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Research Article

In vitro Starch Digestibility and Estimated Glycemic Index of Indonesian Cowpea Starch (*Vigna unguiculata*)

[1,2]Nani Ratnaningsih, [2]Suparmo, [2]Eni Harmayani and [2]Yustinus Marsono

[1]Department of Food Processing Education, Faculty of Engineering, Yogyakarta State University, Kampus Karangmalang, 55281 Yogyakarta, Indonesia
[2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur, 55281 Yogyakarta, Indonesia

Abstract

Background and Objective: Legumes were studied extensively in the past because of their protein contents but they have now become valuable for their low glycemic index (GI), which is attributed to high amylose and resistant starch contents. This study aimed to evaluate the *in vitro* starch digestibility and estimated glycemic index (eGI) of native starches from five varieties of cowpea (*Vigna unguiculata*) cultivated in Indonesia. **Materials and Methods:** Five varieties of cowpea (namely KT4, KT5, KT7, KT8 and KTL) were extracted their starches using wet milling method. The *in vitro* digestibility and eGI of cowpea starches were determined using method of Englyst and Goni. **Results:** Cowpea starches had low RDS content, from 4.09% (KT7) to 7.51% (KT4) but high SDS, from 19.66% (KT5) to 27.07% (KTL) and RS, from 65.75% (KTL) to 76.15% (KT5). The RAG and SAG contents of the cowpea starches ranged from 3.77% (KT7) to 6.79% (KT8) and from 18.56% (KT5) to 25.13% (KT8), respectively. The eGI of cowpea starches varied from 45.46±0.23 (KT5) to 48.14±0.38 (KT8). **Conclusion:** Cowpea starches have high SDS and RS contents and low GI, therefore they are suitable as a dietary carbohydrate alternative for the management of obesity, diabetes mellitus, cardiovascular disease and certain cancers.

Key words: Cowpea starch, estimated glycemic index, *in vitro* starch digestibility

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Corresponding Author: Nani Ratnaningsih, Department of Food Processing Education, Faculty of Engineering, Yogyakarta State University, Kampus Karangmalang, 55281 Yogyakarta, Indonesia Tel: +62 274 586168 Fax: +62 274 586734

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Legumes are the second largest daily dietary foodstuff after cereals. Cowpeas (*Vigna unguiculata* (L.) Walp.) are among the most important food legume crops and are grown in the semi-arid tropics covering Asia, Africa, Southern Europe and Central and South America[1]. Cowpeas are harvested for their immature pod or mature seeds and are generally consumed after a single or combination of processes, including soaking, boiling, milling, roasting, fermentation, puffing and germinating[2,3].

Daily consumption of legumes may restore various physiological states, such as the blood glucose level, blood lipid profile and production of short chain fatty acids in the colon. Therefore, a legume diet has an important role in controlling and preventing various metabolic diseases, such as obesity, diabetes mellitus, coronary heart disease and colon cancer[4].

Cowpea seeds contain approximately 53-66% carbohydrate, most of which is found in the form of starch[5-7]. Starch from legumes, such as cowpea, has high amylose content and C-type starch crystallinity[5,8]. Previous studies on starches from legumes have reported many unique properties, such as high viscosity, high resistance to swelling and rupture, high gelatinization temperature, fast retrogradation, high elasticity of the gel and highly resistant starch content compared to starch from cereal[3,9]. These properties are correlated with the high amylose content of the legume starch.

The glycemic index (GI) is a scale to measure the post-prandial glycemic effect after consumption of carbohydrate-rich foods[10]. The FAO/WHO experts suggested the use of the GI concept for classifying carbohydrate-rich foods to provide a useful means to help people to select the most suitable carbohydrate-containing foods for the maintenance of health and the treatment of several diseases[11,12]. Prior studies indicated that starches from legume have poorer digestibility than those from cereal; therefore, they can promote slow and moderate post-prandial glucose and insulin responses and have low GI values[9,13]. The consumption of low GI foods could prevent the emergence of several diseases, such as obesity, diabetes, cardiovascular diseases and even certain cancers[14].

Previous studies on cowpea starches, mostly from the African region, focused primarily on their structure and physicochemical properties[5-7,15,16]. Studies reported that the *in vitro* starch digestibility and GI of flours and starches from legumes were influenced by their nature, such as their amylose content[17,18]. Ratnaningsih *et al.*[8] reported differences in the composition, microstructure and physicochemical properties of starches from five cowpea varieties cultivated in Indonesia. In the present study, starches from five cowpea varieties that were reported to have high amylose content were evaluated for their *in vitro* starch digestibility and estimated glycemic index (eGI) to provide valuable information about the benefits of cowpea-based diets, especially their ability to prevent several degenerative diseases.

