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Dietary fibre, resistant starch and *in-vitro* starch digestibility of selected elevencommonly consumed legumes (Mung bean, Cowpea, Soybean and Horse Gram) in Sri Lanka

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Abstract

Consumption of dietary fiber rich food has shown many health benefits against a range of disorders including obesity, type 2 diabetes mellitus and colon cancer. Dietary fiber is composed of twocomponent of; soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Legumes are reported to be rich source of dietary fiber (DF) and resistant starch (RS). In addition to that legume starch has low digestibility. This study was aim to screen the high DF and high RS containing legume varieties with intension to develop functional food against non-communicable diseases. Accordingly, eleven legume varieties, mung bean (MI5, MI6), Cowpea (Waruni, MICP1, Bombay, Dhawala, ANKCP1), soybean (MISB1, Pb1) and horse gram (ANK Black, ANK Brown) were analyzed to determine the contents of dietary fibre, resistant starch and predicted glycaemic index (pGI). Among analyzed legume seeds, horse gram and soybean varieties showed the highestdietary fibre contents. Results for RS content exhibited significantly high in ANK Black (10.68±0.55%) followed by ANK Brown (10.45±0.10%), ANKCP1 (9.62 ±0.19%) and Waruni (9.04±1.26%). The values for predicted glycaemic index (pGI) in selected legume varieties were ranged from39.64 ± 0.46 (ANK Brown) to 43.48 ± 0.44(Pb1). The resistant starchcontent of legumes showed inverse correlation with predicted glycaemic index (-0.698; P≤ 0.05).

Keywords: Dietary Fibre, Resistant Starch, Predicted Glycaemic Index, Mung bean, Cowpea, Soybean, Horse bean.

Introduction

Generally, grain legumes play a vital role in the dietary pattern of low income people among developing countries as analternative to meat since it is protein rich source. Hence, interest has grown in the utilization of legumes in forms of flour, protein concentrates and protein isolates rather than consumption of whole seed[1]. In addition to nutritional value, the use of legumes as specially in the form of whole grain provide higher functional value, providing health promoting functional food factors ofresistant starch, dietary fibre, antioxidants, bioactive compounds and certain vitamins, minerals[2]. Moreover, the legume starch also has low digestibility[3]. Considering the advantage of above substantial nutritional and functional properties, functional/ healthy food products could be formulated by using legumes. It is now becoming a highly fascinate food among all over the world as it has remarkable prevention effects on non-communicable diseases.

Among different varieties of legumes, beans/ peas, peanuts, soybeans and lentils are more common in human diets[4]. These legumes are good sources of fibre as well as resistant starch. According to previous study reported by Gunathilake et al, the crude fibre content of mung bean, cowpea and soybean were varying from 3.04 – 7.93%[5]. The high content of crude fibre is mainly due to the presence of seed coats and cell walls. The enzymatic non digestive parts of diet known as dietary fibre can be divided intotwo components of solubleand insoluble forms and those provide plenty of benefits to the human healthy lifestyle. Soluble fibre is able to dissolve in water and is help to regulate blood cholesterol as well as lowering blood glucose level[6]. In contrast, other form called insoluble fibre, does not dissolve in wateris frequently denotedas bulk or roughage since it keep smooth functioning of digestive system. Apart from that, it helps to prevent haemorrhoids, constipation and other digestive difficulties[7].

In addition to dietary fibre, legume starch is high in amylose and resistant starch whichplay major role in designing functional food product. Usually, starch granules of legumes contain 60% to 70% amylopectin and 30% to 40% amylose whereas starchyfood contain 70 to 75% amylopectin and 25% to 30% amylose[3]. Food with high resistant starch and amylosehave shown to exhibit a slow rate of digestibility and reduces energy intake by the intestinal cells, evident by the low GI, providing reduced calorific value[8]. Resistant starch, called as non-digested starch is resistant to human digestive enzymes in small intestine and passes to large intestine or colon[9]. This has enormous health benefits, such as, protection against diabetics, weight control and protection from coronary heart disease[10,11]. In the large intestine, resistant starch is fermented by colonic microbes and produced short chain fatty acids which treat favourable substrate on growth and other functionality of probiotics. This can help to prevent colon cells from colon cancers[12,13].

