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Formulation and Characterization of a Healthy Snack with a Low Glycemic Index

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ABSTRACT

Background: The rate of starch digestion and glycemic response are influenced by the composition of food.
Objectives: To formulate a healthy snack utilizing locally accessible ingredients and to determine the energy and macronutrient composition of the snack, the glycemic index, insulinemic index, and *in vitro* starch digestibility properties of the carbohydrate fractions of the snack and its main ingredients, which may be important in predicting the *in vivo* responses.
Materials & Methods: A healthy snack was formulated using Olu rice, foxtail millet, barley, and chickpeas as main ingredients, together with wheat flour, cinnamon, butter, raisins, egg white, baking powder and vanilla essence. Laboratory analysis was carried out to achieve the objectives.
Results: The proximate compositions of protein, fat, soluble dietary fiber, insoluble dietary fiber and digestible starch in g/100g were 12.35 ± 0.77, 15.00 ± 0.36, 3.47 ± 0.31, 1.8 ± 0.45 respectively with 441.8 kcal energy. The fiber content of the formulated healthy snack had a higher soluble fiber to insoluble fiber ratio. The starch digestion index (SDI) of the four main ingredients ranged from 21.60 to 38.50. The predicted glycemic indices (pGI) of the ingredients varied from 24.69-41.49, whereas the pGI of the formulated snack was 43.69 and the actual glycemic index was 36.5. All these values fell within the low GI category of foods.
Conclusions: A healthy snack with a low glycemic index can be prepared with locally available food items ensuring the cultural acceptability of Sri Lankans.

INTRODUCTION

Snacks often fail to deliver expected standards from a health promotion standpoint. They are often made with refined ingredients with added fiber. Taste, appearance, and texture modifiers are extensively used to meet the healthy snacks palatable and appealing. Furthermore, due to the increase in access to global markets, ingredients may be imported, leaving out local ingredients with inherent healthful properties. The cultural acceptability of a product rests largely on the ingredients used. With increasing prevalence of chronic diseases and their links to increasing energy, fat, sugar and salt consumption, the need for developing healthy snacks is rising.

The benefit of low glycemic index (GI) diets is now well-documented, in both diabetic and non-diabetic populations. The rate of starch digestion and its resulting glycemic response are significantly influenced by the composition of food, such as the content of resistant starch, phosphorylated starch, phytonutrients, dietary fiber, protein, and the fat content (Absar et al, 2009). The interaction of starch with fiber, protein and other food components can affect the diffusion and adsorption of the starch digestive enzymes (Colonna et al, 1992) and will affect the GI following ingestion of the food. Fat in a meal delays gastric emptying and reduces the rate of absorption of glucose and the rise in post-prandial insulin. It reduces starch gelatinization thereby slowing down digestion and absorption of glucose and subsequently lowering the GI (Absar et al, 2009). Hence, the postprandial insulin responses are not always proportionate to the blood glucose concentrations or the total carbohydrate content of a meal. Therefore, it essential to estimate the GI of the composite food made with a mixture of ingredients.

A low glycemic index snack is indicative of one that is more healthful than a high glycemic index snack due to a higher fiber content as well as higher protein, complex starches and will invariably provide more micronutrients. Such a snack would be within recommendations for the diabetic population to improve glycemic control and also for the general population in preventing type 2 diabetes and help in weight loss (Thomas and Elliott, 2010).

In-vitro methods focus on the sensitivity of carbohydrates to digestive enzymes (Englyst and Cummings, 1985). *In-vitro* starch digestibility assays are a good predictor of the *in-vivo* glycemic response of starchy foods (Englyst et al., 2003). *In-vitro* methods can be used to classify starch into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst and Hudson, 1996). The *in vivo* method to determine the GI of foods is laborious, time consuming and requires the co-operation of motivated volunteers. Therefore, several *in vitro* methods which mimic the physiological digestion of carbohydrate foods have been developed. Most of the *in vitro* methods focused on analyzing basic foods (Englyst et al., 1999; Englyst et al., 2000; Englyst et al., 2003; Garsetti et al., 2005). Therefore, the prediction of GI by these *in vitro* methods would be of immense practical use. Other factors that influence glycemic response are the methods of cooking and processing of food and its interaction with other food components.

