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In vitro Starch Hydrolysis Indices of selected Sri Lankan Starchy Tubers

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ABSTRACT

Purpose: *The importance of glycaemic indices (GI) of starchy foods originates from its linkage with type 2 diabetes mellitus. GI of food items is measured by in vivo method which carries a degree of discomfort. We aim to find the in vitro hydrolysis index (HI) of selected starchy tubers in Sri Lanka.*

Research Method: *The degree of hydrolysis for selected tubers was determined by in vitro starch digestion using the proportion of starch converted into maltose. The HI values of six selected tubers were calculated using the ratio between incremental areas under the hydrolysis curve for the tested tubers and the reference food (white bread).*

Findings: *Among the selected tubers, arrowroot had the highest percentage of hydrolysis within the first 30 minutes. There was a significant difference in the percentage of hydrolysis between 60-90 minutes for the selected tubers and white bread. The HI for arrowroot, cassava, potato, purple yam, sweet potato and white yam were 84 ± 6, 114 ± 9, 89 ± 10, 74 ± 4, 104 ± 7 and 69 ± 7 respectively.*

Originality/Value: *The HI of the selected Sri Lankan starchy tubers will be of value in future large-scale studies on traditional tubers and to predict the GI of the starchy tubers.*

Keywords: *arrowroot, cassava, diabetes mellitus, glycaemic index, hydrolysis index, starchy tubers, sweet potato*

INTRODUCTION

The sedentary lifestyle of the modern world drives people towards metabolic diseases which are correlated to dietary patterns (Manton, 1988). Diabetes mellitus (DM) is a major metabolic disorder which occurs as a result of lack of insulin or resistance to insulin ('Diagnosis and Classification of Diabetes Mellitus', 2009). The global prevalence of DM among adults aged 20-79 years was 8.8% in 2017 and for Sri Lanka, it was 8.6% (*IDF Diabetes atlas 8th edition*, 2017). Furthermore, uncontrolled DM can affect almost any part of the human body (Deshpande, Harris-Hayes and Schootman, 2008). Therefore, the optimum level of blood glucose is essential for health. Consumption of high glycaemic index (GI) foods for a longer period is responsible for the development of DM (Wolever *et al.,* 1993). Therefore, the consumption of low GI foods is encouraged (Asif, 2014). This would slow down the rate of glucose absorption into blood (Radulian *et al.,* 2009) and restrain post-meal free fatty acid levels (Radulian *et al.,* 2009).

GI indicates the relative ranking of carbohydrate-containing foods according to their impact on blood glucose level (Jenkins *et al.,* 1981). The GI value of food is measured using *in vivo* methods where it is expressed as a percentage area under the curve for postprandial glucose of a test food to the reference food (white bread or glucose) serving 50g of carbohydrate (Brouns *et*

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al., 2005). However, the present standard method of measuring GI requires six separate finger-prick blood samples from volunteers, and this procedure is comparatively difficult and time-consuming (Brouns *et al.,* 2005). Therefore, *in vitro* methods have been developed to mimic the physiological rate of digestion of carbohydrate-containing foods. These *in vitro* procedures are performed by digestion of foods using brush border and pancreatic enzymes (Englyst *et al.,* 2003)in vitro measures describing the rate of glucose release from foods, are the main determinants of glycaemic index (GI. Several studies have shown a correlation between *in vivo* and *in vitro* measurements of GI. Most of the *in vitro* methods focus on cereal-based foods and legumes (Granfeldt *et al.,* 1992; Woolnough *et al.,* 2008; Hettiaratchi, Ekanayake and Welihinda, 2012)expectorated into a beaker and incubated with pepsin. The incubate was thereafter transferred to a dialysis tubing and incubated with pancreatic alpha-amylase for 3 h. Samples were removed from the dialysate at time intervals and the degree of hydrolysis was calculated as the proportion of the potentially available starch degraded to maltose. A hydrolysis index (HI with only a few focusing on tubers (Ek *et al.,* 2014).