Non communicable diseases (NCD) including diabetes, cardio vascular diseases, hypertension, obesity, stroke, cancers and gastrointestinal diseases are most prominent health issues in worldwide. It is widely believed that the global NCD epidemic is associated with a high intake of carbohydrate and eventually results in developing non communicable diseases. Designing food for leaving nutritional approaches to prevent from non-communicable diseases has become important task in this decade more than any other period. In that case, legume food are by farthe best option in addition to their nutritional properties have spectacular health benefits onnon-communicable diseases[11]. Therefore, the present study was aimed to study the dietary fibre, resistant starch and predicted glycaemic index (GI) in eleven legume seed to determine suitable varieties in order to develop functional healthy food.

Materials and methods

Materials: There are eleven commonly consumed legume varieties from mung bean (MI5, MI6), cowpea (Waruni, MICP1, Bombay, Dhawala, ANKCP1), Soybean (MISB1, Pb1) and Horse bean (ANK Black, ANK Brown) were chosen to analyze for their total dietary fibre, resistant starch and predicted glycaemic index. These legume seeds were collected from Grain Legumes and Oil Seed Crops Research and Development Centre, Agunakolapelessa (GLOSCRD), Sri Lanka.

Enzyme kits for enzymatic assays were purchased from Megazyme international Ireland, Bray Business Park, Bray, Co. Wicklow, Ireland). All the chemicals, standards and reagents were in analytical grade and other materials were provided by reputed regular suppliers.

Sample preparation: Cleaned and dried whole legume seeds were ground with a RETSCH S/S CROSS BEATER Hammer Mill Sk1 to pass through 0.5 mm (500µm) mesh size sieve. The grounded samples were packed in polyethene bags and stored at 10ºC prior to analysis.

Powdered legume samples were analyzed for total dietary fibre, resistant starch and predicted glycaemic index. All these experiments were performed in triplicates and results were expressed as means with standard deviations (SD) on dry weight basis.

Total dietary fibre content: The total dietary fibre contentsof legume seeds were determined by enzymatic gravimetric method as specified in AOAC (2012) - 991.42 official method of analysis[14]. In this protocol, starch and protein were digested with enzymes to small fragments to separate fibre. The soluble dietary fibre(SDF) was removed by filtering and washing residue with water. Remaining residue, insoluble dietary fibre (IDF), was recovered by precipitation with 95% ethanol and washed with acetone.

Resistant starch and non-resistant starch content: Resistant starch (RS) was determined using a kit assay (K-RSTAR, Megazyme international Ireland, Bray Business Park, Bray, Co. Wicklow, Ireland). This procedure has been subjected to inter-laboratory evaluation under the auspices of AOAC International and AACC International and accepted by both associations[15,16].

Hydrolysis and solubilisation of Non-resistant starch: Legume flour samples were accurately measured (100 ±5mg) into screw cap tubes (16×125mm) and mixed with 4ml of pancreatic α-amylase containing amyloglucosidase (AMG-3U/ml). The tubes were tightly capped and incubated at 37°C with continuous shaking (200 strokes/min) for exactly 16h in a shaking water bath.The tubes were removed from the water bath to add 4.0 mL of ethanol (99% v/v) orindustrial methylated spirits (IMS) (99% v/v) with vigorous stirring on a vortex mixer. The tubes were centrifuged (SIGMA 3-16K) at 1,500×g for 10 minutes (non-capped). The supernatants were decanted and the pellets were re-suspended in 2 mL of 50% ethanol or 50% IMS with vigorous stirring on a vortex mixer. Further, 6 mL of 50% IMS was added and the tubes were mixed and centrifuged again at 1,500×g for 10 minutes (Supernant A). The supernatants were decanted and that suspension and centrifugation steps were repeated once more. The supernatants (Supernant B) were decanted and the tubes were inverted on absorbent paper to drain excess liquid.

Measurement of resistant starch: In order to dissolve RS, 2 mL of 2M KOH was added and re-suspended the pellets. The mixtures were gently stirred for 20 minutes in awater/ ice bath using a magnetic stirrer to avoid any lump formation in starch solution. Then 8ml of 1.2M sodium acetate buffer with pH 3.8 was added to each tube and 0.1mL of amyloglucosidase (solution 1: 3300 U/ml) was added immediately. After thatthecontents in tubes were mixed well and placed in a water bath at 50°Cto incubate for 30 minutes with intermittent mixing on a vortex mixer.