The aim of this study was to formulate a healthy snack consisting of locally accessible ingredients and to determine the energy and macronutrient composition of the snack, the glycemic index, insulinemic index, and *in vitro* starch

digestibility properties of the carbohydrate fractions of both the snack and its main ingredients, which may be important in predicting the *in vivo* responses.

MATERIALS & METHODS

Chemicals

5.0 g/L pepsin (Sigma)
0.01M HCl
5.0 g/L guar gum (Sigma)
0.25 M sodium acetate buffer
4.0 g/L Pancreatin (Sigma)
Amyloglucosidase (sigma)
Human glucose liquicolour, complete test kit (Human GmbH)
2 M KOH

Preparation of the healthy snack

A healthy snack was formulated using pre-decided quantities of Olu rice (26 g), foxtail millet (26 g), barley (26 g), and chickpeas (20 g) as main ingredients together with wheat flour (20 g), cinnamon (1 teaspoon), butter (32 g), raisins (40 g), egg white (33 g), baking powder (1 teaspoon) and vanilla essence (1 teaspoon). All ingredients were purchased locally in bulk. The quantity of each ingredient and the final recipe was determined based on maintaining the physical properties of the cookie dough and were fine tuned to maintain the macronutrients within recommendations of EASD (European Association for the study of diabetes). The said ingredients were selected based on scientific reference to these being beneficial to those with type 2 diabetes mellitus (DM) (Narayanan *et al.*, 2016, Minaiyan *et al*., 2014, Nestel *et al.*, 2004).

Olu rice, foxtail millet (*Setaria italic*), barley (*Hordeum vulgare L.*), wheat flour, chickpea (*Cicerarietinum*), cinnamon, baking powder, raisins, butter, vanilla and egg white with water were made into a dough, shaped into balls (8-10 g each) and baked at a temperature of 150^{o}C for 20 minutes.

Protocol for determination of glycemic index and insulinemic index

Participants

Ethical approval (EC 15-069) for the study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo. Informed written consent was obtained from all the participants prior to the study. Twelve healthy volunteers (six males and six females) aged between 25 and 65 years with normal BMI (18.5-24.99 kg/m^2) were selected for the study. Inclusion criteria for the selection of participants were being non-smokers, non-alcoholics, not on any form of medication, non-pregnant or non-lactating, with a normal fasting blood glucose level (70 to 100 mg/dL). Individuals with DM were excluded. Height and weight of the study participants were measured according to the National Health and Nutrition Examination Survey, Anthropometry Procedures Manual (NHANES, 2007).

Determination of GI and insulinemic index

Determination of GI and insulinemic index was carried out according to the method described by FAO/WHO (FAO/WHO, 1998). Following an overnight fast of 10-12 hours, a sample of venous blood was collected for fasting blood sugar testing (2.0 mL blood in a fluoride oxalate tube) and insulin (3.0 mL blood in a plain tube). Subsequently, the participants were given 250.0 mL of glucose solution (55 g of glucose dissolved in 250.0 mL water; corresponding to 50 g available carbohydrate) to be consumed within 10-15 minutes. Venous blood samples were drawn at 15, 30, 60, 90 and 120 minutes after glucose consumption for blood sugar

analysis. After a break of one week, participants were called back for the determination of GI of the test food. Participants were requested to consume the test food containing 50 g available carbohydrate within 10-15 minutes. Blood samples were drawn at 15, 30, 60, 90 and 120 minutes after test food consumption.

All the blood samples were centrifuged (MIKRO 20 Hettich Zentrifugen, Germany) within two hours following collection at 3,500 rpm for 15 minutes and serum was transferred into chilled tubes and immediately stored at -20°C until analysis.

Determination of GI and insulinemic index

Determination of blood glucose concentration

Serum glucose analysis was carried out using the glucose oxidase procedure (Human Glucose liquicolour, complete test kit (Human GmbH) following standard protocol. Two positive controls were assayed daily before each set of serum samples. Inter-assay coefficient of variation (CV) was 0.05% and 0.04% for the respective controls. Each serum sample was analysed in duplicate.

Measurement of serum insulin concentration

Serum insulin concentration was analysed using a solid-phase, enzyme-labelled chemiluminescent immunometric assay on Immulite 1000 automated analyser using standard protocol (Semens Healthcare Diagnostic Products Ltd. USA). Inter-assay CV for the low control was 5.6% and high control was 4.2%. Each serum sample was analysed in duplicate.