Tubers have been an important part of the human diet from ancient times and are second only to cereals among sources for carbohydrate. These crops need minimal amounts of agricultural inputs for growth and can tolerate harsh environmental conditions. Tuber crops can be consumed in different forms (boiled, roasted, fried, or cooked) (Chandrasekara and Josheph Kumar, 2016). Starchy tubers are common in Sri Lankan diets and are rich in nutritional value (Senavirathna *et al.,* 2014). Arrowroot (*Maranta arundinacea* L.) is a medicinally important tuber crop which is rich in carbohydrate, dietary fibre, vitamin B complex and minerals (Chandrasekara and Josheph Kumar, 2016) and it is native to America and cultivated widely in the tropical region (*Maranta arundinacea L - USDA, Agricultural Research Service, National Plant Germplasm System*, 2019). Cassava (*Manihot esculenta*) plays an important role as a primary food for human due to its high carbohydrate content and availability of some bioactive compounds (Blagbrough *et al.,* 2010) and it is widely cultivated throughout the tropics (*Manihot esculenta - USDA, Agricultural Research Service, National Plant Germplasm System*, 2019). Potato (*Solanum tuberosum* L.) is an important global food crop (Blagbrough *et al.,* 2010) which provides carbohydrates, potassium, vitamin A, ascorbic acid and antioxidants (Hesam, Balali and Tehrani, 2012; King and Slavin, 2013; Lee *et al.,* 2016). Potato is cultivated in Africa, America and tropical Asia (*Solanum tuberosum L - USDA, Agricultural Research Service, National Plant Germplasm System.*, 2019). Sweet potato (*Ipomea batatas*) is grown worldwide especially in tropical, and subtropical regions (Scott GJ, Wiersema S, 1991). National Aeronautics and Space Administration selected sweet potatoes for astronauts on space missions (Bovell-Benjamin, 2007). Sweet potatoes are rich in dietary fibre, minerals, vitamins, and bioactive compounds (Chandrasekara and Josheph Kumar, 2016). Yam (*Dioscorea alata*) is a popular staple food in tropical and sub-tropical countries which plays a major role in food security in low income and food-deficit countries (Liu *et al.,* 2007). The GI values of most of the above starchy tubers have not yet been calculated. We aim to find hydrolysis index (HI) values of selected starchy tubers in Sri Lanka using an *in vitro* procedure. The data could be useful in future large-scale studies on traditional tubers.

MATERIALS AND METHODS

Test foods

Arrowroot (*Maranta arundinacea* L.), cassava *(Manihot esculenta)*, potato (*Solanum tuberosum* L.), purple yam (*Dioscorea alata*), red peeled sweet potato (*Ipomea batatas)* and white yam (*Dioscorea alata*) (Figure 01) were collected from Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Sri Lanka and the local market of Anuradhapura, Sri Lanka. Prima brand crust top white bread was used as the reference.

