International Food Research Journal 19 (3): 1153-1159 (2012)

Prediction of glycaemic indices (GI) of meals by starch hydrolysis indices

[1]Hettiaratchi, U.P.K., [1*]Ekanayake, S. and [2]Welihinda, J.

[1]*Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka*
[2]*Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo, Sri Lanka*

Abstract: The glycaemic indices (GI) of food items are determined by an *in vivo* method which is laborious and time consuming. Thus, the aim of this study was to use an *in vitro* method and estimate the rate of hydrolysis of starch in basic foods as well as mixed meals with the intention of correlating these values with the published *in vivo* glycaemic indices of the same foods prepared in the same manner. The basic foods included both cereal based foods (n=5) and legumes (n=4) processed using different conditions. Mixed meals (n=7) contained a starchy staple with other supplementary meal accompaniments. The hydrolysis indices (HI) of basic foods and mixed meals were calculated for each food by taking the ratio between the HI of the test food to that of the standard food (white bread). A significant positive correlation was obtained for the *in vitro* HI and *in vivo* GI values of basic foods and mixed meals ($r=0.949$; $p<0.001$). A relationship of $Y = 1.1367X - 12.38$ was obtained indicating the possibility of calculating the GI of both basic foods and composite mixed meals from the respective hydrolysis indices.

Key words: Glycaemic indices, hydrolysis indices, basic foods, mixed meals, rate of hydrolysis of starch

Introduction

The postprandial glycaemic responses of carbohydrate rich foods and meals vary widely. Thus, the starchy foods are categorized according to their postprandial glycaemic responses with the assistance of Glycaemic Index (GI) values (Jenkins *et al*., 1981). GI expresses the blood glucose response following ingestion of foods. The GI of a food is estimated with the *in vivo* procedure by serving a standard amount of carbohydrate (50 g/ 25 g available carbohydrate portion) of the test food and the standard (Brouns *et al.,* 2005). However, the *in vivo* method that is used to determine GI of foods is laborious, costly, time consuming and requires the co-operation of motivated individuals. In order to overcome the logistical difficulties associated with the *in vivo* procedure several *in vitro* methods that mimic the physiological rate of digestion of carbohydrate foods have been developed. The *in vitro* procedures are established on the rationale that carbohydrate digestion by the digestive enzymes is an essential component of both *in vitro* and *in vivo* digestion procedures. These methods use a variety of digestive enzymes that are present in the human gastrointestinal tract (Englyst *et al*., 2003).

The *in vitro* studies initiate the digestion of starchy foods either by the *in vivo* mastication (Granfeldt *et al*., 1992) or imitating mouth grinding (chewing) process (Englyst *et al*., 1992). The chewing procedure which subjects the food to digestion by α-amylase in the mouth offers certain advantages over the traditional milling or grinding. The chewing time and the physical characteristics of the food will influence the degree of degradation of food particles and the rate of hydrolysis of starch. These methods use a wide variety of enzymes; amylase only (Jenkins *et al.*, 1980.; Snow and O'Dea, 1981) or amylase with other proteolytic enzymes (Colonna *et al.*, 1990; Holm *et al*., 1985). The *in vitro* digestion procedures that follow the digestion of foods using pancreatic and brush boarder enzymes (Englyst *et al*., 2003) had shown high correlations with the *in vivo* glycaemic responses (Brouns *et al*., 2005).

Most of the *in vitro* methods have been focused on analyzing basic foods (Englyst *et al*., 1999; Englyst *et al*., 2000; Englyst *et al*., 2003; Garsetti *et al.*, 2005) but not mixed meals containing several carbohydrate sources.

The objectives of the present study were to analyze the *in vitro* rate of release of starch of selected basic foods (cereal based foods, legumes), mixed meals of South Asian origin and to correlate the hydrolysis indices (HI) of the foods with the published GI values (Hettiaratchi *et al.*, 2009a; Hettiaratchi *et al*., 2009b; Widanagamage *et al.*, 2009; Hettiaratchi *et al*., 2011). The glycaemic indices of the foods were estimated according to standard guidelines (FAO/WHO, 1998). The foods were prepared using a standard recipe for both the *in vivo* and *in vitro* methods.