If samples containing >10% Resistant Starch: The contents of the tube were quantitatively transferred to a 100 mL volumetric flask and adjusted to 100 mL with distilled water. An aliquot of the solution was centrifuged (SIGMA 3-16K) at 1,500×g for 10 minutes.

If samples containing < 10% Resistant Starch: the tubes were directly centrifuged (SIGMA 3-16K) at 1,500×g for 10 minutes.

A0.1mL of aliquots supernatant was transferred into a glass test tube and mixed with 3.0 mL of Glucose Determination Reagent (GOPOD). This content was incubated at 50°C for 20 minutes and the absorbance was measured at 510 nm against the reagent blank (Shimadzu UV - 1601).

Measurement of Non-Resistant (Solubilised) Starch: The supernatant solution, Supernant A and Supernant B inpart (a) were combined and adjusted to 100 ml with 100mM sodium acetate buffer with pH 4.5. The solution was mixed well and added 10µl of diluted AMG solution (300 U/ml) in 100mM sodium maleate buffer (pH 6.0) to 0.1ml aliquots. The solution was incubated for 20 minutes at 50°C. Further, incubated the tubes for a 20 minutes at 50°C by adding 3.0 ml of GOPOD reagent. Finally, the absorbance of the solution was measured at 510 nm against the reagent blank and calculated the NRS in the legume sample.

***In-vitro* starch digestibility (Predicted Glycaemic Index):** *In-vitro* starch digestibility was determined using the method described by Jenkins et al.[17] with some modifications as described by Dahlin and Lorenz[18].

Preparation of DNS reagent: DNS reagent was prepared according to method described by Coughlan and Moloney[19]. According to that, 10 g of dinitrosalicylic acid (DNS) and 300 g of sodium potassium tartrate (Rochelle salt) was added to 800 mL of 0.5 N NaOH and was gently heated to dissolve all the reagents. The volume was then made up to 1.0 L with distilled water.

Building up a standard curve: Known concentration of glucose was prepared from 0.1 mg/mL- 0.8 mg/mL solution by mixing 1mlof glucose solution with 4ml of the DNS reagent. Tubes were placed in boiling water bath for 5 minutes, cooling rapidly and then brought to room temperature by placing them in a water bath at 25ºC. The absorbance was measured at 540 nm, using spectrophotometer (Shimadzu UV – 1601) to work out the standard curve.

In vitro carbohydrate digestibility of legume samples were analysed by placing 1g of available carbohydrate portion from each legume sample into 13cm dialysis bags (cut from 94.5cm length, 4.8 nm pore diameter and 12000 molecular weight cut off). Human saliva was collected daily and stored under refrigerater in sealed vials until use for the experiment. Then 5ml of fresh human saliva and 10ml of distilled water were added into dialysis tube and the slurry was gently to mixed. The dialysis tube was placed in a separate water bath containing 800ml of distilled water at 40ºC with continuous agitation. At each first three hour, 1ml of dialysate was pipetted into a test tube and 4 ml of the DNS reagent was added.

Tubes were placed in boiling water bath for 5 minutes and transferred to an ice bath to cool rapidly and then brought to room temperature by placing them in a water bath at 25ºC. The absorbance was measured at 540 nm, using spectrophotometer (Shimadzu UV - 1601). Previously prepared DNS standard curve was used to calculate actual concentration of the samples.

Statistical analysis: The collected data were statistically evaluated through one-way analysis of variance (ANOVA) and general linear model using Minitab 17. Tukey's multiple comparison was used to determine significant difference between each sample at 5% significant level. Graphical representations of data were done by Microsoft Office Excel 2010.

Results and discussion

Dietary fibre content: According to previous research work, dietary fibre are high in legumes rather than cereals and rich in metabolically active soluble dietary fibre (SDF)[20]. It was reported that the total amount of dietary fibre contained in different legumes varies between 3g and 6g/ 75g serve of cooked legume[21]. The dietaryfibre content of selected locally consume eleven legume varietiesare shown in Table-1. According to that, the mean values for dietary fibre content of selected locally consume eleven legume varieties ranged from the lowest of 13.07 ± 0.51% (cowpea-Bombay) to the highest of 21.38 ± 0.22% (horse bean-ANK Black).

