Effect of Extent of Gelatinisation of Starch on the Glycaemic Responses of Carbohydrate Rich Breakfast Meals

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ABSTRACT

Introduction: Previous studies have shown that roti and pittu, which are South Asian foods, when prepared with the same composition of wheat flour and coconut scrapings had significantly different (p<0.05) glycaemic index (GI) values. The only difference was in the processing where roti (GI 57) was dry-heated (roasted) and pittu (GI 80) was wet-heated (steam cooked). The present study was carried out to investigate the association between GI and the properties of starch during processing for the observed variations of GI values of roti and pittu prepared with different flour varieties. Methods: The characteristics of isolated starch granules, molecular size distribution pattern of carbohydrates, amylose, amyllopectin contents and change in temperature during the cooking of ‘pittu’ and ‘roti’ were analysed. Results: The results indicate that the contribution to GI from starch gelatinisation correlates positively and corroborates with reported data. Thus the significantly low GI values of roti compared to pittu could be mainly attributed to less disintegrated and less swollen starch granules of flour used in the preparation of roti. This was observed irrespective of the variety of flour. Conclusion: This study confirms that wet heat gelatinises starch to a greater extent than dry heat and provides evidence of a possibility that foods processed using dry heat to be associated with lower GI values, than the wet processed foods if other factors are constant.

Key words: Roti, pittu, Glycaemic Index, starch granules, starch gelatinisation

INTRODUCTION

The glycaemic index (GI) has become a major tool in ranking carbohydrate foods and the dietary management of diabetes type 2 and obesity (Thomas & Elliott, 2010). Although diabetes and obesity could be controlled by several methods, dietary management achieved by consumption of low GI foods would be the best solution for patients with mild hyperglycaemia. Hence, identification of low GI foods and studying the factors contributing to low GI have now become an important issue as such information would be of essence in formulating foods with low GI values and in providing dietary advice.

Englyst et al. (2003) demonstrated that foods containing starch, characterised by slow digestibility, produce significantly low (p<0.05) glycaemic responses and insulin

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responses compared to foods that contain starch of higher digestibility. Foods containing starches with ‘Slowly Available Glucose’ (SAG) would result in lower blood glucose responses and consequently low GI. In addition to many other factors, the effect of processing has been shown to be vital in the digestibility of carbohydrates and thus the blood glucose response (Ayodele & Godwin, 2010).

Raw foods contain crystalline starch in hard compact granules which are difficult to digest (Vosloo, 2005). Thus during heating in the presence of water, the starch granules swell, releasing the components amylose and amylopectin into the matrix. Hence, escaped molecules could be easily available and digested by $\alpha$-amylase. However, an intact starch crystalline structure even after cooking could be a reason for low GI values of some processed foods due to less accessibility to pancreatic $\alpha$-amylase (Vosloo, 2005).

According to a previous study on determining GI of Sri Lankan breakfast meals in vivo, the foods roti (consisting of a mixture of flour and coconut scrapings cooked on a heated pan) and pittu (containing a mixture of flour and coconut scrapings cooked by steam) prepared with the same composition and a variety of flours but were wet and dry processed respectively had significantly different GI values ($p<0.05$); wheat flour roti (wheat flour: coconut scrapings 1:1) had a significantly low GI compared to wheat flour pittu made with the same composition (Widanagamage, Ekanayake & Welihinda, 2009).

Though it was observed that the variation in GI values is partly associated with the content of dietary fibre (soluble and insoluble) and protein, the above observation indicates that the processing method, that is, whether wet processed or dry processed, is a determinant in GI being high or low (Widanagamage, et al. 2009).

The endothermic heat transition studies by Resio & Suarez (2001) showed that the temperature for gelatinisation of starch shifts to lower temperature favouring gelatinisation of starch when increasing the water content.

The present research was carried out to study the association of the properties of starch with the variation in GI values of roti and pittu made with a similar composition and other wet and dry heat processed foods. The properties of starch that were studied included chemical nature of starch (amylose: amylopectin ratio), degree of gelatinisation of starch due to dry and wet heat processing, temperature change during processing and change in percentage of starch granules after processing.

**METHODS**

Raw materials such as wheat flour, atta flour (whole wheat), kurakkan (Eleusine coracana) flour, rice flour (AT 353 rice variety of Rice Research Institute at Batalagoda, Sri Lanka), chickpea (Cicer arietinum), mungbean (Vigna radiate), cowpea (Vignaungui culata), olu rice (seeds of Nymphaea lotus) and mature coconut necessary for the preparation of foods were obtained as bulk samples (to minimise variations), and stored at room temperature in air tight containers where possible.