*Corresponding author.
Email: *sagarikae@hotmail.com*

Materials and Methods

Enzymes and chemicals

The enzymes were purchased from Sigma Chemical Company (St. Louis, MO, USA). All the chemicals used were of analytical grade and purchased from BDH (Poole, England) unless otherwise specified. 3'5' dinitrosalicilic acid was purchased from Sigma Chemical Company (St. Louis, MO, USA) and sodium potassium tartrate from AvonChem Ltd (Banbury, Oxon, UK).

Food items

White sliced bread (mass production, available in most parts of the country) purchased from the reputed retail outlets was used as the standard. The details of the basic foods and mixed meals analyzed in the present study are presented in Table 1. Basic foods containing 1 g digestible carbohydrate portions were used for the *in vitro* analysis procedure. The amounts of each basic food used for analysis are presented in Table 1.

Mixed meals

The individual percentages of starch from the staple and of meal accompaniments (starchy sources) used to formulate 1 g of starch in the *in vitro* assay are given in Table 1. The proportions of components of the meal were calculated by taking in to account the palatability and actual edible portion sizes and were similar to that of the portion sizes used for the *in vivo* assay.

Determination of digestible starch

Digestible starch contents of foods were determined by digesting sample (0.500 g) first with α-amylase enzyme at 100ºC and then with amyloglucosidase enzyme at 60ºC (Holm *et al.*, 1986). The liberated glucose concentrations were estimated with the enzymatic kit, GOD-PAP (Biolabo, France).

In vitro hydrolysis of starch

Individuals (n=6) chewed the food items (containing 1 g available carbohydrate portion of standard or test foods) for 15 times, expectorated into a beaker containing 6 mL of 0.05 M Na, K-phosphate buffer and pepsin (50 mg). Subjects rinsed their mouths with 5 mL of water and expectorated into the same beaker. The pH was adjusted to 1.5, incubated at 37°C for 30 mins. The pH was adjusted to 6.9, α-amylase (110 units) added and the volumes adjusted to 30 mL. The contents were transferred to dialysis bags (molecular weight cut off 12-14000 Daltons) and incubated at 37°C for 3 hours in a water bath.

Table 1. Basic foods, mixed meals, preparation methods and portion sizes

Food	**Preparation method**	**Food ppportions (g) containing 1g digestible carbohydrate**
Basic foods **(a) Cereal based foods**		
(i) Bread	Two different types of bread available were purchased from retail outlets	
Wholemeal bread		2.6
Ordinary white bread	Mass production, available in most parts of the country Small scale production	2.4
(ii) Rice		
Red rice (AT 353) obtained from Rice Research Institute, Batalagoda, Sri Lanka was used after dehulling and polishing	Red rice was cooked with water (w/v;50 g/100 mL) under low heat in a rice cooker.	4.1
(iii) *Roti* preparations	Either flour (25 g) were mixed with coconut scrapings (25 g) and 10 mL salt water (saturated salt solution). The dough was prepared by flattening on a plate (13-15 cm) and roasting on a pan for ~ 10 minutes while turning sides.	
Two *roti* varieties were prepared;		
Wheat flour		3.7 (wheat)
Whole wheat flour (atta flour)		3.0 (atta)

