Can bread processing conditions alter glycaemic response?

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A R T I C L E    I N F O
Article history:
Received 7 July 2014
Received in revised form 12 September 2014
Accepted 7 October 2014
Available online 19 October 2014

Keywords:
Bread
Glycaemic response
Processing

A B S T R A C T

Bread is a staple food that is traditionally made from wheat flour. This study aimed to compare the starch digestibility of western baked bread and oriental steamed bread. Four types of bread were prepared: western baked bread (WBB) and oriental steamed bread (OSB), modified baked bread (MBB) made with the OSB recipe and WBB processing, and modified steamed bread (MSB) made with the WBB recipe and OSB processing. MBB showed the highest starch digestibility in vitro, followed by WBB, OSB and MSB. A similar trend was observed for glycaemic response in vivo. MBB, WBB, OSB and MSB had a glycaemic index of 75 ± 4, 71 ± 5, 68 ± 5 and 65 ± 4, respectively. Processing differences had a more pronounced effect on starch digestibility in bread, and steamed bread was healthier in terms of glycaemic response. The manipulation of processing conditions could be an innovative route to alter the glycaemic response of carbohydrate-rich foods.

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1. Introduction

Carbohydrates are quantitatively the most important dietary energy source for humans, typically accounting for 45–70% of total energy intake (Lafiandra, Riccardi, & Shewry, 2014). They play an important role in energy metabolism and glucose homeostasis. Carbohydrate foods that increase blood glucose rapidly are known as high glycaemic index (GI) foods, and those that increase blood glucose gradually are known as low GI foods (Jenkins, Wolever, & Taylor, 1981). They may be divided into low GI (GI < 55), medium GI (56 ≤ GI < 69) or high GI (GI ≥ 70). Diet is known to play a critical role in the aetiology and management of obesity and diabetes (Thomas & Pfeiffer, 2012). A large number of studies, including observational prospective cohort studies as well as randomized controlled trials, show a positive association between consumption of low GI food in the prevention of obesity, diabetes and cardiovascular diseases (Brand-Miller, McMillan-Price, Steinbeck, & Caterson, 2009). The glycaemic response is also influenced by the quantity of carbohydrates consumed. Glycaemic load (GL) is a measure to quantify the overall glycaemic effect of a portion of food, and takes into account the amount of carbohydrates present in a serving (Foster-Powell, Holt, & Brand-Miller, 2002).

Bread is a staple food that is traditionally made from wheat flour. It is consumed in different parts of the world, albeit in different forms due to variations in the choice of ingredients used, and processing techniques employed. The GI value of bread was reported to range from 40 to 97 (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006). The wide-ranging GI values may be due to: (a) differences in molecular configuration of starch present, (b) variations in cooking and processing methods that resulted in differing degrees of starch gelatinization, (c) differences in structure of bread in terms of compactness and viscosity, and (d) possible interactions with other food components, such as starch-protein and starch-lipid interactions, that could impede starch digestibility (Thondre, 2013).

In Asia, notably China and its surrounding regions, steamed bread is a popular staple. In contrast to baked bread, oriental steamed bread is typically made using low to medium protein content wheat flour that undergoes fermentation and is further cooked by steaming, rather than baking. A distinction should be made between steamed bread (“mantou”) and steamed bun (“baozi”). Mantou is plain steamed bread without any filling, whereas baozi is steamed bread containing sweet or savoury fillings made of bean paste or minced meat. Thus, western oven-baked bread and oriental steamed bread vary in both the ingredients used, preparation methods, and heating methods applied. Although glycaemic response of western bread has been researched intensively in recent years, there is a dearth of information related to the glycaemic response and glycaemic index of oriental steamed bread. This
study aimed to compare the in vitro starch digestibility and in vivo glycaemic response of western baked bread and oriental steamed bread. More importantly, the study focused on investigating how differences in macronutrient composition and processing conditions [namely, mixing time, mixing intensity, proofing period and method of cooking] influenced glycaemic response.