MATERIALS AND METHODS

Materials: Cowpea (*Vigna unguiculata*) varieties (KT4, KT5, KT7 and KT8) were obtained from the Indonesian Research Center for Legumes and Tubers, Malang, East Java, Indonesia and a local variety (KTL) was obtained from a local farmer in Yogyakarta, Indonesia. The five cowpeas varieties were selected based on their highest productivity and nutritional composition. All chemical reagents used in this study were analytical grade.

Starch extraction: Cowpea starches were extracted by wet milling according to Ratnaningsih *et al.*[8]. Cowpea seeds were split using a grinder and steeped in distilled water (ratio of seed:distilled water = 1:3) at 4°C for 24 h. The steep water was decanted and the softened pulses were ground using a blender at high speed for 3 min in distilled water (4°C). The slurry was then filtered. The residual pulp was ground using a blender for 3 min in distilled water (4°C) and filtered. The suspensions from the two filter steps were mixed and allowed to settle overnight at 4°C. The supernatant was then drained off. The starch sediment was redissolved in 0.05 M NaOH and kept at 4°C overnight before neutralizing with 2 M HCl to pH 6 at 4°C overnight. The starch sediment was rinsed with distilled water and allowed to settle at 4°C overnight until the settled starch produced a firm and dense deposit on the bottom. The sediment was recovered and dried at 50°C overnight, ground into powder using a blender, sieved through 100 mesh and stored in a sealed container at 4°C until further use.

Determination of *in vitro* starch digestibility: *In vitro* starch digestibility was determined using the method of Englyst *et al.*[19] with modification by Chung *et al.*[20]. Porcine pancreatic α-amylase (0.45 g, E-PANAA, Megazyme Inc., Ireland) was dispersed in sterile distilled water (4 mL) and centrifuged at 1500×g for 12 min. The supernatant (2.7 mL) was transferred to a beaker glass and amyloglucosidase

(0.3 mL, 3260 U mL^{-1}, E-AMGDF, Megazyme Inc., Ireland) and invertase (0.2 mL, 355 U mg^{-1}, E-INVPD2, Megazyme Inc., Ireland) were added to the solution. This enzyme solution was freshly prepared for each determination of starch digestibility.

Starch (100 mg) and 4 mL of 0.5 M sodium acetate buffer (pH 5.2) were added to each test tube. Enzyme solution (1 mL) and 20 glass beads (4 mm diameter) were added to each tube and the tubes were incubated in a shaking water bath (37°C, 120 rpm). After 20 min, aliquots (0.1 mL) were collected and mixed with 1 mL of 80% ethanol. The solution was incubated again in a shaking water bath (37°C, 120 rpm) and an aliquot (1 mL) was collected after 100 min and mixed with 1 mL of 80% ethanol. The shaking of the water bath was not stopped during the sampling period. The aliquot was taken after 20 min and designated as G_{20} (rapidly available glucose, RAG) and that taken after 120 min was designated as G_{120} (slowly available glucose, SAG). The G_{20} and G_{120} were centrifuged at 1500×g for 2 min to obtain a clear supernatant for glucose determination.

The remaining solution was removed from the shaking water bath, shaken vigorously to break up any large particles and incubated in a boiling water bath for 30 min. The test tubes were shaken again and cooled in ice-water for 15-20 min. Thereafter, 10 mL of 7 M KOH was added to the aliquot, mixed well and incubated in a shaking water bath containing ice-water (5-10°C) for 30 min. An aliquot (1 mL) was collected, added to 10 mL of 0.5 M acetic acid and 0.2 mL of amyloglucidase, incubated at 70°C for 30 min, placed in the boiling water bath for 10 min, cooled to room temperature, diluted with 40 mL of distilled water and centrifuged at 1500×g for 5 min. An additional aliquot (0.1 mL) was then collected for Total Glucose (TG) measurement.