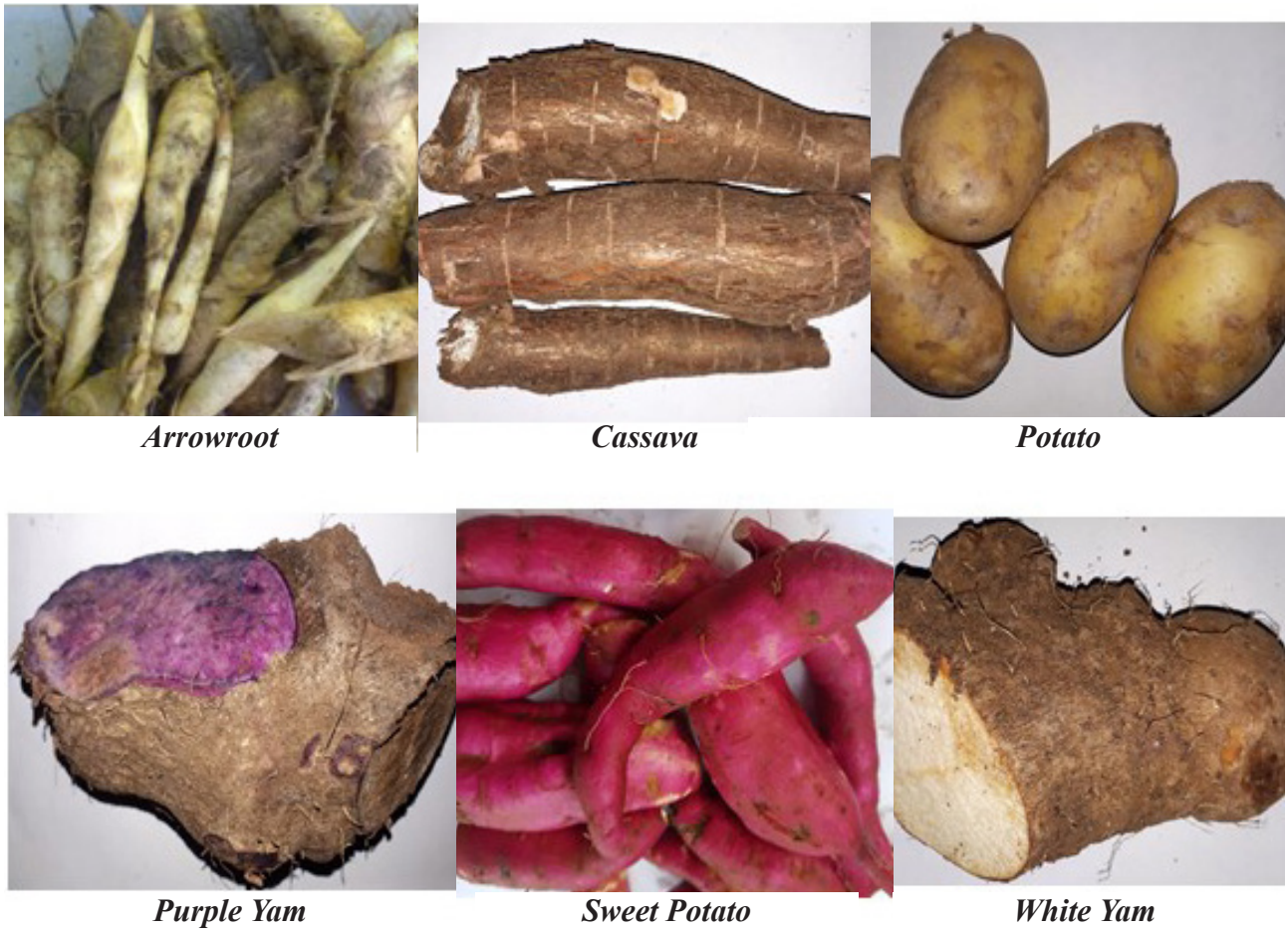

Figure 01: Sri Lankan starchy tubers selected for the study

Preparation of tubers

Boiled tuber powder was prepared to determine the digestible starch of each tuber and white bread. Cleaned tubers from each type were cut into 25g pieces; distilled water was added and boiled for 20-30 minutes until they become soft. Boiled tuber pieces were cut into thin chips (2mm thickness), dried in an electric oven (Gallenkamp CAD OHFO 50.XXL, UK) at 40°C for 3-5 days and powdered using a laboratory-scale grinder and sieved. The flour samples were stored in tightly closed containers until further analysis. Boiled tuber portions which contained 1g of available carbohydrate were used to determine the HI (Granfeldt *et al.,* 1992)expectorated into a beaker and incubated with pepsin. The incubate was thereafter transferred to a dialysis tubing and incubated with pancreatic alpha-amylase for 3 h. Samples were removed from the dialysate at time intervals and the degree of hydrolysis was calculated as the proportion of the potentially available starch degraded to maltose. A hydrolysis index (HI. The moisture content of each sample was also determined. Boiled tuber samples and bread samples were weighed and dried in an electric oven at 105ºC until a constant weight was gained. The weight reduction was taken as moisture content (Helrich, 1990).