(b) Legumes **(i) *Cicer arietinum*** (chickpea), ***Vigna unguiculata*** (cowpea) and ***Vigna radiata*** (mung beans)	These were soaked overnight (~ 10 hours) in excess water (w/v; 50 g /150 mL). All were boiled (50 g) with excess water (chickpea ~ 40 min, cowpea ~ 40 min, mung beans ~ 25 min) and 10 mL salt water (from a saturated salt	7.4 6.9 6.9
(ii) Lentil (*Lens culinaris* medic, S. massoor parippu) curry	Lentils (200 g) were boiled with water (400 mL), chilli powder (2.5 g), curry powder (2.5 g) and turmeric powder (1 g) for 10 min. Coconut milk (1st and 2nd extracts) for lentil curry was prepared as given below: 1st extract - coconut scrapings (100 g) was extracted with water (100 mL) 2nd extract – coconut scrapings left from first extract with water (125 mL) Lentil curry was prepared by first adding 2nd extract (100 mL), salt (20 mL), green chili (10 g), curry leaves (5 g) and subsequently first extract (25 mL). Curry was tempered with chopped onions (10 g) and garlic (5 g).	8.5
Mixed meals Meal 1- Bread meal	(a) wholemeal bread (64% starch) (b) lentil curry (36% starch)	1.6 3.1
Meal 2 - Rice meal	(a) red rice (AT 353) (82% starch) (b) lentil curry (18% starch)	3.3 1.5
Meal 3 - Rice meal	(a) red rice (AT 353), (82% starch) (b) lentil curry (18% starch) (c) *Centella asiatica* (*gotukola*) salad [*Gotukola* (100 g) was mixed with coconut scrapings (50 g), onions (20 g), garlic (10 g), green chilli (10 g), salt powder (10 g) and lime].	3.3 1.5 0.5
Meal 4 – Rice meal	(a) red rice, (82% starch) (b) lentil curry, (18% starch) (c) *Centella asiatica* salad & (d) boiled egg	3.3 1.5 0.5 0.4
Meal 5 – Rice meal	(a) red rice (82% starch) (b) lentil curry (18% starch) (c) *Centella asiatica* salad (twice the amount as in meal 3)	3.3 1.5 1.0
Meal 6 – *Artocarpus heterophyllus* (Jack fruit) meal	(a) jack fruit flesh (boiled) (80% starch) [Flesh was cut into small pieces (800 g), boiled in a large saucepan with water (100 mL) and salt water (20 mL) under high heat for 10 min, and under low heat till all the water was dried] (b) jack fruit seeds (boiled) (20% starch) [Seeds were partially crushed. Outer covers were removed, cut into small pieces (200 g) and boiled with water (200 mL) and salt water (20 mL)] (c) coconut scrapings.	8.0 0.9 0.5

Meal 7 – String hopper meal	(a) string hoppers (wheat flour) (100% starch) [Wheat flour was steam cooked for 30 min. Flour was sifted using a household sieve once cooled. Dough was prepared by mixing flour (500 g), salt water (30 mL) and warm water (600 mL). String hoppers were steam cooked for 10 min]	3.2
	(b) coconut salad [Coconut scrapings (100 g) were grinded with chopped onions (20 g), garlic (5 g), dried chili pieces (10 g), lime and salt powder (10 g)]	0.5
	(c) boiled egg	0.4

Aliquots of the dialysates were analyzed for reducing sugar by 3'5' dinitrosalicylic acid (DNS) method (Miller, 1959) every half an hour. Proportion of the available starch hydrolyzed to maltose was taken as the degree of hydrolysis (Granfeldt *et al*., 1992).

The percentage of starch hydrolysis over the period of 3 hours was plotted (hydrolysis curves) and the area under the curve (AUC) calculated. Hydrolysis indices (HI) of a food per individual is calculated using the following equation:

$$HI = \frac{\text{AUC of test food}}{\text{AUC of standard}} \times 100$$

Statistical analysis

The GI, and HI values are presented as mean ± standard error of mean (SEM). The significance of difference in the parameters tested between test and the standard or between tests were analyzed by Students't test. Differences were considered significant if $p<0.05$. Data were analyzed with Microsoft Excel and Minitab version 14.

Results and Discussion

The present study first determined the rate of *in vitro* hydrolysis of starch of basic foods to study the applicability of this *in vitro* method in analyzing South Asian foods and to correlate the *in vitro* HI values with the reported *in vivo* GI data of the same laboratory (Hettiaratchi *et al*., 2009a; Hettiaratchi *et al*., 2009b; Widanagamage *et al*., 2009). *In vitro* hydrolysis of starch of basic foods (cereal based – 05, legumes - 04) are presented in Figure 1. When considering the two bread varieties analyzed in the present study, 18-20% starch was hydrolyzed to maltose within the first 30 minutes (Figure 1). Wholemeal bread had the highest HI when compared with other bread varieties and other food items (Table 2). In contrast, the *in vivo* GI of wholemeal bread was less than the ordinary bakery bread (Hettiaratchi *et al*., 2009a).