***In-vitro* analysis**

Determination of the proximate compositions of the healthy snack

The baked heathy snack was crushed into small pieces and sun-dried over two days until there was no further weight change to the first decimal place. It was then oven dried at 55°C until no further weight change as measured on an analytical balance, which took a further five hours. It was then ground to a fine powder using a mortar and pestle and 0.5 g of this powder was used for analysis. Standard methods were used to determine digestible carbohydrate (Holm *et al.,* 1986), total starch (solubilizing the sample with 2 M KOH) followed by fat (Croon and Guchs, 1980), protein (AOAC, 1984) and dietary fiber (Asp *et al.,* 1983) of the healthy snack. Each sample was analyzed in triplicate.

In vitro starch digestibility of the healthy snack

In vitro starch digestibility of the healthy snack was analyzed using Englyst's method (Englyst and Hudson, 1996). A sample of 100.0 mg was incubated at 37ºC for 30 mins in a shaking water bath at 250 rpm with 10 mL of pepsin (Sigma) solution (5.0 g/L pepsin dissolved in 0.01M HCl), 5.0 g/L guar gum (Sigma) and 5 glass balls (d=5mm). The pH value was then adjusted to 5.8 using 0.25 M sodium acetate buffer. A mixture of pancreatin (Sigma) (4.0 g/L) and amyloglucosidase (Sigma) (3.0 mL) was then added and incubated for 20 mins. 0.2 mL of the reaction mixture was taken and placed in 1.8 mL ethanol (99.5 %) to inactivate the enzyme. This mixture was then centrifuged at 4696 g for 20 mins and 10 µL of supernatant was taken to determine the glucose concentration (G20) using the glucose oxidase method, to yield RDS values. All samples were analyzed in triplicate.

The same procedure was repeated at 30, 60, 90 and 120 mins of incubation and the glucose concentration was determined, which yielded SDS values for each food in triplicate. The equations of Englyst and Cummings (Englyst HN and Cummings H,1985) for RDS, SDS and the starch digestion index (SDI) used are as follows: RDS = G20*0.9, SDS=(G120-G20)*0.9 and SDI = (RDS/TS)*100.

Predicted glycemic index through starch digestibility of the healthy snack

The starch hydrolyzation using Englyst's (Englyst and Hudson, 1996) method was plotted as glucose concentration against time for 120 minutes for the test food and white bread (standard) in order to calculate the area under the curve in each case. The hydrolysis index (HI) for the calculation of predicted glycemic index was calculated as the ratio between the area under the hydrolysis curve (0 - 120 mins) of the test food and the area under the curve for the standard food (white bread) and expressed as a percentage of total glucose released.

Predicted glycemic index (pGI)

pGI was calculated using the equation, pGI=39.21 + 0.803 (HI) (Odenigbo *et al.*, 2013).

RESULTS

The mean (±SD) proximate compositions of the healthy snack were 12.35 ± 0.77 g/100 g of protein, 15.00 ± 0.36 g/100 g of fat, 3.47 ± 0.31 g/100 g of soluble dietary fiber, 1.8 ± 0.45 g/100 g of insoluble dietary fiber and 61.70 ± 0.48 g/100 g of digestible starch providing 441.8 kcal/100 g of energy (~ 147 kcal/per serving). The macronutrient composition of commercially produced locally available healthy snack and the corresponding percentage contribution to energy is presented in Table 1.

Table 1. Nutrient compositions and % contribution to energy of the health snack

Nutrient	**Formulated healthy snack**	**% Energy contribution**	
		Healthy snack	***EASD recommendation**
Carbohydrate (g/100g)	61.70	11.80	10 - 20
Protein (g/100g)	12.35	30.55	20 - 35
Fat (g/100g)	15.00	58.27	45 - 65
Dietary fiber (g/100g)	5.27		
Energy (kcal)	441.84		

EASD = European Association for the study of diabetes (https://www.easd.org).

Total starch and its fractions, RDS, SDS and RS of the main ingredients (chickpea, barley, foxtail millet and Olu) that were used to prepare the healthy snack, are presented in Table 2. The mean (±SD) starch fractions of the healthy snack as total starch, and its fractions, RDS, SDS and RS were 64.36 g/100 g, 22.75 ± 0.78 g/100 g, 5.82 ± 0.76 g/100 g, and 2.66 ± 0.5 g/100 g, respectively.