Determination of starch hydrolysis indices

The digestible starch content was determined to find the 1g available portion size of the selected tubers and white bread (Holm J, Bjorck I, Drew, 1986). Flour samples (0.5g) were suspended in 15ml of distilled water. Suspended samples were incubated with 40µl of α amylase (Sigma chemical company, St. Louis, MO, USA) and heated in a 100^0C water bath (OLS 200, England) for 25 minutes. Then samples were diluted up to 50ml. 1ml of the diluted sample was mixed with 2ml of sodium acetate buffer (pH=4.75) and 50µl of amyloglucosidase enzyme. Mixture

was incubated for 30 minutes at 60^0C and 1ml of above mixture was diluted up to 10ml. From the above mixture 10µl was added to glucose oxidase solution (Glucose enzymatic kit, GOD – PAP Biolabo, France) and mixed. The reaction mixture was incubated at 37^0C for 10 minutes in shaking water bath. Absorbance of sample was measured using the double beam spectrophotometer (Labomed spectro 2000 RS, USA) at 500nm against a reagent bank. Starch content was determined using following equation: Digestible starch % = [Absorbance of test x 0.9 x 10 x 50 x 100] / [Absorbance of standard x Fresh weight of sample (mg)] where glucose standard (100mg/dL) was used as the standard solution (Holm J, Bjorck I, Drew, 1986). And, 0.9 is the correction factor for conversion to glucose.

In vitro hydrolysis of starch was done according to the Grandfeldt *et al.,* method (Granfeldt *et al.,* 1992)expectorated into a beaker and incubated with pepsin. The incubate was thereafter transferred to a dialysis tubing and incubated with pancreatic alpha-amylase for 3 h. Samples were removed from the dialysate at time intervals and the degree of hydrolysis was calculated as the proportion of the potentially available starch degraded to maltose. A hydrolysis index (HI: Volunteers were requested to fast for 1 ½ hour and rinse the mouth before ingestion of the samples. Then, 15 times of chewing was selected as a reasonable average to digest 1g of digestible starch. Chewed food samples were expectorated into a beaker containing 6 ml of 0.05M sodium-potassium phosphate buffer and 50 mg of pepsin (Sigma Aldrich EC 232-629-3). Subjects rinsed their mouth with 5 ml of water and expectorated it into the same beaker. The pH was adjusted to 1.5 using 1M HCl and incubated at 37^oC for 30 minutes. Then the pH was adjusted to 6.9, and 110 units of α amylase (Sigma Aldrich EC 232-588-1) were added. After adding the enzyme, the final volume of the solution was brought up to 30 ml. The contents were transferred to dialysis bags (molecular weight cut off 12-14000 Daltons) and incubated at 37^oC for 3 hours in a water bath (OLS 200). Aliquots of dialysates were analysed every half an hour for reducing the sugar by 3,5-dinitrosalicylic acid method (Miller, 1959). The proportion of the available starch hydrolysed into maltose was taken as the degree of hydrolysis (Miller, 1959). The hydrolysed starch percentage for each tuber over a period of 3 hours was plotted, and the hydrolysis curves were obtained. The area under the curve (AUC) was calculated, and HI of food per individual was calculated using the equation: HI = (AUC of test food / AUC of standard food) x 100. The HI values were presented as mean ± standard error of the mean (SEM). The significant difference in the parameters tested between the tubers and the white bread or between tests were analysed using one-way ANOVA, and the mean separation was done by post-hoc Turkey LSD HSD where necessary. Data were analysed using Microsoft Excel, Minitab 16 and SAS version 9.

RESULTS AND DISCUSSION

Percentage of starch hydrolysed into maltose at each time point (0 minutes – 180 minutes) was taken as the degree of hydrolysis. Table 01 and Figure 02 depicts the rates of starch hydrolysis and Table 02 shows moisture content of selected tubers for the selected tubers and the reference (white bread).