Although, the hydrolysis of legume starch is reported to be slow compared to other starchy sources (Tovar, 1992), mung beans and lentils had 21% and 18% starch hydrolyzed at 30 minutes as similar to bread varieties. However, when considering chickpea and cowpea, 14% and 15% starch were hydrolyzed at the first time interval (30 minutes) respectively. The differences among legumes in percentages of starch hydrolyzed, could be due to the variations of the sizes of the seeds which in turn give rise to different degrees of degradation of starch during the cooking process. The results of the present study compare well with the hydrolysis of starch of bread varieties (17-24%) and legumes (0-19%) of reported values within the first 30 minutes respectively (Granfeldt *et al*., 1992).

When the *in vitro* HI data of basic foods and *in vivo* GI values were correlated, a significant positive relationship for the two parameters was observed ($r=0.953$; $p<0.0001$) with an equation, $y = 1.1156x - 10.76$. With the above positive correlation for basic foods, we applied the method as a novel approach for mixed meals containing different starchy sources and other meal accompaniments with similar proportions of components of the meal as in the case of *in vivo* determination of GI (Figure 2).

The meal 1 was prepared with wholemeal bread and lentil curry (only two sources of carbohydrates) with a contribution of 64% and 36% starch from the two items respectively. This meal had a GI of 87±6 (Hettiaratchi *et al*., 2009a) and a HI of 81±6 (Table 2). The HI of the meal was comparable with *in vivo* GI of the same meal. A Mexican tortilla and bean mixture ("taco") containing only two starchy sources (each 0.5 g starch) had elicited a HI of 46±2 and a predicted GI of 48 (Tovar *et al*., 2003) thus showing a good correlation between the *in vivo* and *in vitro* values.

A rice mixed meal was given for *in vivo* determination of GI. This included not only starchy sources (rice, lentil curry) but other non-carbohydrate

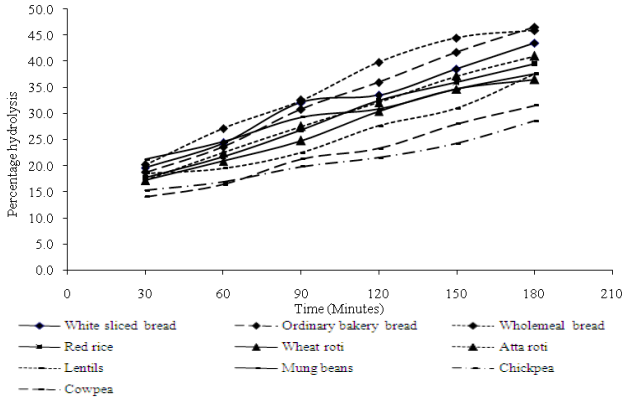

Figure 1. Proportion of starch hydrolysed against time – basic foods (each point represents an average of 6 values).

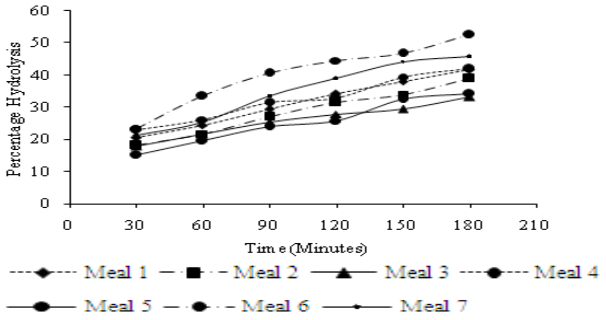

Figure 2. Proportion of starch hydrolysed against time - mixed meals (each point represents an average of 6 values).
Meals 1-7 - The compositions of the meals are presented in table 1.

meal accompaniments [boiled egg, *Centella asiatica* salad and coconut gravy (GI - 60±5)] (Hettiaratchi *et al.*, 2009b) as well. Thus the *in vitro* meals 2-4 were prepared to contain rice as the main starchy staple with other meal accompaniments to resemble the constituents of the meal given for *in vivo* determination of GI.