2. Materials and methods

2.1. Chemicals, reagents and bread ingredients

Sodium hydroxide pellets and concentrated hydrochloric acid (5 M) were purchased from Merck chemicals (Darmstadt, Germany). Pancreatin (P7545, 8X USP specifications), pepsin (800–2500 units/mg) and amyloglucosidase (≥300 U/ml) used in the in vitro digestion protocol were purchased from Sigma–Aldrich Company Ltd. (St. Louis, USA). Amyloglucosidase (E-AMGDF, 3260 U/ml) used for the secondary digestion in the reducing sugar assay was obtained from Megazyme International (Wicklow, Ireland). Absolute ethanol was obtained from Fisher Scientific Company (Fairfield, USA). Milli-Q ultrapure water was used throughout the experiments (Billerica, USA). The maleate (0.2 M/pH 6) and acetate (0.1 M/pH 5.2) buffers were prepared according to previously described methods (Mishra & Monro, 2009). Lancets were purchased from Abbott (Abbott, UK) for in vivo GI testing. Glucotrol (Eurotrol, Sweden) was used for daily quality checking of glucose metres to ensure reliability of results.

High protein wheat flour (Prima bread flour, Singapore), medium protein wheat flour (Hong Kong Bake King Flour, Singapore), vegetable shortening (Bake King, Singapore), yeast (SAF instant, France), salt (Fairprice, Singapore) and sugar (Fairprice, Singapore) were purchased from the local supermarket. Potable water was used for preparation of bread.

2.2. Bread making process

Four types of bread were prepared for this study (Table 1). Western baked bread (WBB) was prepared using standard recipe ingredients (with the use of high protein flour) and processing steps, including baking at 210 °C, and oriental steamed bread (OSB) was prepared using standard recipe ingredients (with the use of medium protein flour) and processing steps, including steaming at 100 °C. The standard recipes for WBB and OSB were adapted from previously described methods (Ananingsih, Gao, & Zhou, 2013; Wang & Zhou, 2004). Modified baked bread (MBB) was prepared using oriental steamed bread recipe ingredients and baked bread processing steps (baking at 210 °C). Modified steamed bread (MSB) was prepared using western baked bread recipe ingredients and steamed bread processing steps (steaming at 100 °C). Bread was freshly prepared on the morning of the study.

2.3. Analytical methods

Protein, fat and moisture contents were determined according to AACC methods 46–11.02, 30–25.01 and 44–01.01 respectively (AACC International, 2000). Protein content was analysed with FOSS Kjeltec Systems (FOSS, Denmark). Fat content was analysed with FOSS Sixtec 2055 (FOSS, Denmark). Dough, proofed dough and bread volume were determined with a VSP 600 Volscan Profiler (Stable Micro System Ltd., UK). The sample was mounted on a stand, and a laser sensor was used to scan the rotating sample to measure the contours of the sample at regular intervals for calculation of volume using the installed computer software. Specific volume was determined by calculating the ratio between the volume of the dough or bread and its weight. These measurements were carried out in triplicates, with two measurements per analysis. The total available carbohydrate content of each type of bread was determined using Megazyme assay kit (Megazyme, Ireland).

2.4. In vitro analysis of starch digestibility

In vitro starch hydrolysis and quantification of sugars released during digestion were carried out according to previously described methodology (Mishra & Monro, 2009; Ranawan & Henry, 2013). About 2.5 g of each type of bread was weighed and analysed. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were quantified. RDS was defined as starch that was rapidly digested within 20 min into pancreatic digestion phase, whereas SDS defined as starch that was digested within 20–120 min of pancreatic digestion phase. Triplicates were carried out for the in vitro analysis of starch digestibility.