**Preparation of food samples**

Table 1 indicates the preparation methods of foods studied with their corresponding GI values (Beals, 2005; Widanagamage et al., 2009). Roti was prepared by a mixture of flour and coconut scrapings (120 g dough) with added salt. The flattened roti dough was spread on a circular pan and subjected to mild heat for 8-10 min turning upside down on the pan at 2-min intervals until the outer surface was brown in colour.

Pittu was prepared by mixing flour and coconut scraping with added salt, loosely packed in a household apparatus (i.e. Pittu Bambuwa) followed by steam cooking. Hopper dough was made by mixing flour,
coconut water and sodium bicarbonate. The mixture was allowed to ferment overnight (10 h). The fermented hopper dough was mixed with salt and first extraction of coconut milk and cooked in a hopper pan for 5 min. After an overnight soak (10 h), chickpea, cowpea and mungbean were prepared by boiling in excess water at 40, 60 and 35 min respectively.

Breadfruit was prepared by boiling pieces of breadfruit in excess water for 45 min. The foods were dried and made into flour. Portions of foods that were used to determine the GI in the previous study (Widanagamage et al., 2009), that is, wheat flour roti, rice flour roti, kurakkan flour roti, atta flour roti, wheat flour pittu, rice flour pittu, kurakkan flour pittu, hoppers, boiled breadfruit and boiled legumes (chickpea, cowpea and mungbean) were used in the present study to determine the properties of starch.

### Effect of properties of starch

#### Determination of amylose/amylopectin ratio

Amylose: amylopectin ratios of the foods were measured (n=6) by the method of Mohammadkhani et al. (1999). The flour sample was digested with 1M NaOH (BDH, England) and 95% ethanol (BDH, England) at 105°C for 45 min in a sand bath to solubilise the starch. The pH (6.9-7.0) of the mixture was adjusted with 1M citric acid (BSH, England) in order to maintain the stability of the final amylose-iodine complex. The amylose contents were determined by reacting the solubilised starch with I$_2$-KI mixture (0.08 g of I$_2$ with 0.8 g of KI) and diluted to 100 mL to form an amylose-iodine complex and absorbance measured at 620 nm against an amylose standard curve. The amylose standard curve was drawn using potato amylose. A stock of standard amylose solution was prepared by dissolving amylose (100mg) in NaOH (1M, 4.5mL) and

### Table 1. The glycaemic indices of Sri Lankan breakfast meals with the composition and the preparation methods

<table>
<thead>
<tr>
<th>Food</th>
<th>Composition</th>
<th>Preparation method</th>
<th>GI ±SEM **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet heat processed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoppers</td>
<td>Rice flour and coconut milk</td>
<td>Frying batter</td>
<td>95±6*a</td>
</tr>
<tr>
<td>Rice flour pittu</td>
<td>Rice flour and coconut scrapings</td>
<td>Steaming</td>
<td>81±6*a</td>
</tr>
<tr>
<td>Wheat flour pittu</td>
<td>Wheat flour and coconut scrapings</td>
<td>Steaming</td>
<td>80±7*a</td>
</tr>
<tr>
<td>Kurakkan flour pittu</td>
<td>Kurakkan flour and coconut scrapings</td>
<td>Steaming</td>
<td>67±5*b</td>
</tr>
<tr>
<td>Breadfruit</td>
<td>Pealed breadfruit (cubes of 3 cm x 3cm)</td>
<td>Boiling</td>
<td>51±6*b</td>
</tr>
<tr>
<td>Mungbean</td>
<td>Whole grain</td>
<td>Boiling</td>
<td>45±4*c</td>
</tr>
<tr>
<td>Cowpea</td>
<td>Whole grain</td>
<td>Boiling</td>
<td>39±6*c</td>
</tr>
<tr>
<td>Chickpea</td>
<td>Whole grain</td>
<td>Boiling</td>
<td>23±4*c</td>
</tr>
<tr>
<td>Dry heat processed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat flour roti</td>
<td>Wheat flour and coconut scrapings</td>
<td>Roasting on a pan</td>
<td>57±5*b</td>
</tr>
<tr>
<td>Kurakkan flour roti</td>
<td>Kurakkan flour, wheat flour and</td>
<td>Roasting on a pan</td>
<td>55±6*b</td>
</tr>
<tr>
<td></td>
<td>coconut scrapings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice flour roti</td>
<td>Rice flour, wheat flour and</td>
<td>Roasting on a pan</td>
<td>55±6*b</td>
</tr>
<tr>
<td></td>
<td>coconut scrapings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atta flour roti</td>
<td>Atta flour and coconut scrapings</td>
<td>Roasting on a pan</td>
<td>53±7*c</td>
</tr>
</tbody>
</table>