Comparatively, horse bean and soybean contained significantly higher amounts ($P \leq 0.05$) of dietary fibre rather than cowpea and mung bean. In this context, no significant difference ($P > 0.05$) showed between horse gram and soybean except Pb1. Similar values for the dietary fibre contents of 16.8±1.3 and 12.14±0.12 in Horse gram were reported by Sreerama et al. and Marimuthu and Krishnamoorthi respectively[22,23]. When consider the mean values of soybean, Amalraj and Pius reported that the TDF content of the raw undehulled soybean flour (*Glycine max*) was 28.40 ± 1.50% and it was 30.18% according to the Perera et al. [22,25]. In the present study, dietary fibre content of cowpea varieties ranged from 13.07 ± 0.51% to 15.99 ± 0.49%. These findings are in agreement with previous research works. Sreerama et al. reported that TDF content of *Vignaunguiculata L* was 14.1 ± 0.3%[22]. But it was recorded as 21.4 ± 1.5% by Amalraj and Pius and 18.2% by Khan et al.[24,26]. Regarding the dietary fibre content of mung bean, current results are not resemble with the findings of Amalraj and Pius, 25.90±1.80% for raw undehulled mungbean flour (*Vigna radiate*)[24].

Resistant starch and non-resistant starch content: According to the results shown in Table-1, the mean values for resistant starch content of selected legume varieties ranged from 0.01 ± 0.00% (Pb1) to 10.68 ± 0.55% (ANK Black). Among them, horse gram (ANK Black and ANK Brown) and three cowpea varieties (ANKCP1, Bombay and Waruni) show comparatively higher values for RS and those are not significantly differ ($P > 0.05$) from each other. It is interesting to note that the contents of resistant starch in some cowpea varieties (MICP1-3.55 ± 1.29% and Dhawala-3.24 ± 0.28%) are lower than the values of mung bean (MI5-5.95 ± 0.32% and MI6-5.70 ± 0.08%). However, soybean exhibits the lowest values for resistant starch and no significant difference ($P > 0.05$) was found between in their RS contents of Pb 1 (0.01 ± 0.00%) and MISB 1 (0.1 ± 0.01%) in their RS contents.However RS values for the soya bean are lower due to the presence of low content of carbohydrates and high contents of protein and fat.

Table-1: Contents of Dietary fibre, Resistant Starch, Non-resistant Starch and Predicted Glycaemic Index (PGI) of mung bean, cowpea, soybean and horse bean (values are on dry weight basis).

	Mean (g/100g of sample)± SD			
	Dietary Fibre	Resistant Starch	Non-resistant starch	Predicted GI
Mung Bean				
MI5	15.49 ± 0.62^{cd}	5.95 ± 0.32^{b}	41.31 ± 0.63^{a}	41.54 ± 0.25^{b}
MI6	13.64 ± 1.15^{de}	5.70 ± 0.08^{bc}	38.07 ± 1.70^{bc}	42.05 ± 0.09^{b}
Cowpea				
Waruni	13.60 ± 0.15^{de}	9.04 ± 1.26^{a}	34.11 ± 0.36^{ef}	41.72 ± 0.06^{b}
MICP1	15.31 ± 0.62^{cd}	3.55 ± 1.29^{cd}	35.91 ± 0.83^{de}	42.23 ± 0.25^{b}
Bombay	13.07 ± 0.51^{e}	9.06 ± 1.91^{a}	39.04 ± 0.36^{b}	41.73 ± 0.10^{b}
Dhawala	14.56 ± 0.41^{cde}	3.24 ± 0.28^{d}	38.03 ± 0.02^{bc}	41.94 ± 0.40^{b}
ANKCP1	15.99 ± 0.49^{c}	9.62 ± 0.19^{a}	38.70 ± 0.43^{b}	41.34 ± 0.46^{b}
Soybean				
MISB1	19.67 ± 1.00^{a}	0.10 ± 0.01^{e}	6.63 ± 0.09^{g}	41.38 ± 0.29^{b}
Pb1	18.51 ± 0.67^{b}	0.01 ± 0.00^{e}	6.11 ± 0.13^{g}	43.48 ± 0.44^{a}
Horse Bean				
ANKBlack	21.38 ± 0.22^{a}	10.68 ± 0.55^{a}	36.48 ± 0.81^{cd}	39.83 ± 0.38^{c}
ANKBrown	21.35 ± 0.95^{a}	10.45 ± 0.10^{a}	32.69 ± 0.20^{f}	39.64 ± 0.46^{c}

Values are expressed as an average (mean ± SD) of three determinations (n=3). Means within the same column with different letters are significantly different (P < 0.05).

