that bound more polyphenols were hydrolyzed to a lesser extent. These observations suggested that polyphenol-starch interactions influenced hydrolysis by possibly hindering the enzymes. This mechanism also could have contributed to the reduced starch digestibility observed in this study.

Ragaee and others (2011) reported that, despite higher fiber contents, rapidly and slowly digestible starch fractions of fortified breads were not different from control. In addition, Wolever (1990) reported no significant correlation between soluble dietary fiber contents and glycemic index. This suggests that fortification of starchy foods with polyphenol-rich extracts
could be more effective at reducing starch digestibility than addition of whole grains or fibers alone.

Since sponge cakes contain a substantial amount of sucrose, it is important to consider the contribution of sucrose to the glucose release. Figures 4.2B and 4.2D show that polyphenols have a different effect on total glucose release as opposed to glucose release from starch alone (Figures 4.2A and 4.2C). In fact, trends were found to be different. While addition of 4% or 6% of tea extract to sponge cake had different effects on glucose release from starch, both levels of GT had a similar effect on total glucose release. Sponge cake with 9% of tea exhibited the lowest glucose release. Different trends observed in Figures 4.2C and 4.2D suggest that polyphenols have different effects on starch degrading enzymes and invertase. In fact, it appears that green tea phenolics are more effective at reducing breakdown of starch than that of sucrose.

Glycemic index

All three levels of green tea fortification resulted in a similar, but significant, reduction of eGI from 85 (control) to approximately 80 (Table 4.2). While polyphenols, especially condensed tannins, have been found to contribute to reduced glycemic response (Thompson and others 1984), consumption of tea may not have a significant effect on glucose control (MacKenzie and others 2007). In addition, no significant effect of tea polyphenols on glycemic response in mice was reported when polyphenols were added to normal or waxy corn starches (Liu and others 2011). Interestingly, the authors did find that addition of polyphenols to high amylose corn starch resulted in higher glycaemic response with a delayed blood glucose peak.
These inconsistent results indicate that more work is needed to investigate how different polyphenols, along with different starches and other carbohydrates, influence GI. Nevertheless, our results show that even a small addition of green tea extract may result in a product with reduced GI.

Texture

Addition of green tea to sponge cake formulation affected the textural properties of the final products (Table 4.3). The most affected attributes include hardness, gumminess, and chewiness, as shown in Figure 4.3. Addition of 4%, 6%, or 9% of tea increased hardness by 64%, 97%, and 110%; gumminess increased by 63%, 87%, and 93%; and chewiness increased by 62%, 80%, and 86%, respectively. The largest changes were observed for GT4 and GT6 cakes while the addition of 9% of tea resulted in changes only slightly bigger than that of GT6. Other textural properties were also affected but to a lesser extent. Springiness, cohesiveness, and resilience were all decreased. These observations are in agreement with Lu and others (2010), who replaced 10%, 20%, or 30% of flour with powdered green tea leaves. The authors attributed the increase in hardness to a decrease of volume as a result of tea addition. In the current study, addition of tea did not affect the cakes’ weight and dimensions (data not shown). Therefore, the reason for increased hardness likely lies in the differences between the tea extracts used by Lu and others (2010) and the present study. Our extract was a dried polyphenol-rich green tea concentrate, which contained no leaves. It is possible that polyphenols from tea interacted with proteins in the cake creating a firmer, chewier, and gummier structure. Wang and others (2007) reported that addition of green tea extract to
breads increased their hardness but the authors attributed this to reduced amylase and yeast activity.

Above texture results support the starch digestibility findings. For example, increase in hardness, gumminess, and chewiness had positive correlations with reduction in starch digestibility at 20 min ($r^2$ of 0.76, 0.70, and 0.68, respectively). In addition, the changes in these texture attributes correlated positively with reductions in total glucose release (at 20 min) with $r^2$ of 0.86, 0.83, and 0.84 for hardness, gumminess, and chewiness, respectively.