* Data adopted from Widanagamage et al., 2009; **GI±SEM determined against white bread and converted relative to glucose; a high GI foods, b medium GI foods and c low GI foods (Beals, 2005)
neutralising with citric acid (~2.2 mL) followed by dilution to 10 mL with distilled water. The absorbance values (620 nm) were measured (n=6) using different volumes of the stock amylose (10, 20, 25, 30, 50 μL) mixed with iodine/ KI solution (1000 μL) and diluted to 10 mL followed by standing for 20 min (2-8°C). The iodine/ KI solution was prepared freshly by dissolving I₂ (0.08 g) with KI (0.8 g) and diluted to 100 mL and used in amylose quantification.

The Spearman’s correlation coefficient was calculated using SPSS 13 software to study the correlation between the GI and amylose: amylopectin ratios of the foods studied.

Microscopic studies

The effect of extent of the gelatinisation of starch granules on GI was determined by studying the isolated starch. Isolation of starch was carried out by suspending food flour (20 g) in distilled water (50 mL) and homogenising (Ultra-Turrax®, T 25 basic, 17,500 rpm). The pH was adjusted to 1.5 and digested with pepsin (E.C. 3.4.23.1; Sigma, USA, 500 mg) at 40°C in a water bath (1 h). Then the mixture was centrifuged (2000 rpm, 20 min). The top white layer was separated and the sediment was washed with 60% ethanol and centrifuged (2000 rpm, 10 min). The clear white precipitate was then dried at room temperature (Ekanayake, Baboo & Asp, 2006). The isolated starch of food flour was observed under the light microscope (Olympus model CX21FS1) with and without iodine staining (iodine/ KI) and the extent of disruption of starch granules was observed (10 x 10 and 10 x 40). The length and breadth of the starch granules of raw and cooked food flour were measured in different microscopic fields (n=50) using a stage micrometer and an eye piece graticule. A mature starch granule per microscopic field was selected for measurement. The percentage increases in length and breadth were calculated compared to raw starch granules.

Molecular size distribution of carbohydrates

Molecular size distribution of carbohydrates of wheat flour pittu, wheat flour roti and chickpea was studied according to the method described in Ekanayake et al. (2006). Food (wheat flour roti, wheat flour pittu and boiled chickpea) flour sample containing starch (60 mg) was digested with pepsin (10 mg, pH 1.5, 40°C, 30 min), dissolved in dimethyl sulfoxide (90%, 4.5 mL), heated in a water bath (80°C, 6 h) and followed by centrifugation (Kubota™ 5100, 2000 r.p.m, 10 min). The supernatant was dissolved in KOH (0.1 M, 1 mL) and introduced into a Sepharose™ CL-6B (Amersham Biosciences, Sweden) column (height 30 cm, column diameter 2.5 cm). Potassium hydroxide (0.1 M) was used as the mobile phase (1.7 mL/min). The void volume and the bed volume were determined by Blue Dextran and glucose respectively. The starch of wheat flour roti, wheat flour pittu and boiled chickpea were introduced into the column and the elution profiles were determined by analysing the sugar contents in each fraction by phenol-sulphuric acid reagent. The sugar contents were measured using an internal standard of glucose (0.05 g) (Dubois et al., 1956).

Temperature vs time plot of wheat flour roti and wheat flour pittu

The probe of a calibrated thermometer (Digithermo model AZ-668shr, Australia) was inserted into the centre of the pittu and roti preparations which consisted of 1:1 ratio of wheat flour and coconut. The temperature readings were recorded at 1 min intervals during processing in order to verify the temperature changes that occur during processing.

Change in moisture during heat treatment of wheat flour roti and pittu

The ratio of water: starch of foods was studied to investigate the change in the water
contents of processed dough, in order to correlate the extent of gelatinisation with processing temperature. The water: starch ratios of the initial dough and the wheat flour roti and pittu after processing were calculated using corresponding moisture and digestible starch contents (Widana-gamage et al., 2009).

**Statistical analysis**

The percentage increases in starch granular sizes are expressed as mean of 50 determinations and the Student t-test ($\alpha = 0.05$) was used for comparison of starch granules of different foods. MINITAB 14 software package was used in calculations. Correlation of GI to amylose: amylopectin ratio was calculated with Spearman’s $p (\alpha = 0.05)$ using SPSS 13 software package.