Meal 4 contained rice and all other accompaniments of rice mixed meal as given for *in vivo* determination of GI. This resulted in a HI of 65±7. Meal 3 contained rice and the other accompaniments of the rice mixed meal except for a portion of egg. Having included this combination a HI of 71±6 (Table 2) was obtained. Meal 2 was given by only including the starchy sources of the meal (82% starch from boiled rice and 18% starch from the lentil curry) similar to the proportions of starch included in the in vivo meal and a HI of 84±8 was obtained (Table 2). The HI of the three rice meals (meal 2, 3, 4) were not significantly different from each other ($p>0.05$). These observations clearly indicate that a HI which will reflect the GI closely can be obtained when a composite mixed meal is analyzed.

In order to study the effect of dietary fibre on hydrolysis of starch, a meal with double the amount of dietary fibre (by means of including twice the

Table 2. Portion sizes, GI (n=10) and HI (n=6) of basic foods and mixed meals

Food	Portion size (g)	GI (mean±SEM)	HI (mean±SEM)
Basic foods			
Wholemeal bread	2.6	[1]103 ± 11	113 ± 6
Ordinary bakery bread	2.4	[1]114 ± 9	102 ± 7
Red rice	4.1	[2]99 ± 10	89 ± 9
Roti (wheat flour)	3.7	[3]72 ± 6	70 ± 9
Roti (atta flour)	3.0	[3]67 ± 9	79 ± 7
Lentils	8.5	ND	52 ± 4
Chickpea	7.4	[3]29 ± 5	37 ± 5
Mung beans	6.9	[3]57 ± 6	58 ± 6
Cowpea	6.9	[3]49 ± 6	58 ± 6
Mixed meals			
Meal 1			
Wholemeal bread, lentils	1.6, 3.1	[1]87 ± 6	81 ± 6
Meal 2			
Red rice, lentils	3.3, 1.5	ND	84 ± 8
Meal 3			
Red rice, lentils , *gotukola* salad	3.3, 1.5, 0.5	ND	71 ± 6
Meal 4			
Red rice, lentils, g*otukola* salad, egg	3.3,1.5,0.5, 0.4	[2]60 ± 5	65 ± 7
Meal 5			
Red rice, lentils, *gotukola* salad	3.3, 1.5,1.0	ND	74 ± 8
Meal 6			
Jack fruit flesh, seeds, coconut scrapings	8.0, 0.9, 0.5	75 ± 11	85 ± 8
Meal 7			
String hopper (wheat), egg, coconut salad	3.2, 0.4, 0.5	[2]104 ± 7	98 ± 8

SEM-Standard error of mean; ND-Not determined; Source:[1]Hettiaratchi *et al.,* 2009a, [2]Hettiaratchi *et al.,* 2009b, [3]Widanagamage *et al.*,2009.

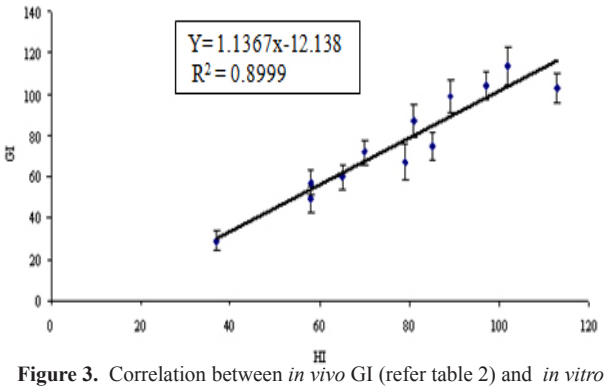

Figure 3. Correlation between *in vivo* GI (refer table 2) and *in vitro* HI values of basic foods and mixed meals.

amount of *Centella asiatica* salad) than meal 3 was given (meal 5). Meal 5 had a HI of 74±8 (Table 2) which was not significantly different from meal 3. This reflected that inclusion of additional quantity of fibre as given in this case might not have an effect on lowering HI.