Table 2. Weight of rapidly digestible starch (RDS), slowly digestible starch (SDS), total starch (TS) and resistant starch (RS) present in 100 g of the main ingredients and in the formulated healthy snack

Parameter	Ingredients in the formulated healthy snack				Healthy Snack
	Chickpea	Barley	Foxtail millet	Olu rice	
RDS(g/100g)	17.53	24.78	13.90	21.24	22.75 ± 0.78
SDS(g/100g)	3.51	2.53	9.69	5.31	5.82 ± 0.76
TS(g/100g)	62.64	72.61	71.44	69.45	64.36
RS(g/100g)	4.541	3.511	4.588	2.80	2.66 ± 0.5

The enzymatic hydrolysis curves for the standard food (white bread) and the healthy snack are depicted in Figure 1. The hydrolysis Index (HI) calculated from the hydrolysis curves and the corresponding pGI was 41.17 and 43.69 respectively. Starch digestion index is a measure of the relative rate of starchdigestion, and it was 34. Starch digestion index, HI and the corresponding pGI of the ingredients are depicted in Table 3.

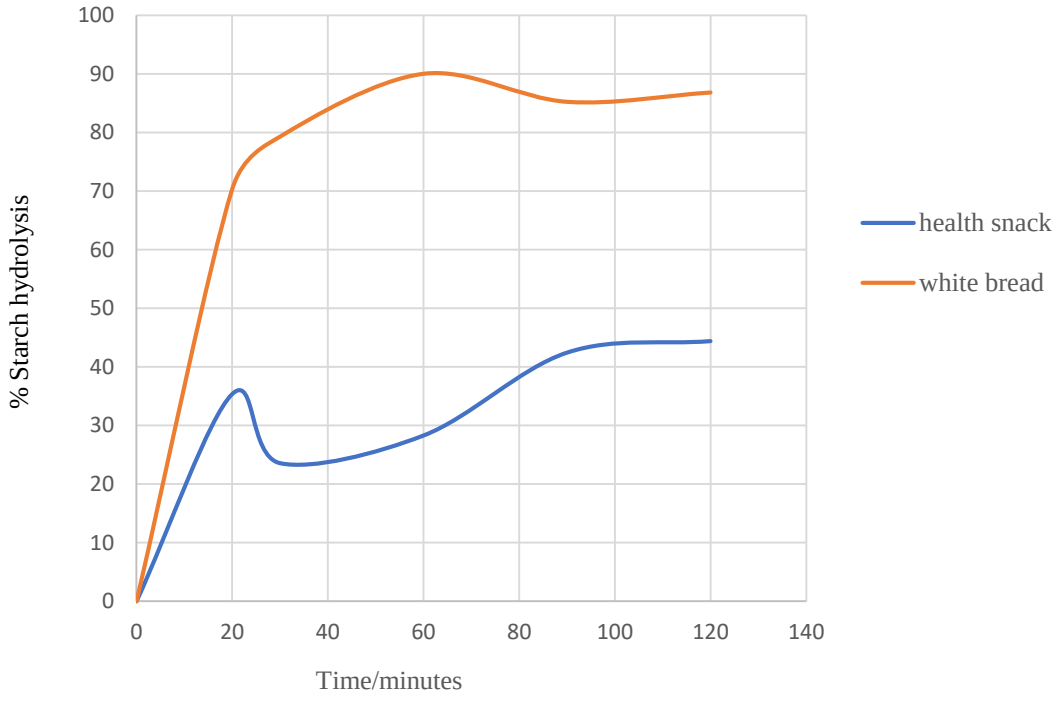

Figure 1. Hydrolysis curves for the standard food and test food

Table 3. Hydrolysis index (HI), predicted glycemic index (pGI) and starch digestion index (SDI), of the main ingredients present in the snack.

	Chickpea	Barley	Foxtail millets	Olu rice
HI	41.99	74.01	43.40	58.45
pGI	23.91	41.49	24.69	32.95
SDI	38.50	21.60	33.00	36.92

The blood glucose and insulin curves for the standard food (glucose) and test food are shown in Figure 2. The glycemic index and the insulinemic index of the healthy snack were 36.5 and 47.79, respectively. Serving size was determined to be 33 g, which provides 147 kcal.