**RESULTS**

Figure 1 shows the amylose: amylopectin ratio of the different foods studied against the GI of different foods. A significant correlation was not observed between GI and amylose: amylopectin ratio (Spearman’s $p = -0.248; p \geq 0.05$) of roti and pittu made with wheat flour or any other food studied.

Table 2 indicates percentage increase in starch granules with different processing methods when compared against raw granules. When starch granules of wheat flour roti and pittu were compared, the lengths of the mature granules (mean±SD) of wheat flour roti (59±5 mm) were significantly lower (6.3% lower; $p<0.05$) than that of wheat flour pittu (63±4 mm). The same was observed for the percentage differences in breadths (5.7% lower; $p<0.05$) of starch granules of wheat flour roti (50±6 mm) and pittu (53±6 mm). Furthermore the starch granules of rice flour pittu, kurakkan flour pittu and hoppers prepared using wet heat were swollen and disintegrated to a higher extent compared to corresponding roti made with same starch source (i.e. rice flour roti and kurakkan flour roti) preparations.

![Figure 1. Correlation between GI vs amylose: amylopectin ratios of the foods studied](image-url)
The light microscopic appearances of starch granules of wheat flour roti and pittu are shown in Figure 2. This also indicates highly disintegrated starch granules in wheat flour pittu (wet heat processed) compared to that of wheat flour roti (dry heat processed). A similar observation was made when considering other wet heat processed foods. A phenomenal observation was made with boiled legumes as the starch granules were highly swollen, but remained intact and cell-enclosed. Boiled breadfruit starch granules were completely disintegrated.

During processing, the temperature at the centre of pittu (steam processing) was 98.6 °C (tap water boiled at 98.6 °C). In roti,
the temperature in the middle of the roti was 97.6 °C and the surface temperature of the heating pan registered 208 °C. The moisture contents of roti and pittu dough before processing was 35%. Upon processing the water: starch ratio in roti decreased from 1.1 to 0.6 g water/g starch and in pittu increased from 1.1 to 1.2 g water/g starch.

Figure 3 depicts the molecular size distribution of carbohydrates by gel filtration chromatography in wheat flour roti, wheat flour pittu and boiled chickpea. Wheat flour roti contained eight times higher high molecular size (Kav < 0.2) carbohydrate fraction compared to wheat flour pittu (Table 3). Likewise wheat flour pittu consisted of three times higher low molecular size (0.8 < Kav < 1.0) carbohydrates compared to wheat flour roti (Table 3). When carbohydrates of boiled chickpea were studied (Figure 3), a high percent of low-molecular size starch (41%) which was 5 times higher compared to wheat flour roti and 1.7 times higher compared to wheat flour pittu was observed (Table 3).

**DISCUSSION**

In the present study, the effect of swelling power, solubility, particle sizes and the specific surface area of raw starch granules were not investigated as the comparisons were carried out with foods prepared with the same composition of raw flour (Riley, Wheatley & Asemota, 2006).

![Figure 3. Molecular size distribution of carbohydrates of wheat flour roti, wheat flour pittu and chickpea](image)

**Table 3. Distribution of different molecular weight size carbohydrates in the sepharose gel column**

<table>
<thead>
<tr>
<th>Food</th>
<th>% HMWC (Kav &lt; 0.2)</th>
<th>% MMWC (0.2 &lt; Kav &lt; 0.8)</th>
<th>% LMWC (0.8 &lt; Kav &lt; 1.0)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour roti</td>
<td>8</td>
<td>86</td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>Wheat flour pittu</td>
<td>1</td>
<td>75</td>
<td>24</td>
<td>71</td>
</tr>
<tr>
<td>Chickpea</td>
<td>6</td>
<td>53</td>
<td>41</td>
<td>96</td>
</tr>
</tbody>
</table>

* Percentage of total starch content for each food distributed in the column; HMWC- high molecular weight carbohydrates; MMWC- medium molecular weight carbohydrates; LMWC- low molecular weight carbohydrates.
The significant difference in GI of different foods could not be attributed to the contents of amylose and amylopectin as there was no significant correlation between GI and the amylose:amylopectin (Figure 1) in the present study although it has been reported that this ratio affects GI (Granfeldt, Drews & Bjorck, 1995).

The degree of swelling of starch granules of foods indicates that the starch granules of wet heat processed foods (wheat flour pittu, rice flour pittu, kurakkan flour pittu and hoppers), are more highly gelatinised than that of dry heat processed (wheat flour roti, rice flour roti and atta flour roti) foods (Table 2). The wheat starch granules were swollen to a lesser extent in dry heat processed foods studied (Table 2). This indicates that wet heat gelatinises starch granules to a greater extent compared to dry heat.