Meal 6 and 7 were given for *in vitro* assay using the same components and proportions as *in vivo* determination of GI. The GI and HI data of those two meals were also not significantly different ($p>0.05$). The *in vivo* GI values of both basic foods and mixed meals were correlated with *in vitro* HI data and a significant positive correlation ($r=0.949$; $p<0.001$) was obtained for the two parameters (Figure 3). A relationship of Y=1.1367X-12.138 was also obtained for all the foods analyzed in the present study enabling the calculation of GI values of foods from the respective HI values.

Thus the present *in vitro* method which estimates the rate of hydrolysis of starch could be used to analyze not only meals containing only starchy sources (one or two) but mixed meals comprising a starchy staple, other starch sources and non starch accompaniments (i.e Rice and different curries). However, when non-starchy meal accompaniments (containing protein and dietary fibre sources) are included in a meal, these might affect the breakdown of the food and reduce the digestion by salivary α-amylase. This might lead to a reduced HI compared to a meal containing only starchy sources. Although it is reported that the *in vitro* method does not experience the physiological effect of fat (Latge *et al*., 1994), protein (Jenkins *et al*., 1981; Bornet *et al*., 1987) and dietary fibre (Bjorck *et al*., 1994; Liljeberg and Bjorck, 1994) on a starchy meal, the present results indicates a reduction in HI which could be due to less digestion of starch. Thus, the *in vitro* HI data would be useful in calculating GI of not only basic foods but mixed meals with a wider range of combinations (of starchy staples and meal accompaniments). Furthermore, this method has the added advantage of being less costly compared to a standard GI determination procedure.

Conclusions

The present *in vitro* method can be applied to predict the glycaemic response of a basic starchy food as well as the glycaemic responses of mixed meals containing a basic starchy staple and other meal accompaniments thus indicating the usefulness and practicality of the method. The GI of foods can be calculated by using the HI values of the foods prepared in the same manner with the equation Y = 1.1367X - 12.38. However, prior to analyzing the foods using the *in vitro* method, the actual edible meals in terms of compositions, percentages of starch (if there are several sources of starch) and edible proportions of meal accompaniments should be planned to obtain the true practical benefits.

Acknowledgements

The authors acknowledge the financial assistance given by the grants; National Science Foundation, Sri Lanka; NSF/RG/2005/AG/10, National Research Council, Sri Lanka; NRC-05-03 and International Programme in Chemical Sciences; IPICS SRI 07, Sweden.

Programme in Chemical Sciences; IPICS SRI 07, Sweden.

References

Bjorck, I., Granfeldt, Y., Liljeberg, H., Tovar, J. and Asp, N. 1994. Food properties affecting the digestion and absorption of carbohydrates. American Journal of Clinical Nutrition 59: 699S-705S.

Bornet, F.R.J., Costagliola, D., Rizkalls, S.W., Blayo, A., Fontvieille, A., Haardt, M. Letanoux, M., Tchobroutsky, G. and Slama, G. 1987. Insulinemic and glycemic indexes of six starch-rich foods taken alone and in a mixed meal by type 2 diabetics. American Journal of Clinical Nutrition 45: 588-595.

Brouns, F., BJorck, K., Frayn, N., Gibs, A.L,, Lang, V., Slama, G. and Wolever, T.M.S. 2005. Glycaemic index methodology. Nutrition Research Reviews 18: 145-171.

Colonna, P., Barry, J., Cloarec, D., Bornet, F., Gouilloud, S. and Galmiche, J. 1990. Enzymic susceptibility of starch from pasta. Journal of Cereal Science 11: 59-70.

Englyst, E., Englyst, H., Hudson, G., Cole, T.J. and Cummings, J. 1999. Rapidly avaialble glucose in foods: an in vitro measurement that reflects the glycemic response. American Journal of Clinical Nutrition 69: 448-454.

Englyst, H., Kingman, S. and Cummings, J. 1992. Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46: S33-S50.