The determination of Free Glucose (FG) was conducted as follows: Starch (400 mg) and 5 mL of 0.5 M sodium acetate buffer (pH 5.2) were added to screw-cap test tubes, shaken well, incubated in a boiling water bath for 30 min and cooled to room temperature. An aliquot (1 mL) was collected and 2 mL of 80% ethanol was added, shaken well and centrifuged at 1500×g for 5 min. The supernatant (1 mL) was taken and 5 mL of distilled water was added and shaken well for the determination of FG. The hydrolyzed glucose content was measured using glucose oxidase-peroxidase reagent (K-GLUC, Megazyme Inc., Ireland). Aliquots (0.1 mL) were collected and 3 mL of GOPOD reagent was added, incubated at 40-50°C for 20 min and cooled at room temperature. Then, the absorbence was measured at 510 nm.

Starch classification based on the rate of hydrolysis included rapidly digestible starch (RDS, digested within 20 min), slowly digestible starch (SDS, digested between 20 and 120 min) and resistant starch (RS, undigested after 120 min). The digestible starch fractions and available starch fractions were calculated as follows:

$$\text{RAG} = G_{20} \quad (1)$$

$$\text{SAG} = G_{120}\text{-}G_{20} \quad (2)$$

$$\text{TS} = (\text{TG-FG})\times 0.9 \quad (3)$$

$$\text{RDS} = (G_{20}\text{-FG})\times 0.9 \quad (4)$$

$$\text{SDS} = (G_{120}\text{-}G_{20})\times 0.9 \quad (5)$$

$$\text{RS} = \frac{\text{TS}-\text{RDS}-\text{SDS}}{\text{TS}}\times 100\ (\%) \quad (6)$$

Determination of the estimated glycemic index (eGI): Determination of the eGI of cowpea starch was conducted using the method of Goni *et al*.[21]. Starch (50 mg) and 10 mL of HCl-KCL buffer (pH 1.5) were added to conical tubes and 0.2 mL of pepsin solution (1 g of pepsin (0.7 FIP-U mg^{-1}, EC 3.4.23.1, Merck Inc., Germany) in 10 mL of HCl-KCl buffer; pH 1.5) was added to each sample and incubated at 40°C for 1 h in a shaking water bath. The volume was brought to 25 mL with tris-maleate buffer, pH 6.9. Then, 5 mL of pancreatic α-amylase solution (Sigma A3176, Sigma-Aldrich Inc., US) in tris-maleate buffer containing 2.6 UI was added to each sample and incubated at 37°C in a shaking water bath. Aliquots (0.1 mL) were collected from each sample after every 30 min from 0-180 min and placed in a tube at 100°C and were then refrigerated until the end of the incubation time. Sodium acetate buffer (1 mL, 0.4 M, pH 4.75) was added to each aliquot and 30 µL of amyloglucosidase (Sigma A9913, Sigma-Aldrich Inc., US) was added to hydrolyze the digested starch into glucose after incubating at 60°C for 45 min in a shaking water bath. The hydrolyzed glucose content was measured using the glucose oxidase-peroxidase reagent (K-GLUC, Megazyme Inc., Ireland). The glucose was converted into starch by multiplying to released glucose weight.

The rate of starch digestion was expressed as the percentage of TS hydrolyzed at different times (0, 30, 60, 90, 120 and 180 min). The total starch hydrolysis (%) of cowpea starches at different times were calculated as follows:

$$\text{Total starch hydrolysis}(\%) = \frac{\text{Released glucose weight}\times 0.9}{\text{Total starch weight}}\times 100 \quad (7)$$

The kinetics of *in vitro* starch digestion were calculated using the non-linear model established by Goni *et al.*[21]. The first-order equation is:

$$C = C_{\infty}(1\text{-}e^{-kt}) \tag{8}$$

where, C is the percentage of starch hydrolyzed at time t (min), C_{∞} is the equilibrium percentage of starch hydrolyzed after 180 min and k is the kinetic constant. The parameters C_{∞} and k were estimated for each treatment based on data obtained from the *in vitro* starch digestion. The area under the hydrolysis curve (AUC) was calculated using the following equation:

$$AUC = C_{\infty}\left(t_f - t_o\right) - \left(\frac{C_{\infty}}{k}\right)\left[1 - \exp^{\{-k(t_f - t_o)\}}\right] \tag{9}$$

where, C_{∞} is the equilibrium percentage of starch hydrolyzed after 180 min, t_f is the final time (180 min), t_0 is the initial time (0 min) and k is the kinetic constant.

