Combined effects of added beta glucan and black tea in breads on starch functionality

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ABSTRACT

Bread and tea are usually consumed separately but there may be different food matrix interactions and changes in starch characteristics when they are combined in bread. This study developed breads (white bread, WF; black tea, BT; beta glucan, βG; beta glucan plus black tea, βGBT) and determined their starch functionalities. Breads were developed using a standard baking recipe and determined their starch characteristics. There was no significant difference in starch hydrolysis between BT and WF but βGBT reduced early (10 min) starch hydrolysis compared with βG. The starch granules in βG and βGBT were elliptical and closely packed together. These results suggest that the addition of beta glucan and black tea to bread preserved the elliptical starch granules and lowered short-term starch hydrolysis.

Keywords: beta glucan bread, starch granules, starch hydrolysis, food matrix

Introduction

Tea is one of the most common beverages consumed around the world. Long-term intake of tea has been associated with risk reduction for cardiovascular diseases, cancers, hypertension and diabetes mellitus (Gardner et al., 2007; Khan & Mukhtar, 2012). The health effects of tea have been attributed to the presence of bioactive polyphenols (Rothwell et al., 2012). Acute intake of tea also reduced postprandial glycaemia in humans (Bryans et al., 2007).

Beta glucan is a soluble dietary fibre with mixed β-(1→3) and β-(1→4) linkages. The effects of beta glucans on post-prandial glycaemic and insulinaemic responses in humans have been well established (Ostman et al., 2006). The European Food Safety Authority (ESFA) approved a health claim in which 4 g of beta glucan from either oats or barley per 30 g available carbohydrate is recommended to reduce glycaemic response without disproportionally increasing postprandial insulinaemic response (Agostoni et al., 2011). Hence, beta glucan can be used as an active ingredient in formulating products aiming at reducing postprandial blood glucose.

Bread is the most popular starchy food in Europe with an average intake of 50 kg bread per person per year (Bakers, 2014). Bread contains gluten and starch whose properties are directly influenced by different stages of bread making (mixing ingredients, proofing and baking) (Rosell, 2011). Breads are a good target for further development to improve their functional properties (Hayta & Gamze, 2011). In addition, they have been used as a model to study food matrix interactions (Juntunen et al., 2002; Juntunen et al., 2003). Food constituents can interact in several ways when eaten separately but if cooked together in a product such as bread, these interactions may be more complex and influence other components during food processing. The addition of polyphenols and/or dietary fibres to

foods may directly or indirectly modify the properties of the starch components, their digestibility, and antioxidant properties (Juntunen et al., 2002; Juntunen et al., 2003; Rosen et al., 2011; Gawlik-Dziki et al., 2013). The addition of guar gum (guar galactomannan) to bread reduced *in vitro* starch hydrolysis by forming a physical 'barrier' to starch-alpha-amylase interactions (Brennan et al., 1996). The mechanism was due to the layer of galactomannan mucilage that coated the starch granules and bread matrix. Tea polyphenols (epigallocatechin gallate and epigallocatechin) remain stable during bread making (Wang & Zhou, 2004) and thus may confer further health benefits when incorporated into bread. Studies have shown that starch hydrolysis was directly related to glycemia and insulinaemic response in acute human feeding trials (Ekstrom et al., 2013). In this study we hypothesized that the addition of black tea and/or beta glucan will change the characteristics and functionality of wheat bread.

2. Materials and methods

2.1 Materials

White wheat flour and easy bake yeast were purchased from Allinson (Peterborough, United Kingdom), unsalted butter from Morning Fresh (Caerphilly, United Kingdom), dried skimmed milk powder form WM Morrisons Supermarkets PLC (Bradford, United Kingdom), barley beta glucan concentrate (Glucagel^{TM}) from DKSH (containing 17.9% carbohydrate, 9.9% moisture, 4.9% protein and other nitrogenous compounds, 1.9% lipid and 2% ash (according to manufacturer data) (Quai du Rhône, France) and freeze-dried pure tea granules from Tata Global Beverages GB LTD (containing 452.17 ± 18.93 mg gallic acid equivalents/100 ml) (Greenford, United Kingdom).