When consider the previous research works, there were slight variations with the results of current study. According to Sreerama et al. RS values of *Vignaunguiculata L.* (cowpea) was 2.5± 0.10% whereas resistant starch content of raw cowpea flour was 12.65% as reported by Chen et al.[22,27]. These variations can be encountered by methodology difference, varietal difference, different climatic conditions etc.In the case of mung bean similar values were observed by other scientists. Vatanasuchart et al. reported that the RS content of the raw undehulledmungbean flour (*Vignaradiate)* was 22.90±0.00%[8]. The findings of Chen et al. shows that RS content of mung bean was 16.66% and the raw undehulled soybean flour (*Glycine max*) was 3.42%[27]. The RS content is low due to high fat content in soybean. These fats create amylose-lipid complexes. Therefore available amylose to interact with external chains of amylopectin to form resistant starch is very low. As a result of that resistant starch synthesis in soybean is very low[28]. These observations for horse gram are not agree with those reported by Sreerama et al., as their findings the RS content for horse gram was 2.20±0.20[22]. According to the Marimuthu and Krishnamoorthi, it was 2.15±0.20[23]. Present finding regarding RS content of mung bean, cowpea, soybean and horse gram are in variations with values described in previous literature, this may be due to method used to analysis or genotype and environmental conditions[29]**.**

***In-vitro* starch digestibility (Predicted Glycaemic Index-pGI):** According to the results mentioned in Table-1, the mean values for predicted glycaemic index content of selected eleven legume varieties ranged from low value of 39.64 ± 0.46% (ANK Brown) to high value of43.48 ± 0.44% (Pb 1). It is not observed significant difference (P>0.05) between values of mung bean, cowpea and soybean varieties (except Pb1). But horse gram shows marginally less values, ANKBlack-39.83 ± 0.38% and ANKBrown-39.64 ± 0.46%. Present results are in-line with several investigations. Sreerama et al. has reported pGI of

Vignaunguiculata L. was 41±10% and Chen et al. mentioned that the pGI of cowpea is ranged between 33% and 50%[22,27]. Also, Vatanasuchart et al. reported that the pGI of mungbean (*Vigna radiate*) was 45.00±1.00%[8]. The findings of Chen et al. shows that pGI of mung bean is 31%[27]. In terms of soybean, Chen et al. have found that comparatively lower value forpGI of soybean (*Glycine max*), 18-25%[27]. It may be because of very low content of resistant starch content (MISB1, 0.10 ± 0.01- Pb1, 0.01 ± 0.00) in the soybean varieties. Turning to horse gram, observations are severely deviated those reported by Sreerama et al., as it was 51±11%[22]. The variations of values obtained in current study may be varied due to the different factors such as the reference food, portion sizes, method of calculating the area under the curve, type of test used etc[30].

Predictable Glycaemic Index for all studied legume varieties is in the range of 39-44. Under the classification of GI those values fall under the low GI category that is less than 55[31]. Therefore, those varieties can be recommended as low GI food. Therefore in utilizing local legume varieties in preparation of functional foods will be a good opportunity to recover the NCDS.

Starch hydrolysis % of legumes: Starch hydrolysis percentage was analyzed for all legumes varieties and the readings were taken for glucose release an each and every hour for 3 hours period. Results are graphically represented in Figure-1. According to that soybean varieties (Pb1 and MISB1) have by far the highest starch digestibility relative to other legume varieties. That explains soybean contribute far more on increase blood glucose level than other legumes. Whereas, lower the starch digestibility / hydrolysis, the glycemic index is lower. Based on these observations, the varieties with lower starch digestibility have a beneficial effect on health.

Table-2: Starch Hydrolysis % of legumes (mung bean, cowpea, soybean and horse gram) over 3 hours.

Legume	Variety	0h	1h	2h	3h
Mung Bean	MI5	0.0000	0.0000	0.1205	0.2015
	MI6	0.0000	0.0000	0.1570	0.1900
Cowpea	ANKCP1	0.0000	0.0000	0.1310	0.1820
	MICP1	0.0000	0.0000	0.1080	0.1810
	Bombay	0.0000	0.0000	0.1385	0.1900
	Waruni	0.0000	0.0000	0.1205	0.1785
	Dhawala	0.0000	0.0000	0.1155	0.2500
Soy Bean	Pb1	0.2655	0.3300	0.5915	0.7265
	MISB1	0.1150	0.1655	0.4575	0.6600
Horse Gram	ANKBlack	0.0000	0.0000	0.1205	0.1595
	ANKBrown	0.0000	0.0880	0.1915	0.2260