The hydrolysis index (HI) represents the rate of starch digestion and the predicted GI indicates the digestibility of the cowpea starch in relation to the digestibility of starch in a reference material, white bread. The HI, a good predictor of glycemic response, was calculated by dividing the AUC of each treatment by the AUC of a reference (white bread). The GI was then estimated using the following equation of Goni *et al.*[21]:

$$GI = 39.71+0.549HI \tag{10}$$

Statistical analysis: All experiments were performed in duplicate and were expressed as the Mean±Standard Deviation. Data were analyzed using analysis of variance. Duncan's multiple range tests were conducted to assess significant differences among experimental mean values ($p<0.05$). The Pearson correlation was used to evaluate the correlation between the digestible starch fraction, available glucose fraction and eGI of cowpea starches.

RESULTS AND DISCUSSION

Total starch, digestible starch fractions and available glucose fractions of cowpea starch: The cowpea starch characterization results (TS, RDS, SDS, RS, RAG and SAG) are shown in Table 1. The total starch content ranged from 80.86-85.42%, which was lower than those reported by Huang *et al.*[6] and Adebooye and Singh[15]. The RDS is starch that is rapidly and completely digested in the small intestine and is associated with rapid elevation of post-prandial plasma glucose[22]. The RDS content of various Indonesian cowpea varieties varied significantly and ranged from 4.09% (KT7) to 7.51% (KT4). These values were similar to those reported by Sandhu and Lim[13] for starches from black gram, chickpea, field pea, lentil, mung bean and pigeon pea but lower than those reported for other legume starches, such as common bean, pinto bean, red kidney bean, black bean and navy bean[14,23]. Ambigaipalan *et al.*[9], Kaur *et al.*[24] and Hughes *et al.*[25] reported that the starches from chickpea, faba bean, black bean, pinto bean and mung bean contained higher RDS than cowpea starch. The RDS represents the hydrolysis of starch chains at or near the vicinity of the granule surface and was measured chemically during 20 min of enzyme digestion[22]. Therefore, the period was not sufficient for all of the hydrolytic enzymes to enter the granule interior since diffusion into the substrate must occur prior to the hydrolytic event[9]. The difference in RDS content among the cowpea starches reflects the interplay between the surface characteristics and the extent of molecular order at the granule surface[8].

The SDS content, which is digested more slowly, varied significantly among varieties of Indonesian cowpea starches, with the highest content in KTL (27.07%) and the lowest in KT5 (19.66%). These values were higher than those reported in previous studies for other legume starches[14,24] but lower than those reported by Chung *et al.*[20], Ambigaipalan *et al.*[9] and Liu *et al.*[18]. The low SDS in cowpea starches reflects the higher crystalline stability and denser packing of double helices within the crystalline domains (both of which restrict the

Table 1: Total starch, digestible starch fractions and available glucose fractions of cowpea starches

Cowpea varieties	TS (%)	RDS (%)	SDS (%)	RS (%)	RAG (%)	SAG (%)
KT4	81.46±2.62[b]	7.51±2.40[a]	22.87±0.94[c]	69.62±1.46[c]	6.77±1.95[a]	20.72±0.76[b]
KT5	85.42±2.88[a]	4.19±1.12[b]	19.66±1.78[d]	76.15±2.67[a]	4.09±1.06[b]	18.56±2.43[c]
KT7	80.96±2.35[c]	4.09±0.69[b]	24.37±0.48[b]	71.54±0.21[b]	3.77±0.65[b]	21.92±0.54[b]
KT8	84.81±0.36[a]	7.20±2.18[a]	26.67±2.82[a]	66.13±2.00[d]	6.79±2.09[a]	25.13±1.38[a]
KTL	81.58±0.57[b]	7.18±0.38[a]	27.07±0.73[a]	65.75±1.11[d]	6.51±0.39[a]	24.54±0.42[a]

Values are Means±Standard Deviations and different superscripts in the same column are significantly different ($p<0.05$), TS: Total starch, RDS: Rapidly digestible starch, SDS: Slowly digestible starch, RS: Resistant starch, RAG: Rapid available glucose, SAG: Slowly available glucose accessibility of amylolytic enzymes towards glycosidic linkages). The SDS is generally considered the most desirable form of dietary starch and is beneficial for the management of several diseases; such as obesity, diabetes, cardiovascular diseases and some cancers[26].