Among the selected tubers, arrowroot had the highest percentage of hydrolysis within first 30 minutes whereas purple yam had the lowest. There was a significant difference in the percentage of hydrolysis between 60-90 minutes. However, there was no significant difference in the percentage of hydrolysis between other time intervals (0-30, 30-60, 90-120, 120-150 and 150-180) for the tested tubers and white bread. Also, a fast first part of hydrolysis from 0 to 90 min and a slow second part from 90 to 180 min are seen for most of the tubers. The HI for arrowroot, cassava, potato, purple yam, sweet potato and white yam were 84 ± 6, 114 ± 9, 89 ± 10, 74 ± 4, 104 ± 7 and 69 ± 7 respectively.

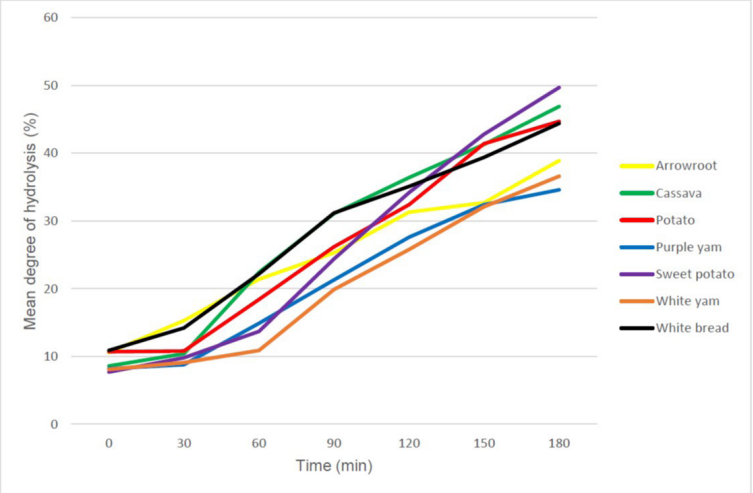

Figure 02: Hydrolysis of selected tubers and white bread

Table 01: Hydrolysis of selected tubers and white bread

Sample (n = 6)	Mean degree of hydrolysis % (± SEM)							Hydrolysis Index ± SEM
	0 min	30 min	60 min*	90 min*	120 min	150 min	180 min	
Arrowroot	10.6 ± 0.9^{ab}	15.3±1.79^{a}	21.4±3.78^{a}	25.3± 1.24^{bc}	31.3 ±1.97^{abc}	32.7± 2.81^{bcd}	38.9±4.04^{bcd}	84 ± 6.2^{cd}
Cassava	8.6 ± 0.27^{bc}	10.4±1.27^{c}	22.4±0.60^{a}	31.1± 1.02^{a}	36.4± 2.78^{a}	41.3± 2.87^{abc}	46.9±2.53^{ab}	114 ± 7.8^{a}
Potato	10.7 ± 1.19^{ab}	10.8 ±1.71^{bc}	18.4 ± 1.41^{ab}	26.2±3.54^{b}	32.4±3.85^{abc}	41.4±4.49^{ab}	44.7±5.17^{abc}	89 ± 10^{bc}
Purple yam	8.2 ± 0.6^{c}	8.8 ± 0.79^{c}	14.9 ± 1.55^{bc}	21.3 ± 1.35^{cd}	27.6 ± 2.71^{bc}	32.4 ± 2.04^{cd}	34.6± 2.4^{d}	74 ± 3.9^{cd}
Sweet potato	7.7 ± 0.05^{c}	9.8 ± 0.66^{c}	13.7±0.87^{bc}	24.4±1.40^{bcd}	34.2 ±1.37^{ab}	42.8 ±4.31^{a}	49.7±1.78^{a}	104 ± 7.3^{ab}
White yam	8.1 ± 0.23^{c}	9.1±1.27^{c}	10.9 ±0.60^{c}	19.9± 1.02^{d}	25.8 ± 2.78^{c}	32.1± 2.87^{d}	36.6± 2.53^{cd}	69 ± 7^{d}
White bread	10.9 ± 1.19^{a}	14.2±1.33^{a}	22.2±0.88^{a}	31.2± 1.23^{a}	35.1±1.96^{a}	39.4 ± 2.21^{abcd}	44.4±0.33^{abc}	NA