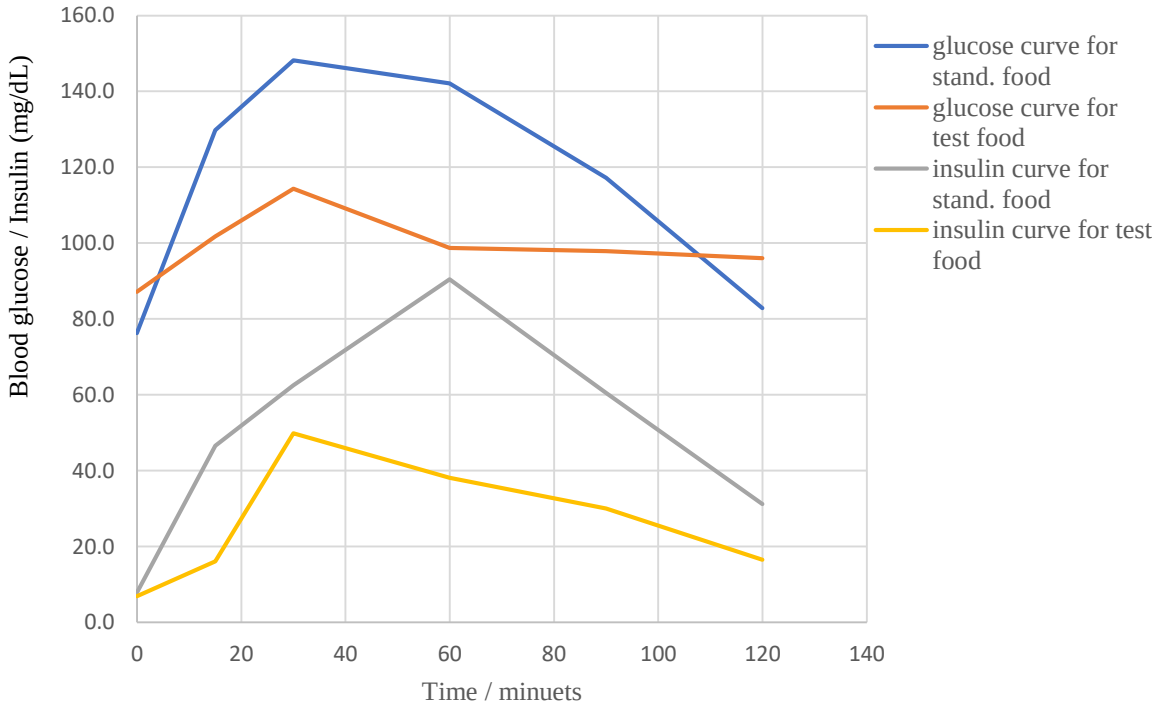

Figure 2. Blood glucose curves and insulin curves for the standard food (glucose) and test food.

DISCUSSION

The contribution to energy from macronutrients, protein, carbohydrate and fat of the formulated healthy snack fell within the recommendations of the European Association for the Study of Diabetes (EASD) (EASD,2004). The percentage contribution from the macronutrients to total energy of the formulated snack compared well with the Nigerian diabetic snacks formulated by Onyechi *et al.*, 2013.

The glycemic index of the healthy snack fell within the low glycemic index range (≤55), as defined by the American Diabetes Association (American Diabetes Association, 2013). The predicted glycemic indices (pGI) of the ingredients varied from 23.91-41.49, whereas the pGI of the formulated snack was 43.69 and the actual glycemic index was 36.5. All these

values fell within the low GI category of foods. Prolonged or increased postprandial insulinemia has been shown to play a role in the development of insulin resistance and associated disease (Blaak *et al.,* 2012). The insulinemic index of the formulated snack was low. The estimation of the insulinemic index of foods is both theoretically and practically significant as it will be important in the treatment of DM. The formulated healthy snack reported a lower GI, to that of snacks available in the local market.

Soluble dietary fiber is recognized as one of the major factors that can significantly decrease the blood sugar response and thus promotes a lower glycemic index (Hallfrisch and Behall, 2000). This effect is due to the viscous nature of soluble fiber which is capable of thickening the food in the digestive tract thereby slowing down the action of digestive enzymes on starch. The fiber content of the formulated healthy snack contains a higher soluble fiber to insoluble fiber ratio and is possibly a one reason for its lower glycemic index.