The RS content of cowpea starches varied significantly and ranged from 65.75% (KTL) to 76.15% (KT5). These values were similar to the values reported in previous studies for other legume starches[14,18] but higher than those reported by Kaur *et al.*[27] and Ambigaipalan *et al.*[9]. However, the RS content of all cowpea starches was higher than corn starch (24.5%) but lower than potato starch (84.5%)[14]. Goni *et al.*[28] classified foodstuff based on RS content as follows: Negligible ≤1%, low 1-2.5%, intermediate 2.5-5.0%, high 5.0-15.0% and very high >15%. According to this classification, cowpea starches were categorized as very high RS foodstuff. The RS content of native starches could be influenced by many factors, such as amylose content, crystallinity, crystalline perfection and amylopectin structure[5]. Ratnaningsih *et al.*[8] reported that the amylose contents of cowpea starches from Indonesia varied from 39.09-42.78% and had C_A-type crystallinities. The high RS content of cowpea starches suggests its potential as a functional ingredient for the development of cowpea-based functional food products. The health benefits of RS in the diet are related to its role as a substrate for probiotic microorganisms growth and its effects on hypoglycemia, hypocholesterolemia, increased absorption of minerals and colon cancer prevention[5,22,29].

The RAG content of cowpea starches varied significantly from 3.77-6.79%. These values were considerably lower than those of microwave-baked and water-blanched potatoes, which were 20 and 21/100 g, respectively[30] and boiled and microwave-cooked taros, which were 11.6 and 15.6/100 g, respectively[12]. Englyst *et al.*[31] showed that the RAG content is strongly correlated with GI and that it could be a major determinant of the magnitude of GI for most foods that contained carbohydrate. Therefore, food with a lower RAG content has a lower GI and vice versa. The RAG is another index for the evaluation of starch digestibility that reflects the total amount of glucose released into the blood stream from a certain portion of food[31]. The RAG value includes both the RDS and FG and it was reported to be a good indicator of the blood glucose and insulin response of foods[12,31].

The SAG content of cowpea starches varied significantly from 18.56-25.13%. Englyst *et al.*[32] reported that the SAG content of cereal products varied from 0.6-13.8/100 g. Therefore, cowpea starches have a higher content of SAG than cereal products. A high content of SAG corresponds to a low GI, which is rich in slowly released carbohydrates for the maintenance of blood glucose and the insulin response[32].

***In vitro* hydrolysis rate of cowpea starches:** *In vitro* starch hydrolysis rates of cowpea starches are shown in Fig. 1. The hydrolysis of cowpea starches and white bread increased with digestion time. Cowpea starches showed a lower starch hydrolysis rate than white bread at all times. The starch

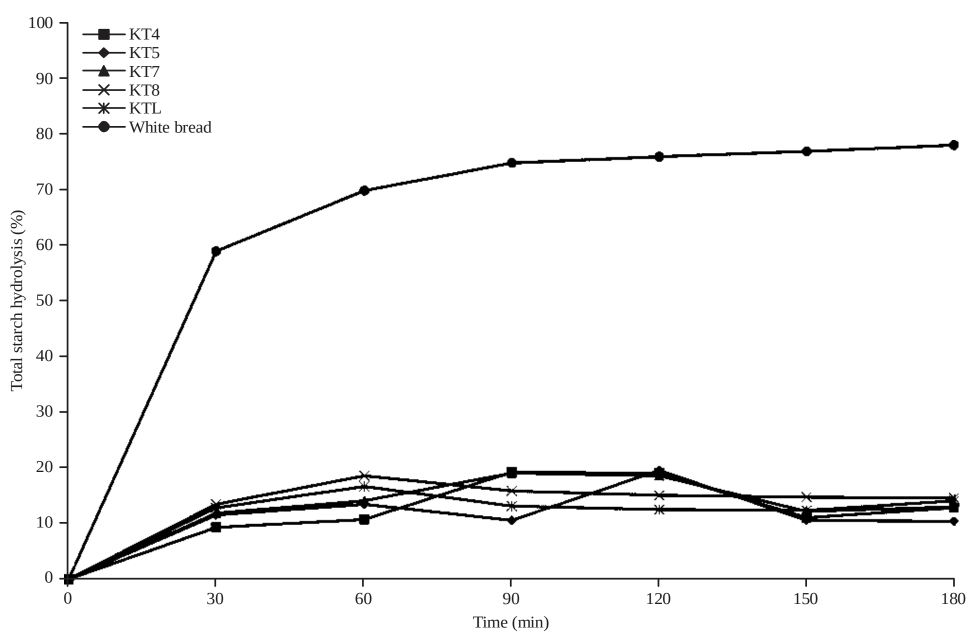

Fig. 1: *In vitro* starch digestibility of cowpea starches from different cowpea varieties. Values are the mean of two replicates