2.5. In vivo analysis of glycaemic response

2.5.1. Subjects

Thirteen healthy subjects (seven male and eight female; age 24 ± 5 years old; BMI 21.2 ± 1.8 kg/m²; values expressed as means ± SD) participated in the study. The inclusion criteria for healthy subjects were: age between 21 and 50 years old, BMI values ranging between 18.0 and 24.9 kg/m², blood pressure values <120/80 mmHg, and fasting blood glucose levels <6.0 mmol/l. Subjects who smoked, had metabolic diseases or took part in sports at competitive levels were excluded from the study. Ethics approval was given by the National Healthcare Group Domain Specific Review Board. Written informed consent was obtained from subjects prior to participation in the study.

2.5.2. Study design

The study protocol used was in accordance with procedures recommended by the FAO/WHO/ISO (FAO, 1998). The subjects were instructed to avoid vigorous exercise and excessive alcohol the day before the test, and to consume dinner in standard portions the evening before in order to avoid the second meal effect (Wolever, Jenkins, Ocan, Rao, & Collier, 1988). Subjects were requested to fast for at least 10 h prior to the test, and to report to the testing site between 0800 and 0900 h on the day of the test session. Bread was presented to subjects in a randomized order on four separate test sessions. Each portion of bread served was equivalent to 50 g of total available carbohydrate content to account for differences in recipe formulation. The reference food was 50 g of anhydrous glucose dissolved in 250 ml of potable water. The mean glycaemic response of reference food, calculated as the average glycaemic response from three separate test sessions of consumption of 50 g glucose, was used for the calculation of GI of test bread. This was done to account for inter-day variability of subjects. There was at least a 1 day gap between GI measurements to minimise carry-over effects. The test and reference food were served with plain drinking water. Subjects were instructed to finish the test food within 15 min, and physical activity was kept to a minimum during the test.

Fasting blood samples were taken at –5 min and 0 min. Fingers were gently massaged prior to finger pricking. Baseline values were calculated as a mean of the two values with coefficient of variation ≤4%. The test food was consumed after taking baseline measurements, and timing started with the first bite of the test food. Further blood samples were taken at 15, 30, 45, 60, 90 and 120 min. Blood glucose was measured with a HemoCue Glucose 201+ RT analyser (Hemocue® Ltd., Sweden). The glucose metres were checked daily using Glucotrol to ensure reliability of the measurements. Incremental area under blood glucose curves (IAUC) was calculated geometrically (Brouns et al., 2005). GI value of
Table 1
Ingredients and processing parameters for WBB, MSB, OSB and MBB.

<table>
<thead>
<tr>
<th></th>
<th>WBB</th>
<th>MSB</th>
<th>OSB</th>
<th>MBB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Western baked bread</strong></td>
<td>Modified steamed bread made with conventional baked bread recipe</td>
<td>Modified baked bread made with steamed bread recipe</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ingredients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flour (g)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Water (g)</td>
<td>590</td>
<td>590</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>40</td>
<td>40</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Salt (g)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Yeast (g)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Shortening (g)</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixing</td>
<td>- Mix dry ingredients at level 1 intensity for 1 min</td>
<td>- Mix dry ingredients at level 1 intensity for 1 min</td>
<td>- Mix dry ingredients at level 1 intensity for 1 min</td>
<td>- Mix dry ingredients at level 1 intensity for 1 min</td>
</tr>
<tr>
<td></td>
<td>- Add water, mix at level 1 intensity for 1 min, followed by level 3 intensity for 7 min</td>
<td>- Add water, mix at level 1 intensity for 1 min, followed by level 3 intensity for 7 min</td>
<td>- Add water, mix at level 1 intensity for 1 min, followed by level 2 intensity for 4 min</td>
<td>- Add water, mix at level 1 intensity for 1 min, followed by level 3 intensity for 7 min</td>
</tr>
<tr>
<td>Resting</td>
<td>15 min</td>
<td>15 min</td>
<td>15 min</td>
<td>15 min</td>
</tr>
<tr>
<td>Proofing</td>
<td>70 min</td>
<td>40 min</td>
<td>40 min</td>
<td>70 min</td>
</tr>
<tr>
<td>Cooking</td>
<td>Baking at 210 °C, 11 min</td>
<td>Steaming at 100 °C, 10 min</td>
<td>Steaming at 100 °C, 10 min</td>
<td>Baking at 210 °C, 11 min</td>
</tr>
</tbody>
</table>

*Resting was carried out at room temperature.