**Conclusion**

This study investigated the effect of green tea extract on nutritional qualities of sponge cake. GT6 and GT9 significantly reduced starch digestibility while GT4 had no effect. GT6 and GT9 reduced rapidly and slowly digestible starch fractions and increased the residual starch fraction. Total glucose (glucose from both starch and sucrose) release was also measured. While GT9 was the most effective treatment, the reduction in total glucose was not as high as the reduction in glucose release from starch. This suggested that green tea polyphenols are more effective at reducing breakdown of starch than that of sucrose. While eGI was significantly reduced by the green tea extract, more work is needed to determine whether certain phenolic compounds may be more effective at reducing total glucose release. This would help improve the nutritional qualities of products containing substantial amounts of starch and/or sucrose.
Tables and Figures

Table 4.1: Formulation of sponge cakes

<table>
<thead>
<tr>
<th>Ingredient (g)</th>
<th>Control</th>
<th>GT4</th>
<th>GT6</th>
<th>GT9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cake flour</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Sucrose</td>
<td>129</td>
<td>129</td>
<td>129</td>
<td>129</td>
</tr>
<tr>
<td>Whole egg</td>
<td>129</td>
<td>129</td>
<td>129</td>
<td>129</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Nonfat dry milk</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Water</td>
<td>42</td>
<td>35</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Reconstituted green tea extract (% of total weight)</td>
<td>0 (0%)</td>
<td>20 (4%)</td>
<td>27 (6%)</td>
<td>40 (9%)</td>
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</table>
Table 4.2: Bioactive components, antioxidant activity, and expected glycemic index of sponge cakes

<table>
<thead>
<tr>
<th>Composition</th>
<th>Control</th>
<th>GT4</th>
<th>GT6</th>
<th>GT9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary Fiber (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insoluble</td>
<td>1.3±0.2a</td>
<td>2.1±0.0b</td>
<td>2.1±0.0b</td>
<td>2.2±0.0b</td>
</tr>
<tr>
<td>Soluble</td>
<td>1.1±0.0a</td>
<td>1.1±0.1a</td>
<td>1.0±0.0a</td>
<td>0.9±0.2a</td>
</tr>
<tr>
<td>Total</td>
<td>2.4±0.1a</td>
<td>3.3±0.1b</td>
<td>3.1±0.0b</td>
<td>3.2±0.2b</td>
</tr>
<tr>
<td><strong>Resistant starch (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.90±0.04a</td>
<td>1.04±0.00b</td>
<td>1.06±0.00b</td>
<td>1.10±0.07b</td>
</tr>
<tr>
<td><strong>Phenolic compounds (ug/g)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free</td>
<td>119±10a</td>
<td>3631±75b</td>
<td>5048±127c</td>
<td>8078±86d</td>
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<tr>
<td>Bound</td>
<td>N/A</td>
<td>525±71a</td>
<td>732±91a</td>
<td>974±6b</td>
</tr>
<tr>
<td><strong>Antioxidant activity (% DDPH scavenged)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1±0a</td>
<td>54±2b</td>
<td>71±1c</td>
<td>92±0d</td>
</tr>
<tr>
<td><strong>eGI</strong></td>
<td>85±0a</td>
<td>80±1b</td>
<td>81±0b</td>
<td>80±1b</td>
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</table>

*a-c* Means with different letters in each row are significantly different (p<0.05).
Table 4.3: Texture profiles of the sponge cakes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hardness (g)</th>
<th>Springiness</th>
<th>Cohesiveness</th>
<th>Gumminess</th>
<th>Chewiness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>768±72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>644±57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>636±60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GT4</td>
<td>1259±16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1050±43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1031±33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.48±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GT6</td>
<td>1513±62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.95±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.80±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1206±53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1147±47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.47±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GT9</td>
<td>1614±96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.95±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.77±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1243±67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1186±96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means with different letters in each column are significantly different (p<0.05).
Figure 4.1: Starch digestibility (A) of sponge cakes and rapidly digestible (B), slowly digestible (C) and residual (D) starch fractions. Values in A represent % reductions in starch digestibility relative to control at 20, 60 and 120 min. Values from top to bottom represent values for GT4, GT6 and GT9, respectively.
Figure 4.2: Glucose released from sponge cakes measured by Englyst method without invertase (A) and with invertase (B). C and D represent rapidly available glucose from figures A and B, respectively. Values in A and B represent % reductions in glucose release relative to control at 20, 60 and 120 min. Values from top to bottom represent values for GT4, GT6 and GT9, respectively.
Figure 4.3: Percent change of texture attributes of sponge cakes with different levels of green tea relative to the control.
Chapter 5: Conclusions

Starch is a major carbohydrate in many food products including white bread, cakes, cookies, rice, and potato. Chronic consumption of foods rich in carbohydrates can lead to a number of health related complications, such as obesity and diabetes. Therefore, strategies are being researched to reduce the glycemic index of such products. One of strategies to achieve this is to use phenolic compounds to reduce starch digestibility. This approach was investigated by the above studies.