Englyst, K., Hudson, G. and Englyst, H. 2000. Starch analysis in food. R.A.Meyers (Ed.), Encyclopedia of Analytical Chemistry. Chichester: John Wiley & Sons Ltd.

Englyst, K., Vinoy, S., Englyst H.N. and Lang, V. 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. British Journal of Nutrition 89: 329-340.

Englyst, K., Vinoy, S., Englyst, H. and Lang, V. 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. British Journal of Nutrition 89: 329-340.

Garsetti, M., Vinoy, S., Lang, V., Holt, S., Loyer, S. and Brand-Miller, J.C. 2005. The Glycemic and Insulinemic index of plain sweet biscuits: Relationships to in vitro starch digestibility. Journal of the American College of Nutrition 24: 441-447.

Granfeldt, Y., Bjorck, I., Drews, A. and Tovar, J. 1992. An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products. European Journal of Clinical Nutrition 46: 649-660.

Hettiaratchi, U.P.K., Ekanayake S. and Welihinda, J. 2009a. Glycaemic indices (GI) of three Sri Lankan wheat bread varieties and a bread-lentil meal. International Journal of Food Science and Nutrition, 6 (Supplement 4): 21-30

Hettiaratchi, U.P.K., Ekanayake S. and Welihinda, J. 2009b. Do Sri Lankan meals help decrease blood glucose response? Ceylon Medical Journal 54: 39-43.

Hettiaratchi, U.P.K., Ekanayake S. and Welihinda, J. 2011. Nutritional assessment of a jackfruit (*Artocarpus heterophyllus*) meal. Ceylon Medical Journal 56: 54-58.

Holm, J., Bjorck, I., Asp, N., Sjoberg, L. and Lundquist, I. 1985. Starch avaialbility *in vitro* and *in vivo* after flaking, steam cooking and popping of wheat. Journal of Cereal Sciences 3: 193-206.

Holm, J., Bjorck, I., Drews, A. and Asp, N. 1986. A rapid method for the analysis of starch. Starch/Starke 3:, 224-226.

Jenkins, D.J.A., Wolever, T.M.S., Taylor, R.H., Ghafari, H., Jenkins, A., Barker, H. and Jenkins, M. 1980. Rate of digestion of foods and postprandial glycaemia in normal and diabetic subjects. British Medical Journal 281: 14-17.

Jenkins, D.J.A., Wolever, T.M.S., Taylor, R.H., Barker, H., Fielden, S.R.D.H., Baldwin, J.M., Bowling, A.C., Newman, H.C., Jenkins, A.L., and Goff, D.V. 1981. Glycaemic index of foods: a physiological basis for carbohydrate exchange. American Journal of Clinical Nutrition 3: 362-366.

Latge, C., Thouvenot, P. and Kedzierewicz, F. 1994. The influence of a lipid loading on gastric emptying and glycemia. American Journal of Clinical Nutrition 59(Suppl.): 182S.

Liljeberg, H. and Bjorck, I. 1994. Bioavaialbility of starch in bread products. Postprandial glucose and insulin responses in healthy subjects and *in vitro* resistant starch content. European Journal of Clinical Nutrition 48: 151-163.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Biochemistry 31: 426-428.

Snow, P. and O'Dea, K. 1981. Factors affecting the rate of hydrolysis of starch in food. American Journal of Clinical Nutrition 34: 2721-2727.

Tovar, J. 1992. Bioavailability of starch in processed legumes, University of Lund, Lund, Sweden.

Tovar, J., Sáyago-Ayerdi, S.G., Peêalver, C., Paredes-López, O. and Bello-Pérez, L.A. 2003. *In vitro* starch hydrolysis index and predicted Glycemic index of corn tortilla, black beans (*Phaseolus vulgaris* L.) and Mexican "taco". Cereal Chemistry 80: 533-535.

Widanagamage, R. D., Ekanayake, S. and Welihinda, J. 2009. Carbohydrate-rich foods: glycaemic indices and the effect of constituent macronutrients. International Journal of Food Science and Nutrition, 60 Supplement 4: 215-223.