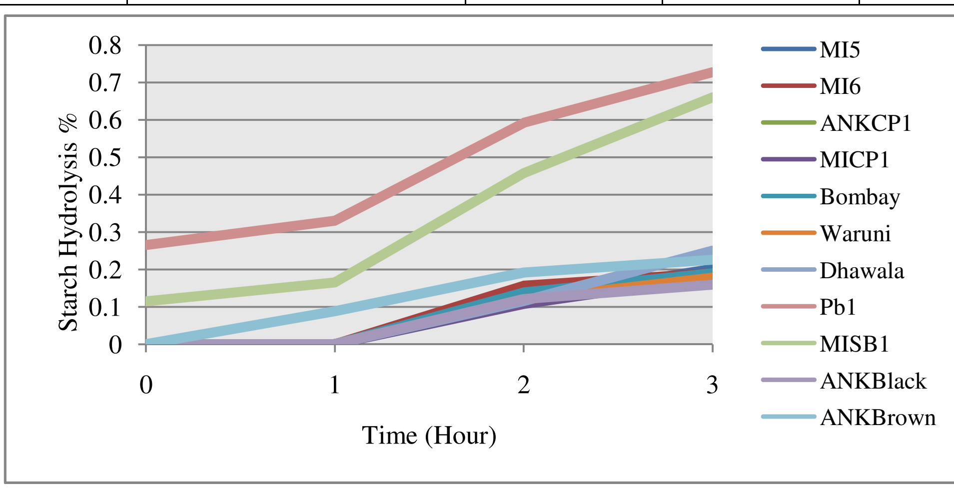

Figure-1: Starch Hydrolysis % of legumes (mung bean, cowpea, soybean and horse gram) over 3 hours.

Correlation between resistant starch (RS) content and predicted glycemic index (pGI) content of legumes: Resistant starch (RS) is an important measurement to characterize starch digestibility. Current protocol for RS analysis involved 16 hours enzymatic incubation for digestible starch hydrolysis before the treatment and hydrolysis of RS pellet. The figure 2shows that there is an inverse (negative liner) relationship between resistant starch and pGI (with -0.659 of pearson correlation). Present study showed that there is a significant relationship between RS and pGI (p≤0.05). This relationship is significant between RS and pGI (p≤0.05). According to the findings of Alonso et al. (1998) the increasing resistant starch content resulted the lower the predicted GI value[32].

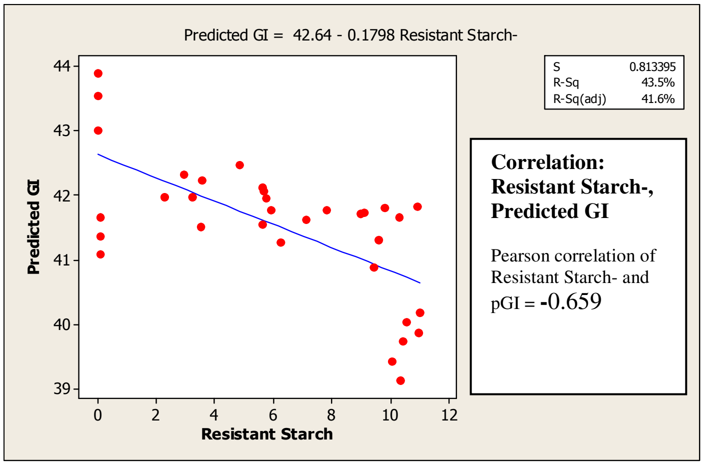

Figure-2: Correlation between resistant starch content and predicted glycemic index in selected legume varieties (n=3).

Conclusion

Conclusively, the highest dietary fibre content was in horse gram among selected legumes, i.e. ANKBlack21.38±0.22%. Results for RS content demonstrated significantly highest amount in ANKBlack10.68±0.55%. The lowest predicted glycemic index(pGI) was observed in ANKBrown39.64±0.46%. The RS content of legumes had an inverse correlation with pGI (-0.698; P<0.05). Therefore, horse gram can be used as an alternative source to develop health promoting functional food preparations.Since, legumes contain lot of anti- nutritional factors whichmay have both positive and negative effects. Hence, prior to do more research on functional food with legumes, further analysis of antinutrititional factors are recommended.

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