2.2 Bread making

Breads were prepared using standard bread making techniques (Burton-Freeman, 2010). All ingredients (**Table 1**) were weighed (in triplicate) on a digital kitchen scale (Brabanita, UK) into a baking pan and mixed manually before being placed in a domestic bread maker (Morphy Richards Ltd, South Yorkshire, UK). White bread (WF) and black tea bread (BT) were prepared using standard program 8 (bread mixes, 150 min) while beta glucan (βG) and beta glucan plus tea (βGBT) bread using program 5 (French bread, 210 min). The addition of beta glucan competed with gluten for water; therefore, more water was needed to compensate for water uptake by beta glucan (Jacobs et al., 2008). Additional proofing time allows extended fermentation by yeast to take place and this improved dough quality. The latter program allows extended proofing time and is crucial for the development of both βG and βGBT breads. Fully developed bread was allowed to cool for a total of 60 min which included 30 min in bread pan and 30 min at room temperature. The breads were individually weighed and sliced into 1.5 cm thickness. All breads were analysed in triplicate and used for each analysis. The structure of the breads was visualised using digital still camera at 4x power (Sony Cybershot, Sony Corp, Japan).

2.3 Proximate analysis

2.3.1 Protein content (Kjedahl method)

One gram of bread was weighed in a digestion flask for protein analysis. The sample was digested at 450°C for 1 h using Foss Tecator Digestor (Foss Tecator AB, Höganäs, Sweden), then distilled and titrated in an automated Kjeltec 2300 Analyzer (Foss Tecator AB,

Höganäs, Sweden). A specific Jones factor for bread of 5.70 was used for calculation of protein content.

2.3.2 Fat content (solvent extraction, Soxtec method)

One gram of bread sample was used for the determination of fat content. Seventy ml of petroleum ether (Fisher Scientific, USA) was added to the sample in an aluminium thimble which was carefully loaded into a Soxtec 2050 Automatic System (Foss Analytical AB, Höganäs, Sweden).

All analyses were done in triplicate and nutrient contents are expressed as g/100 g fresh weight.

2.4 Resistant starch (RS)

2.4.1 Hydrolysis and solubilisation of non-resistant starch

Resistant starch (RS) was determined using a Resistant Starch kit (Megazyme International Wicklow, AOAC Method 2002.02 and AACC Method 32-40). Samples (100 mg) were incubated with pancreatic α-amylase (EC 3.2.1.1) containing amyloglucosidase (AMG) (EC 3.2.1.3) at 37°C with continuous shaking (200 strokes /min) for 16 h. The tubes were centrifuged at 3,000 rpm for 10 min, the supernatant was carefully decanted and the pellets re-suspended in 2 ml of 50% ethanol with vigorous stirring on a vortex mixer. A further 6 ml 50% ethanol was added, mixed and centrifuged again at 3,000 rpm for 10 min. The supernatant was decanted and the extraction repeated followed by centrifugation.

2.4.2 Measurement of resistant starch

2 M KOH (2 ml) was added to each tube to re-suspend the pellets and stirred using a magnetic stirrer for 20 min in an ice water bath. Thereafter, sodium acetate buffer (pH 3.8) was added to each tube and incubated with 0.1 ml of AMG at 50°C for 30 min. An aliquot of the solution was transferred into glass test tubes and 3.0 ml of glucose oxidase/peroxidase (GOPOD) reagent added, incubated at 50°C for 20 min and absorbance measured at 510 nm against the reagent blank (BioMate 3, Thermo Electron Corporation, Madison, USA).

2.4.3 Measurement of digestible (solubilised) starch

The supernatant obtained above was pooled in a volumetric flask and 0.1 ml incubated with 10 µl of dilute AMG solution for 20 min at 50°C. Finally, 3.0 ml of GOPOD reagent was added, incubated at 50°C for 20 min and absorbance read at 510 nm against a reagent blank.

All analyses were done in triplicate and nutrient contents are expressed as g/100 g fresh weight.