The light microscopic studies confirm the fact that dry heat gelatinises the starch granules to a significantly lesser extent than wet heat, as the starch granules in wheat flour pittu (wet heat processed) were highly disintegrated compared to that of roti (dry heat processed) as indicated in Figure 2. Other wet heat processed foods (boiled legumes, and boiled breadfruit) also indicated a higher degree of gelatinised starch granules, confirming this finding. Hence the microscopic studies of starch granules qualitatively and micrometrically as well as quantitatively reveal that wet heat processing causes starch granules to swell and disintegrate to a higher extent than dry heat processing and makes starch more susceptible to digestion when ingested.

In the processing of pittu, the steam carries the heat directly to the starch granules and the starch granules experience uniform heating at 98.6 °C. Whereas in roti, the temperature in the middle (97.6 °C) was due to heat transfer mainly via conduction and steam which is generated by the heating water inside the dough of roti. During preparation, roti dough had been inverted on the pan every 2 min making the cooking process efficient and even. Thus both wheat flour roti and wheat flour pittu have been processed at similar temperatures irrespective of the method of heating. However, the extent of gelatinisation of starch is also affected by the water content present during processing (Resio & Suarez, 2001). Although roti and pittu dough had similar moisture contents initially (35%), roti lost moisture (from 1.1 to 0.6 g water/g starch) upon roasting and pittu gained moisture (from 1.1 to 1.2 g water/ g starch) on steaming. This indicated that the roti had been cooked at continuously decreasing water content and with an increase in gelatinisation-temperature while pittu experienced the opposite conditions. According to Eliasson (1980), in wheat flour, the first endotherm occurs at ~ 60 °C with the endothermic heat flow decreasing with the water content. Thus after initiating the processing, wheat flour roti could be expected to be gelatinised to a lower extent compared to that of wheat flour pittu due to lower water content.

In demonstrating the extent of gelatinisation of wheat flour roti and pittu, the molecular size distribution of isolated starch obtained from both the wheat flour roti and pittu was studied. Table 3 indicates that wheat flour roti consisted of eight times higher proportion of high molecular size carbohydrate fractions compared to wheat flour pittu indicating a lower extent of gelatinisation. Moreover wheat flour pittu had three times higher low molecular size carbohydrates compared to wheat flour roti which clearly indicates that wet heat processing has contributed to a higher extent of gelatinisation of starch compared to dry heat processing.

Hence the significant reduction of GI values in roti preparations (dry heat processed) compared to pittu preparations (wet heat processed) studied could be mainly associated with the differences in the processing methods. Thus the categorisation of foods into low, medium and high GI (Widanagamage et al., 2009) correlates well with the processing method (Table 1). The
study of molecular size distribution patterns and microscopy employed in this study were novel attempts at understanding the effect of properties of starch on variation of GI of foods.

Although it was previously observed that the boiled legumes had highly swollen starch granules (Widanagamage et al., 2009), the low GI values of boiled legumes could be due to cell/matrix-enclosed (Tovar, Bjorck & Asp, 1992) starch granules which prevent amyllose and amylopectin molecules escaping into the matrix. This was also supported by the molecular size distribution studies which indicated a high percent of low-molecular starch in boiled (wet processed) chickpea (Table 3), the release of which from cell/matrix-enclosed granules could have been facilitated by the pepsin digestion step involved in the starch isolation procedure. Cell/matrix enclosure could also be partly associated with low GI values of legumes apart from the effect of protein, IDF and SDF (Widanagamage et al., 2009).

Boiled breadfruit, a medium GI food (Widanagamage et al., 2009) contained completely disintegrated starch granules and it could be postulated that this could be due to the presence of substance(s) that could stimulate insulin secretion or inhibit glucose absorption. However, further studies are needed before a definite conclusion is reached.

CONCLUSION

The results of the present study suggest that the method of processing, namely dry heat or wet heat, could be a significant factor associated with the variation in GI values in South Asian basic starchy foods in addition to the effects of other nutrients. This observation is also consistent with the general hypothesis that wet heat contributes to an increase in the GI. However, this has not been shown in relation to boiled legumes studied in the present study. The extensively gelatinised starch of legumes trapped in the cell/matrix enclosed granules contributed to a decrease in GI in addition to high protein and dietary fibre. It is recommended that when using wet heat in food processing, due control is to be exerted so as not to cause extensive starch gelatinisation in order to control the glycaemic response. These findings would be highly beneficial for the choice of processing or production at household or industrial level in producing low GI foods.

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Conflict of interest

The authors declare no conflict of interest related to this study or research article.

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