**Columns showing significant difference (p<0.05); [a, b, c, d] Different superscripts within a column are significantly different (p<0.05); NA – not applicable, SEM - standard error of the mean*

Table 02: Moisture content of selected tubers

Type of food	Moisture content (wet basis %)
Arrowroot	77.98 ± 2.11c
Cassava	67.62 ± 1.64e
Potato	82.72 ± 1.01a
Purple yam	78.67 ± 1.03bc
Sweet potato	74.52 ± 1.22d
White yam	80.91 ± 0.89ab
White bread	35.68 ± 1.41f

Grouping from Tukey's test - mean values that do not share a letter are significantly different at a probability level of 5%.

Arrowroot had a high hydrolysed percentage than the white bread within the first 60 minutes. Therefore, arrowroot showed initial rapid digestion compared to other tubers. Amylose: amylopectin ratio of arrowroot is low compared to other tubers (Senavirathna *et al.,* 2014). Amylose molecules are harder to digest than amylopectin molecules (Brand-Miller J, Wolever TMS, Foster-Powell K, 2003). Less availability of amylose could cause rapid initial digestion. However, arrowroot contains a high level of insoluble dietary fibres leading to slower digestion at the end (Senavirathna *et al.,* 2014). The GI of arrowroot from previous literature was 82 ± 8 (Senavirathna *et al.,* 2014) compared to the HI value of 84 ± 6.

Cassava (89 ± 10) and Potato (74 ± 4) recorded high HI values similar to the previously published literature (Garcia - Alonso and Goni, 2000; Leeman, A.M, Barstroem,L.M.,Bjoerck, 2005). However, a prior study reported no significant difference in digestion rates of potatoes by processing methods (Kingman, S and Englyst, 1994). Less availability of dietary fibre and less protein content could have led to the rapid digestion of both Cassava and Potato (U. P. K. Hettiaratchi, Ekanayake and Welihinda, 2009). GI values of Cassava and Potato were 120 ± 2 (U P K Hettiaratchi, Ekanayake and Welihinda, 2009) and 90 ± 6 (Pirasath, 2015) in previous literature.

Purple (74 ± 4) and white (69 ± 7) yam had a low HI value similar to previously published GI value of 64 ± 8 and 69 ± 4 (Senavirathna *et al.,* 2014) respectively. Yam starch is digested and absorbed at a slower rate and products are released slowly. Yam tubers mainly consist of type B starch granules which lead to lower gelatinisation compared to other tubers (Widanagamage, Ekanayake and Welihinda, 2009). Also, light microscopic studies revealed that starch granules are enclosed by cell (protein) starch granules. Legumes also show a lower glycaemic response for similar reasons (Kim, H. S., Huber, 2008).

A considerably low value of obtained HI (104 ± 7) was recorded than the published GI value of 140 for Sweet potato (Waidyarathna GRNN, Ekanayake S, Chandrasekara GAP, 2018). The difference could be attributed to the location of growth, maturity and the variety. This study used *Wariyapola red* variety of sweet potato, and glycaemic response varies among the varieties of sweet potatoes (Allen JC, 2012). The resistant starch content of the *Wariyapola red* variety is higher than other tested sweet potato varieties available in Sri Lanka (Senanayake *et al.,* 2013). It could be a reason for the low HI value compared to the published GI value. Presence of a low level of total starch content and high level of the crude fibre content of *Wariyapola red* can also be attributed to low digestibility (Senanayake *et al.,* 2013). However, digestibility of starch can vary according to the chemical nature of the starch, physical form, the presence of possible inhibitors, physical distribution of starch and dietary fibre components such as cellulose level (Snow and O'Dea, 1981).