Although the *in-vivo* digestion process is considered a better method, compared to *in-vitro,* the *in-vivo* method is very complex and exact replications are not possible. However, studies done by Holm *et al,* 1988 and Yoon *et al,* 1983 have shown a strong correlation between *in-vivo* and *in-vitro* starch digestibility. The RDS is the amount of starch hydrolyzed within the first 20 mins of incubation with digestive enzymes. It is rapidly hydrolyzed, therefore results in a quick rise in blood glucose and insulin response (Ells *et al,* 2005). The SDS is the amount of starch hydrolyzed between 20-120 minutes of incubation, it is slowly hydrolyzed by digestive enzymes and is absorbed slowly, therefore results in a slow and steady rise in blood glucose. In this study, incubation time was fixed at 30 min to standardize and allow for comparison of the different ingredients and the test food (Englyst and Hudson, 1996). The SDS value for the formulated snack was high (5.82 ± 0.76 g/100g).

In understanding the properties of the formulated snack, the SDI, SDS and RDS of the four main ingredients were also determined. The SDI was found to range between 21.60 to 38.50 for the four ingredients. The SDS of the ingredients were highest for foxtail millet followed by Olu rice, chickpea and barley. Foxtail millet had the highest amount of SDS and the lowest amount of RDS compared to Olu rice, barley and chickpeas. Results for hydrolyzation percentages at 30 min identified that barley (48.97 %) was the most rapidly hydrolyzed ingredient followed by chickpea (24.3%), Olu rice (34.4%) and foxtail millet (22.9%). It is interesting that the formulated healthy snack achieved a hydrolyzation rate similar to the ingredient with the lowest rate, foxtail millet and was 23.57%, which indicates that the SDS fraction is higher than the RDS fraction in the snack. The importance of formulating a food which retains the starch digestibility properties of the ingredients used as demonstrated in this study is paramount, as there is increasing evidence for the link between processing of food and chronic disease.

Differences in the digestibility of starch among species is due to factors such as the source of starch (Ring *et al.,*1988), granular size (Snow and O'Dea 1981) amylose/amylopectin ratio (Hoover and Sosulski, 1985), degree of crystallinity (Hoover and Sosulski, 1985), and the type of crystalline polymorphic sites (Jane *et al.,* 1997). It is known, as demonstrated by Snow & O'Dea (1981) as early as 1981, that reducing particle size increases the surface area which results in a higher starch hydrolysis rate as they demonstrated through grinding rice (both brown and white). Chickpeas, barley, foxtail millet and Olu rice were selected for the formulation of the snack as they have documented benefits in the management of DM.

A study done by Naismith *et al.*, 1991, showed that diabetic rats fed with diets containing barley or wheat exhibited a significantly lower blood glucose concentration, and weight loss. A diet formulated with foxtail millet by Jali *et al.*, 2012 showed that a daily consumption of 80 g of foxtail millet lowered HbA_{1c}, fasting blood glucose and homocystine concentrations and increased the insulin concentration in blood. In another study by Thathola *et al.*, 2011, showed a significant reduction of serum glucose, cholesterol and LDL levels with foxtail millet biscuits. Yang *et al.*, 2007 have shown that dietary chickpeas improved insulin resistance and reversed impaired glucose intolerance in long term high-fat fed animals. These ingredients demonstrate health benefits, some of which can be explained by their starch digestibility properties and some of which have not yet been fully explained. The present study demonstrated that a combination of these ingredients in a healthy snack retains the beneficial properties related to glycemic index and insulinemic index. While these indices have been used predominantly in the management of patients with DM. There is now increasing evidence that healthy individuals are also benefitted with the food items having these properties. It is therefore increasingly important to develop such products retaining the properties of the individual ingredients by low levels of processing as in the current study. A key strength of this study is that it offers a new perspective in formulating healthy snacks. Further, it researched both on the insulinemic index and the GI of the formulated snack. The main limitation of this study is the lack of information on moisture content, ash and total sugars.

CONCLUSIONS

The predicted glycemic indices (pGI) of the ingredients varied from 24.69 - 41.49, whereas the pGI of the formulated snack was 43.69 and the actual glycemic index was 36.5 and fell within the low GI category of foods. A low glycemic index healthy snack, with recommended quantities of protein, carbohydrate and fat and a high quantity of soluble dietary fiber was formulated using the main ingredients Olu rice, foxtail millet, barley, and chickpeas. Further, we effectively formulated a healthy snack which retains a major proportion of the properties of the main ingredients used. A healthy snack with a low glycemic index can be prepared with locally available food items/ingredients ensuring the cultural acceptability of Sri Lankans.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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