2.5 Beta glucan content

A Mixed-Linkage Beta-Glucan kit (McCleary method) from Megazyme International Ireland (Wicklow, Ireland) was used for the determination of beta glucan. Sodium phosphate buffer (4.0 ml, 20 mM, pH 6.5) was added to 1 g bread samples and stirred on a vortex mixer. Lichenase (EC 3.2.1.73) was added and incubated for 1 h at 50°C. Sodium acetate buffer (5.0 ml, 200 mM, pH 4.0) was added and mixed on a vortex mixer. The tubes were centrifuged at 3,500 g for 10 min. Aliquots (0.1 ml) were incubated with β-glucosidase (EC 3.2.1.21) in 50

mM sodium acetate buffer (pH 4.0) at 50°C for 10 min. Finally, 3.0 ml of GOPOD reagent was added, incubated at 50°C for 20 min and absorbance measured at 510 nm against a reagent blank. The glucose content was calculated using Megazyme-Calc, a calculation sheet provided by the manufacturer. All analyses were done in triplicate and nutrient contents are expressed as g/100 g fresh weight.

2.6 Starch hydrolysis of breads

Starch hydrolysis was determined using a commercially available assay (Megazyme International, Wicklow, Ireland). Breads containing 50 mg available carbohydrate were added to 4 ml pancreatic α-amylase (5 mg/ml). Tubes were incubated at 37°C and the reaction was stopped by adding 0.7 ml of ethanol (99% v/v) at different time points (0, 10, 30, 60, 90, 120, 150 and 180 min). Sodium acetate buffer (100 mM, pH 4.5) was added and incubated with 10 µl of dilute AMG solution for 20 min at 50°C. Finally, 3 ml of GOPOD reagent was added, incubated for 20 min at 50°C and absorbance read at 510 nm against a reagent blank. The glucose content was calculated using Megazyme-Calc, a calculation sheet provided by the manufacturer. Glucose content was converted to starch using a 0.9 multiplying factor. Results are expressed as percentage (%) of total hydrolysed starch at different time.

2.7 Microscopic study of breads structures

Breads were sampled from the centre of the bread loaf and processed using a standard protocol of 70% alcohol (1 h), 90% alcohol (1 h), absolute alcohol (6 h 30 min), xylene (2 h 30 min) cycles and fixed in paraffin. The sections were cut into 2.5 µm thickness with a microtome (Shandon Finesse E, Thermo Scientific, Runcorn, United Kingdom) and dried in

150 an oven for 1 h at 60°C. The bread sections were stained with Lugol's iodine solution
151 [(0.33% I_2, w/v) and 0.67% KI (w/v)] (Sigma Aldrich, Steinheim, Germany) for 2 min
152 followed by 0.1% Light Green (Gurr, BDH Ltd., Poole, United Kingdom) for another 2 min.
153 The slides were visualised under light microscopy at the magnification power of 40x. Under
154 light microscopy, amylopectin stains brown, amylose stains dark brown (appears in the centre
155 of starch) and gluten stains light green.

156 *2.8 Statistical analysis*

157 Data are presented as the mean ± standard deviation. One-way ANOVA (SPSS version
158 21.0, SPSS Inc., Chicago, USA) and *post hoc* LSD test were used to determine the mean
159 differences between groups. General Linear Model (Repeated Measures) was used to
160 determine the effect of time interactions. Values are considered significantly different at the
161 level of $p < 0.05$. Coefficient of variation (CV) was calculated as standard deviation/mean x
162 100.

3. Results

3.1 Bread characteristics

White bread (WF) and black tea bread (BT) had significantly ($p < 0.05$) lower bread volumes than beta glucan (βG) and beta glucan plus black tea (βGBT) bread (**Table 2**). All breads showed similar length ranging from 12.1 to 13.4 cm. βG and βGBT breads had significantly lower height than WF and BT breads (8.1 - 8.7 cm and 13.0 - 13.6 cm, respectively; $p < 0.05$). The cross-section of the bread structure is shown in **Figure 1**. Both BT and βGBT appeared darker because of the black tea.