*Proofing was carried out at 40 °C, 85% relative humidity.*
bread for each subject was calculated by expressing the IAUC of bread as a percentage of IAUC of the mean of the three glucose tests. The GI of each type of bread was calculated as the mean GI for all the subjects. The GI of each type of bread was calculated by: (GI x 50 g total available carbohydrate)/100.

2.6. Statistical analysis

Data processing was carried out using SPSS software (version 16, USA). Data was presented as means ± standard deviation (SD) or means ± standard error of mean (SEM), as indicated. The specific volume results were analysed using Kruskal–Wallis test, and post hoc comparisons were carried out using independent samples t-test. Statistical significance was set at p < 0.05 for all cases.

3. Results and discussion

3.1. Specific volume of bread

The specific volume of dough, proofed dough and bread were measured to compare the development of bread structure (Table 2). MBB dough had the highest specific volume, and specific volume for other types of dough did not differ significantly (p > 0.05). Increased protein content of flour has previously been found to have a positive effect on bread specific volume (Barak, Mudgil, & Khatkar, 2013). WBB had the highest specific volume for both proofed dough and baked bread, followed by MBB, OSB and MSB. The higher protein content in WBB (Table 3) may result in the formation of a more extensive gluten network during dough development which enabled the more effective retention of gas during proofing, thereby resulting in a higher specific volume as compared to MBB. On the other hand, the specific volumes of MSB proofed dough and bread were found to be significantly lower than OSB, despite having higher protein content. This suggested that specific volume of bread was not only dependent on protein content, but was also influenced by processing parameters.

During optimal dough development, gluten proteins are hydrated and undergo dis-aggregation, in which glutenins align and form cross links with gliadins, and cross-linked protein sheets are layered one over another to form the gluten network (Kontogiorgos, 2011). Energy input during dough development increased with mixing intensity and duration. The gluten network has been shown to be weakened and ruptured with excessively high energy input, resulting in reduced dough visco-elasticity (Peighambardoust, Fallah, Hamer, & van der Goot, 2010). Increasing the length of fermentation time also had a positive effect on bread volume for optimally developed dough, in which the expansion of gas cells brought about an increase in porosity of bread crumb. The use of higher mixing speed, longer mixing times and longer proofing period during the WBB and MBB bread making procedure resulted in WBB and MBB with significantly higher specific volume for both proofed dough and bread, as compared to OSB and MSB. In addition, bread typically experiences oven-spring upon heating, due to further expansion of gas volumes at elevated temperatures (Mondal & Datta, 2008). The use of higher temperatures during baking (210°C), as compared to steaming (100°C) may have resulted in a faster rate of temperature increase of dough for baked bread as compared to steamed bread, resulting in higher specific volumes for WBB and MBB, as compared to OSB and MSB. MSB had the lowest specific volume, possibly due to under-development of dough. High protein flour was used for preparation of MSB, but underwent a short mixing period with low intensity, and this could have resulted in insufficient development of the gluten network. The combination of low energy input during dough development, as well as a shortened fermentation period, resulted in MSB having the least porous structure as compared to other types of bread.

3.2. Starch digestibility

3.2.1. In vitro starch digestibility

Bread was subjected to in vitro enzymatic digestion under controlled conditions to quantify the amount of rapidly digestible starch (RDS) and slowly digestible starch (SDS) (Fig. 1). In a previous study, RDS had been found to show good correlation with in vivo glycaemic response, and it could therefore be a proxy indicator of GI value (Englyst, Vinoy, Englyst, & Lang, 2003). RDS was found to be the predominant starch fraction in all four types of bread, as most of the starch was fully gelatinized, and bread typically has an open structure, rendering starch highly accessible to hydrolysis by amylase (Ranawana & Henry, 2013; Ronda, Rivero, Caballero, & Quílez, 2012). Comparing MBB with WBB (different recipe ingredients and same processing procedures), it was found that MBB had significantly higher RDS content than WBB. Similarly, comparing OSB with MSB (different recipe ingredients and same processing procedures), OSB had significantly higher RDS content than MSB. Although more sugar was used in the recipe
for preparing WBB and MSB, the medium protein content wheat flour used for preparing OSB and MBB had a higher available carbohydrate content as compared to the high protein content wheat flour used for preparing WBB and MSB (Table 3). Even though less sugar was used in the recipe for OSB and MBB, it resulted in higher RDS amounts being detected during in vitro studies.