Calculated HI of selected tubers showed a significantly positive correlation (r=0.907: p=0.013) with their previously published GI values mentioned in the previous paragraph of the discussion (Pearson correlation analysis). Published GI values obtained for glucose reference was converted to white bread reference, by a conversion factor of 1.4 (Foster-Powell, Holt and Brand-Miller, 2002). A relationship of GI=1.57HI – 45.27 was obtained indicating the possibility of calculating the GI of starchy tubers available in Sri Lanka from the respective hydrolysis indices. Sri Lankan mixed meals *($r = 0.949$; $p<0.001$; GI = 1.1367HI - 12.38)* (Hettiaratchi, Ekanayake and Welihinda, 2012), Sweden cereals and legumes *($r=0.877$; $p<0.0001$)* (Granfeldt *et al.,* 1992)expectorated into a beaker and incubated with pepsin. The incubate was thereafter transferred to a dialysis tubing and incubated with pancreatic alpha-amylase for 3 h. Samples were removed from the dialysate at time intervals and the degree of hydrolysis was calculated as the proportion of the potentially available starch degraded to maltose. A hydrolysis index (HI, Greece food items *($r=0.800$; $p=0.010$)* (Argyri *et al.,* 2016) and Taiwanese rice *($r = 0.946$; $P\leq0.001$; GI = 0.717HI + 28.778)* (Lai *et al.,* 2016) had similar results. However, the found equation was from the correlation values of selected tubers limiting its applicability. Moreover, previously established

GI values were used to derive the equation which might result in inaccurate predictions. However, similar to previous literature, the selected tubers were harvested at their commercial maturity level and from the same agro-climatic zones (Senavirathna *et al.,* 2014; Waidyarathna GRNN, Ekanayake S, Chandrasekara GAP, 2018). Consideration of food structure, quantity, crop variety and physiological aspects in the equation could produce a more accurate estimation of GI of Sri Lankan tubers. Further, important data such as proximate composition, starch content, amylose and amylopectin contents, dietary fibre content, resistant starch content, available carbohydrate, the curve of total starch hydrolysis, the kinetic constant of selected tubers are essential to explain the differences in the hydrolysis and glycaemic indices of the tubers.

In vivo physiological processes such as effects on fat, protein and gastric emptying are not reflected by *in vitro* procedures (Jenkins *et al.,* 1981; Granfeldt *et al.,* 1992; Latage, Thouvenot and Kedzierewicz, 1994)expectorated into a beaker and incubated with pepsin. The incubate was thereafter transferred to a dialysis tubing and incubated with pancreatic alpha-amylase for 3 h. Samples were removed from the dialysate at time intervals and the degree of hydrolysis was calculated as the proportion of the potentially available starch degraded to maltose. A hydrolysis index (HI. The rate of gastric emptying depends on acidity, osmolality, volume and concentration of sugar and soluble fibre. However, the influence of these factors is not reflected by the *in vitro* procedure (Foster-Powell, Holt and Brand-Miller, 2002). Also, the amylose and amylopectin in a solution leads to rapid digestion by the formation of a mixture of linear oligosaccharides and branched alpha-limit dextrins. However, starch is usually present as solid structures in food items (Colonna, Leloup and Buleon, 1992).

CONCLUSIONS

The *in vitro* hydrolysis method produced valuable data on hydrolysis index Sri Lankan starchy tubers. The above data could be useful in future large-scale studies on traditional tubers. It is worthy to conduct experiments to find both GI and HI values in the same varieties of starchy tubers in view of developing an equation to predict GI using *in vitro* HI of Sri Lankan starchy tubers.

Authors' contributions

IS conceived the idea of the study and all authors participated in designing the study. SG and IS were involved in laboratory procedures while SG and DR were involved in data analysis. All authors were involved in data interpretation. SG drafted the manuscript while DR, IS and MK critically revised it. All authors approved the final manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this published article.

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