3.2 Nutrient composition

The total available carbohydrate of WF and BT was significantly ($p < 0.05$) higher than that of βG and βGBT (**Table 2**). There was no significant difference in resistant starch content. Digestible starch was lower ($p < 0.05$) in βG and βGBT than WF and BT. The protein content ranged from 7.0 - 9.1 g/100 fresh weight and was similar between the different types of bread. Fat content was significantly ($p < 0.05$) higher in WF bread than the other breads. Moisture content of bread added with beta glucan was significantly ($p < 0.05$) higher compared to WF and BT. The addition of beta glucan significantly ($p < 0.05$) reduced total energy content of βG and βGBT.

3.3 Microscopic study of bread structures

The bread microstructures, starch granules and protein (gluten), were studied and visualised under a light microscope (**Figure 2**). Amylopectin granules stained brown, amylose dark brown (appears in the middle of starch granule) and gluten light green. Starch

granules in WF and BT were swollen and sheared into small circular structures. In contrast, starch granules in βG and βGBT were elliptical and closely packed to each other. The green-stained gluten area was embedded in between starch granules and the porous (irregular white structures) area of the breads. The gluten networks were more prominent and appeared as a more continuous matrix in βG and βGBT compared with WF and BT.

3.4 Starch hydrolysis of breads

Standard flour (SF) showed the lowest starch hydrolysis from 10 to 120 min (**Figure 3**). There were significant ($p < 0.05$) time interactions for SF at 90, 120, 150 and 180 min compared with 10 min. There was no significant difference in starch hydrolysis between WF and BT from 0 to 180 min. Both WF and BT showed significantly ($p < 0.05$) higher starch hydrolysis at 10 min compared with βG and βGBT. Combination of beta glucan and black tea in βGBT significantly ($p < 0.05$) reduced early (10 min) starch hydrolysis by 25% compared with βG. There were significant ($p < 0.05$) time interactions for βGBT at 60, 90 and 150 min compared with 10 min.

4. Discussion

The aim of this study was to develop breads to determine food matrix interactions of two food components with established health effects. We determined the effect of adding beta glucan and/or black tea on starch structures and the impact of these interactions on starch characteristics and functionality. This is the first study to our knowledge of the development of a bread with combination of beta glucan and black tea on starch hydrolysis and microscopic study of starch granules. The composition of protein (gluten), starch and water play an important role in making good quality bread (Flander et al., 2007). Gluten is responsible for dough formation while starch is important in textural properties and stability of the bread. Water hydrates and expands gluten forming a viscoelastic protein network. The added beta glucan competed with gluten for water and decreased rising of the dough during proofing; therefore, more water was needed to compensate for water uptake by beta glucan; this was necessary to improve dough quality (Hager et al., 2010; Jacobs et al., 2008). The presence of mixed linkage (1→3)(1→4)-β-D-glucans stabilizes the air cells in bread dough and improves coalescence (Wang et al., 1998). Others have demonstrated that the addition of sorghum flour in flat bread lowers the proportion of rapidly digestible starch (RDS) and starch digestibility by 29% and 25%, respectively (Yousif et al., 2012).

In vitro studies demonstrated that black teas reduced *in vitro* starch hydrolysis (Zhang & Kashket, 1998; Guzar et al., 2012). Mechanistically, a structural relationship of flavonoids and α-amylase activity has been described with hydrogen bonding as well as the formation of a conjugate that stabilized the interaction with the active site of α-amylase (Lo Piparo et al., 2008). This effect may have been mediated through inhibition of amylase activity. However, in our study the addition of black tea in bread (BT) did not reduce starch hydrolysis. This

may be explained by differences in experimental design. In our study, tea was added to breads as an ingredient during baking. Therefore, we would anticipate that this would lead to a different effect than the one observed in previous studies because of complex food matrix-interaction between gluten, black tea and starch in bread. In bread, the addition of apple pectin and polyphenols extracts, from kiwi, blackcurrant or apple in dough development and bread baking, directly influenced the cross-linking of gluten polymers which could lead to more water holding and softer bread (Sivam et al., 2011). The type of polyphenols used may have different mechanisms in forming complexes with bread protein (gluten). Highly polar phenolic acids (*i.e.* the caffeic acid present in kiwi) are more mobile in bread than low polarity polyphenols (*i.e.* anthocyanins and proanthocyanins in blackcurrant and apple). Caffeic acid is attracted to charged components in protein and/or directly incorporates into the protein mesh work with less steric hindrance (Sun-Waterhouse et al., 2009; Sivam et al., 2011). Hence, we proposed that the presence of black tea polyphenols could form cross-linking with gluten which leads to softer bread, exposing starch granules to α-amylase activity and increased starch hydrolysis.