Processing conditions had a marked effect on in vitro digestibility. Baked breads (WBB and MBB) were found to have higher RDS content. This could be attributed to the higher specific volume and porosity, resulting in increased accessibility of amylases to starch granules, rendering starch more susceptible to hydrolysis. A previous study has shown that an increase in degree of mixing in dough results in higher amounts of RDS content, possibly due to a weakened gluten matrix that renders starch granules more accessible to enzymatic digestion (Parada & Aguilera, 2011). WBB and MBB were found to have significantly higher SDS content than MSB and OSB \((p < 0.05)\). Rapid evaporation of water from the outermost region of dough in the presence of dry heat resulted in incomplete gelatinization of starch granules (Primo-Martín, van Nieuwenhuijzen, Hamer, & van Vliet, 2007). Digestibility has been shown to reduce with decreased gelatinization, as limited swelling and hydration decreases the chemical reactivity of starch granules towards amylolytic enzymes (Parada & Aguilera, 2009). The use of moist heat during steaming did not result in crust formation, accounting for the significantly lesser SDS content found in OSB and MSB.

3.2.2. In vivo glycaemic response

The postprandial blood glucose responses to steamed and baked bread showed different temporal profiles as shown in Fig. 2. In agreement with a previous study (Brouns et al., 2005), there were no significant differences in IAUC and GI between male and female subjects. Peak glycaemic response for OSB and MSB was observed at 30 min, whereas WBB and MBB showed a delayed peak glycaemic response at 45 min. WBB and MBB gave rise to higher peak glucose concentrations than OSB and MSB, and the higher blood glucose concentrations were sustained until 120 min. This resulted in higher IAUC for WBB and MBB, but the differences were not significant amongst the four types of bread. Physical structure was found to be an important factor in determining glycaemic response of bread, as reported in previous studies (Burton & Lightowler, 2006; Lioger et al., 2009). The manipulation of physical structure, and in turn, starch digestibility, was brought about by differences in processing procedures. A more compact bread structure could have hindered the accessibility of amylase to starch granules, resulting in a slower rate of glucose release, and reduced glycaemic response in OSB and MSB.

The total IAUC of MBB was slightly higher than WBB, with GI values of 75 and 71 respectively (Table 4). MBB and WBB were prepared using different recipe ingredients, but underwent the same processing procedure. A similar trend was observed for OSB and MSB, which had GI values of 68 and 65, respectively. The modest differences in glycaemic response in this study suggested that
macronutrient composition played a minor role in digestibility of starch. Digestion of starch is known to be hindered in the presence of proteins and lipids, as proteins may encapsulate starch granules to form a protective barrier against enzymatic hydrolysis, and starch, particularly amylose, may form complexes with lipids to resist breakdown. The lower protein and fat content in MBB and OSB, as compared to WBB and MSB (Table 3), did not change postprandial glycaemic response markedly. MBB showed a slightly higher glycaemic response than WBB in vivo, and had significantly higher in vitro starch digestibility than WBB. Despite having a lower specific volume, the greater extent of starch digestibility of MBB, both in vitro and in vivo may be partly attributed to the lower protein content in the flour used for the preparation of MBB. MBB dough was subjected to intense energy input during dough development, and the weakened gluten network in MBB, as seen from the lower specific volume of bread, as compared to WBB, could have resulted in reduced resistance during enzymatic digestion, and correspondingly a higher glycaemic response.

