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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, amylopectin 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 α-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 α-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,

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

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

* 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)

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, 4.5 mL) and 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 I_2 (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-6 B (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 (Digi-thermo 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 (Widanagamage *et al*., 2009).

Statistical analysis

The percentage increases in starch granular sizes are expressed as mean of 50 determinations and the Student *t*-test (α = 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* (α = 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.

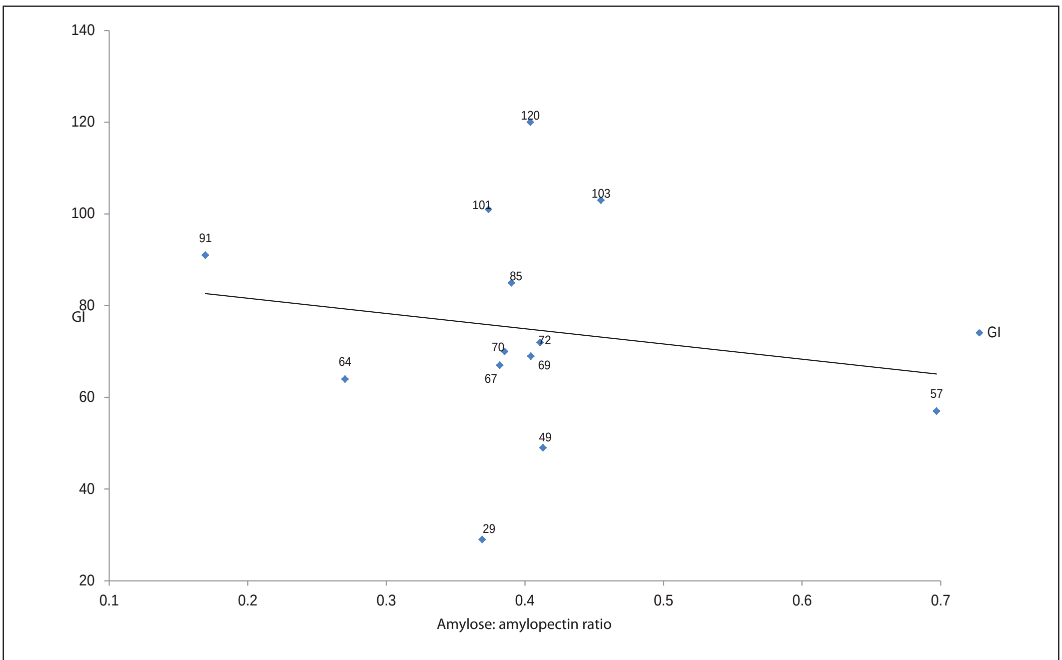

Figure 1. Correlation between GI vs amylose: amylopectin ratios of the foods studied

Table 2. Percentage increase in the sizes of dry heat and wet heat processed starch granules compared to the corresponding raw flour starch granules*

	Wheat starch granules		*Rice starch granules*		*Kurakkan starch granules*		*Atta starch granules*	
	Length	*Breadth*	*Length*	*Breadth*	*Length*	*Breadth*	*Length*	*Breadth*
Dry heat								
Wheat flour *roti*	35[a]	30[d,e]	-	-	-	-	-	-
Rice flour *roti*	25[b]	26[d]	>100	88	-	-	-	-
Kurakkan flour *roti*	31[a]	31[e,f]	-	-	>100	>200	-	-
Atta flour *roti*	-	-	-	-	-	-	30	27
Wet heat								
Wheat flour *pittu*	48[c]	49[f]	-	-	-	-	-	-
Rice flour *pittu*	-	-	DI	DI	-	-	-	-
Kurakkan flour *pittu*	-	-	-	-	DI	DI	-	-
Hoppers	-	-	DI	DI	-	-	-	-

* n =50. Percentage increases are calculated by the difference of the means of 50 measures; DI – disintegrated; Values with the same superscripts are not significantly different.

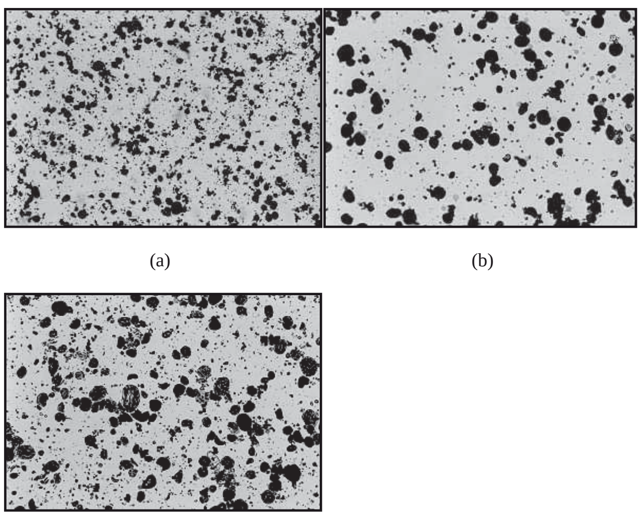

Figure 2. Light microscopic pictures of (10 × 10)(a) raw wheat flour (b) wheat flour *roti*-dry heat processed (c) wheat flour *pittu*-wet heat processed / steam cooking.

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(K_{av}<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<K_{av}<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).

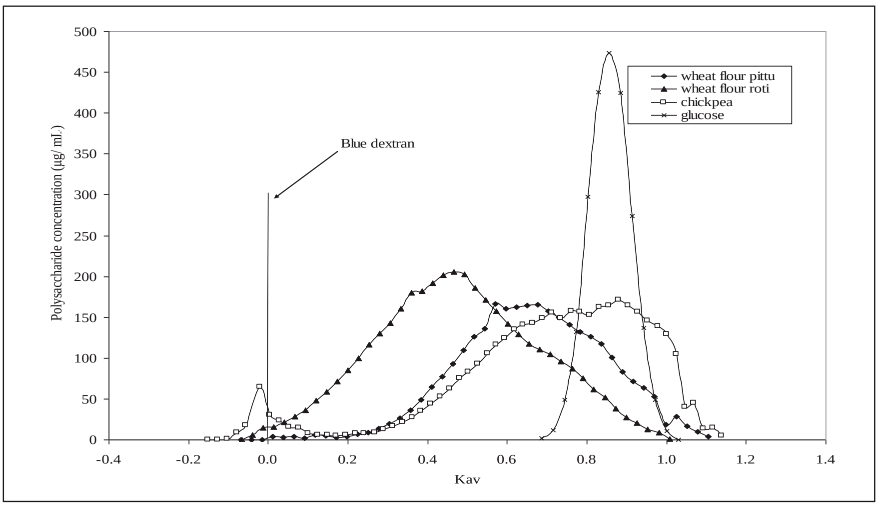

Figure 3. Molecular size distribution of carbohydrates of wheat flour *roti*, wheat flour *pittu* and chickpea

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

Food	*% HMWC (K_{av}<0.2)*	*% MMWC 0.2<K_{av}<0.8*	*% LMWC 0.8<K_{av}<1.0*	*% Recovery*
Wheat flour *roti*	8	86	8	90
Wheat flour *pittu*	1	75	24	71
Chickpea	6	53	41	96

* 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 quantitativelyreveal 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 amylose 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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