In guar gum wheat bread, the presence of galactomannan coated the starch granules and protein matrix during bread making process and subsequently reduced starch hydrolysis (Brennan et al., 1996). Our study showed that black tea had an additional effect when added together with beta glucan. The incorporation of black tea and beta glucan significantly reduced short-term (10 min) starch digestibility in βGBT compared with βG bread. The microscopy suggested that both beta glucan and black tea preserved the starch granule structure, which resulted in lower short-term starch hydrolysis. Black tea contains higher levels of high molecular weight theaflavins and thearubigins compared to the other types of teas (Shao et al., 1995). We propose that there is a food-matrix interaction between black tea

polyphenols, beta glucan and starch granules in the bread. The presence of black tea polyphenols could form complexes with the gluten-network while beta glucan preserved the starch structure and/or decreased the surface area for starch digestion by α-amylase and hence reduced starch hydrolysis.

The blunted early starch hydrolysis could translate to benefits for human health by various mechanisms. Firstly, it could reduce post-prandial glucose and insulin response *in vivo*. It has been previously demonstrated that the supplementation of guar bread reduced post-prandial insulin response in healthy individual (Ellis et al., 1991). Secondly, the polyphenol-linked beta glucan will be passed undigested and metabolised by gut microbiota in the large intestine to short chain fatty acids and phenolic acids. Tea has been metabolised by microbiota in the large intestine into phenolic acids and short chain fatty acids (Stalmach et al., 2009; Stalmach et al., 2010). Beta glucan was fermented by intestinal microbiota and significantly increased propionate giving an acetate:propionate:butyrate production ratio of 51:32:17 which was considered as propionate-rich (Hughes et al., 2008). It has been demonstrated that phenolic acid metabolites and short chain fatty acids decreased the levels of reactive oxygen species, modified gut microbial balance and promote satiety (Beloborodova et al., 2012; Parkar et al., 2013; Arora et al., 2011). The incorporation of propionate in foods could induce hypophagic effects through inhibition of cholesterol synthesis, reduced lipolysis and delayed gastric emptying rate (Arora et al., 2011). Hence, thirdly, the addition of beta glucan in bread will increase the level of propionate by the action of gut microbiota and promoting greater satiety. A further mechanistic study is warranted to study the structural relationship between polyphenols and dietary fibre in reducing starch hydrolysis and their fermentability by gut microbiota.

5. Conclusions

This study showed that the addition of beta glucan alone or combination of black tea and beta glucan influenced starch functionality in bread. The combination of black tea and beta glucan reduced the hydrolysis of rapidly digestible starch. This finding suggests a food matrix effect of beta glucan and black tea resulting in preserved elliptical starch granules and lowered short-term starch hydrolysis. The applicability of these newly developed breads as functional foods is promising.

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Declaration of interest

The authors would like to declare that there is no conflict of interest.

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Figure 1. Detail of bread structure. The structure of bread was visualised at 4x power. (a) WF, white bread; (b) BT, black tea bread; (c) βG, beta glucan bread; (d) βGBT, beta glucan plus tea bread.

Figure 2. Microscopic figures of bread structures. (a) WF, white bread; (b) BT, black tea bread; (c) βG, beta glucan bread; (d) βGBT, beta glucan plus tea bread. Amylopectin granule stains brown, amylose dark brown (appears in the middle of starch granule) and gluten light green.

Figure 3. Starch hydrolysis based on percentage (%) of total hydrolysed starch of different breads. WF, white bread; BT, black tea bread; βG, beta glucan bread; βGBT, beta glucan plus tea bread; SF, Standard flour (provided by supplier). Values are expressed as mean ± standard deviation. Values with different letters are significantly different at the level of $p < 0.05$ within same time point. Asterisk (*) indicates significant ($p < 0.05$) time interactions vs 10 min. Coefficient of variation (CV) is less than 32.0%.