In vitro starch digestibility and in vivo glycaemic response results were in agreement and showed the same ranking for the four types of bread. MBB was found to have the highest glycaemic response, followed by WBB, OSB and MBB. This indicated that digestibility of starch and the release of glucose were consistent in both studies. Bread was predominantly made up of RDS, and the amount of SDS was comparatively too low to significantly impact on the glycaemic response. Hence, the higher SDS contents did not contribute to reduced glycaemic response in this case. The physical structure of bread could be manipulated by processing conditions (namely mixing time and duration, fermentation period and method of cooking), resulting in pronounced changes in glycaemic response. When ascertaining whether macronutrient composition or processing parameters had a greater impact on glycaemic response, the latter was found to play a more pivotal role in this study, as both types of steamed bread, OSB and MSB, demonstrated lower starch digestibility in vitro and reduced glycaemic response in vivo. It has been reported that the risks of developing cardiovascular diseases is associated with an elevated blood glucose levels in healthy individuals (Braun, Bopp, & Faeh, 2013). Modest increases in postprandial blood glucose levels could also reduce the long-term risk of developing Type 2 diabetes (Braun, J., Bopp, M., & Faeh, D., 2013). Blood glucose may be an alternative to starch in reducing the long-term risk of developing Type 2 diabetes (Buyken, Mitchell, Ceriello, & Brand-Miller, 2010). In this study, steamed bread emerged as a "healthier" alternative to baked bread.

Glucose tolerance is known to decrease with age due to impaired insulin secretion and action. Higher postprandial glucose concentrations after consumption of a mixed meal has been observed in elderly adults as compared to younger individuals (Basu et al., 2006). Subjects within the age band of 19–50 years are typically recruited for GI studies (Wolever et al., 2008), however, the mean age of the subjects recruited for this study was 24 years. It is important to recognise that the results in elderly adults, compared to the relatively young adults in our study, could be different. Future studies could be carried out to assess the glycaemic response and insulinemic index in elderly adults.

4. Conclusion

This study demonstrated for the first time that even with the use of identical bread recipe ingredients, the application of varied processing conditions, namely mixing time, mixing intensity, proofing period and method of cooking, resulted in lower starch digestibility in vitro and reduced glycaemic response in vivo as compared to baked bread. Processing played a major role in affecting the physical structure of bread. It has been customary to use food ingredients such as β-glucan, galactomannan, non-starch polysaccharides and polyols to reduce the glycaemic index of high GI foods (Thondire, 2013). The observation that processing parameters could impact on glycaemic response of wheat-based foods provides us with a new approach to manipulate the glycaemic index of carbohydrate-rich foods. Work is in process to elucidate the differences in the microstructure of bread to understand the potential links between food microstructure and glycaemic response.

Conflict of interest

None of the authors declare any conflict of interest.

Acknowledgements

This research was funded by the A+STAR Health and Lifestyle Grant (Grant No. 112 177 0033). We would also like to thank Dr. Tan Sze Wee, Deputy Director of Biomedical Research Council, for his continued support.

References


Table 4

Postprandial blood glucose characteristics of WBB, MSB, OSB and MBB.

<table>
<thead>
<tr>
<th></th>
<th>WBB</th>
<th>MSB</th>
<th>OSB</th>
<th>MBB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max incremental peak rise (mmol/L)</td>
<td>3.2 ± 0.2*</td>
<td>2.7 ± 0.2*</td>
<td>2.9 ± 0.3*</td>
<td>3.2 ± 0.2*</td>
</tr>
<tr>
<td>GI</td>
<td>71 ± 5*</td>
<td>65 ± 4*</td>
<td>68 ± 5*</td>
<td>75 ± 4*</td>
</tr>
<tr>
<td>GI</td>
<td>36*</td>
<td>33*</td>
<td>34*</td>
<td>38*</td>
</tr>
</tbody>
</table>

Values are represented as means ± SEM. Values within a row with same superscript letters are not significantly different (p > 0.05).