Different starches (wheat, rice, corn, and potato) were cooked in the presence of green or black tea extracts (Chapter 3). It was found that either tea extract was able to reduce the hydrolysis of starches pre-treated with the extracts. The observations raised a question of the mechanism of inhibition: did starch-polyphenol interactions reduce the starch’s accessibility to enzymes (α-amylase and amyloglucosidase) or did the polyphenols inhibit the enzymes? After measuring the free phenolic content (FPC) in the supernatant obtained during starch hydrolysis, a trend was observed between the black tea FPC and reduction in starch hydrolysis. For example, rice starch had the highest FPC values and had the lowest reduction in hydrolysis. Conversely, potato starch had the lowest FPC but also had the highest reduction in hydrolysis. This suggested that the more black tea polyphenols bound by a starch, the more resistant to breakdown it became. However, this trend was not as clear for starches cooked with green tea. For example, corn, wheat, and potato starches had similar supernatant FPC contents but the reduction in hydrolysis of potato starch was lower than that of wheat and corn starches. These
observations led to a conclusion that structural differences between the starches may impact the extent of interactions and the resulting hydrolysis. It was also suggested that starch-polyphenol interactions may reduce starch hydrolysis by possibly hindering the enzymes. In addition, by comparing the products of the two enzymes (α-amylase and amyloglucosidase), we found that the reduction in hydrolysis of wheat, corn, and rice starches was likely due to reduced activity of amyloglucosidase and not α-amylase. The reduction in hydrolysis of potato starch, however, was due to the decreased activity of both enzymes. This may be attributed to potato starch having longer chains, which may have enhanced starch-polyphenol interactions, thus reducing the number of available sites for α-amylase, which in turn reduced the amount of substrate for amyloglucosidase. These observations also supported our conclusion that starch structure might play a role in starch-polyphenol interactions.

Furthermore, we investigated the effect of green tea extract on starch digestibility in a product system. Sponge cakes were prepared with three different levels of reconstituted tea extract: 4% (GT4), 6% (GT6), or 9% (GT9). GT6 and GT9 significantly reduced starch digestibility, which was evident by changes in three starch fractions: rapidly digestible (RDS), slowly digestible (SDS), and residual starch (RS) fractions. Both RDS and SDS were reduced, and RS was increased. GT4, however, had no effect on starch digestibility. Since sponge cakes contain a substantial amount of sucrose, the contribution of sucrose to glucose release was considered as well. We observed that polyphenols had a different effect on total glucose (glucose from both sucrose and starch) release as opposed to glucose release from starch alone. While GT9 was the most effective treatment, the reduction in total glucose release was not as high as the reduction in glucose release from starch. This indicated that green tea phenolic compounds are
more effective at reducing breakdown of starch than that of sucrose. Nevertheless, addition of all three levels of tea extract to sponge cake significantly reduced its expected glycemic index.

While above studies showed that starch hydrolysis may be reduced by starch-polyphenol interactions, this effect depends on the structures of both starch and polyphenols. Therefore, more work is needed to characterize the nature of starch-polyphenol interactions. In addition, polyphenols from other sources, such as fruits, should be considered because of great diversity of their structures and properties. Furthermore, future research should also investigate whether some natural phenolic compounds could be used to reduce the glucose release not only from starch but also from sucrose and how that would impact the glycemic index of food products. Such research has a potential of providing an effective alternative to synthetic compounds for treating blood glucose related health issues, such as diabetes.
References


Guzar I., Ragaee S., & Seetharaman K. 2012. Mechanism of hydrolysis of native and cooked starches from different botanical sources in the presence of tea extracts. *Journal of Food Science: In Press*


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Appendix

List of relevant publications and presentations

Publications


Presentations

Ontario Cereal Industry Research Council (OCIRC) Vth Annual Meeting

- presented a talk titled ‘Effect of Tea Polyphenols on Starch Hydrolysis’

American Association of Cereal Chemists Annual International Meeting

- presented a poster titled ‘Effect of phenolic compounds on starch hydrolysis by pancreatic amylase’