hydrolysis rate of cowpea starches was similar to that of raw mung bean starch[24] but lower than faba bean, black bean, pinto bean starches[9] and pea starches[18]. Hoover *et al.*[5] reported that native legume starches were more digestible than potato or high amylose corn starch but less digestible than cereal starches. The lower digestibility of native legume starches has been attributed to the absence of pores on the granule surface, the high content of amylose, B-type crystallites and strong interactions between amylose chains[5]. The high content of amylose and RS, C_A-type crystallinity, smooth surface of the granules and mean granule diameter (Z average, 7.91-15.51 μm) of cowpea starches have been associated with reduced susceptibility to enzymatic hydrolysis[8]. Differences in the digestibility of native starches among species have been attributed to the interplay of multiple factors, such as the starch source, granule surface organization (e.g., pores), granule size and architecture, amylose/amylopectin ratio, retrogradation of amylose, amylose-lipid complexes, amylose chain length, molecular structures of amylopectin, degree of crystallinity, type of crystalline polymorphic forms (A, B or C), the amount of B-type crystallites in C-type starches and the presence of compound granules[5,18].

Estimated glycemic index (eGI) of cowpea starches: The kinetic constant, HI and eGI of cowpea starches are shown in Table 2. The kinetic constant of the starch hydrolysis of cowpea starches varied from 0.0261±0.0020 to 0.0328±0.0002, which was higher than those reported by Goni *et al.*[21]. The HI represents the digestibility of starch in foods in relation to the digestibility of starch in a reference food, namely, white bread[13]. The HI of cowpea starches varied significantly from 10.47±0.42% (KT5) to 15.36±0.69% (KT8). These values were in the range reported by Kaur *et al.*[27] for lentil starch and by Kaur *et al.*[24] for mung bean starch but much lower than bean starch[23] and oat starch[33].

The eGI of the cowpea starches varied significantly from 45.46±0.23 (KT5) to 48.14±0.38 (KT8). These values were similar to those reported by Sandhu and Lim[13] and Kaur *et al.*[27,24] but much lower than those reported by Chung *et al.*[23]. Foster-Powell *et al.*[11] classified the glycemic index of foods as follows: Low (GI<55), medium (GI 56-69) and high (GI>70). According to this classification, all of the cowpea starches were categorized as low GI. The low GI of cowpea starches has been attributed to the high content of amylose and RS, C_A-type crystallinity, the smooth surface of the starch granules and the strong interactions between amylose chains. The reported health benefits of low-GI starchy foods include improved blood glucose control, reduced insulin demand, reduced blood lipid levels in healthy adults and patients with diabetes and hypertriglyceridaemia, improved satiety and increased colonic fermentation[34]. Therefore, consumption of low-GI foods could play an important role in the management and prevention of several degenerative diseases, such as obesity and diabetes.

Table 2: Kinetic constant (k), calculated hydrolysis index (HI) and estimated glycemic index (eGI) of cowpea starches

Cowpea varieties	k	Calculated HI	Estimated GI
KT4	0.0328±0.0002[a]	13.74±0.43[b]	47.25±0.23[b]
KT5	0.0261±0.0020[c]	10.47±0.42[c]	45.46±0.23[c]
KT7	0.0328±0.0005[a]	13.81±0.90[ab]	47.29±0.49[ab]
KT8	0.0308±0.0006[ab]	15.36±0.69[a]	48.14±0.38[a]
KTL	0.0286±0.0004[bc]	14.62±0.34[ab]	47.74±0.19[ab]

Values are Means±Standard Deviations and different superscripts in the same column are significantly different (p<0.05)

Table 3: Pearson correlation among starch digestible fraction, available glucose fraction and estimated glycemic index of cowpea starches

Parameters	RDS	SDS	RS	RAG	SAG
SDS	0.337				
RS	-0.689*	-0.914**			
RAG	0.996**	0.331	-0.683*		
SAG	0.321	0.984**	-0.895**	0.330	
eGI	0.480	0.797**	-0.819**	0.456	0.768**

*,**Significant correlation at p<0.05 and p<0.01, respectively, SDS: Slowly digestible starch, RS: Resistant starch, RAG: Rapid available glucose, SAG: Slowly available glucose, eGI: Estimated glycemic index

Correlation between the starch digestible fraction, available glucose fraction and estimated glycemic index: The correlations among the starch digestible fraction, available glucose fraction and eGI of cowpea starches are shown in Table 3. The RDS and SDS were negatively correlated with RS (r = -0.689 (p<0.05) and r = -0.914 (p<0.01), respectively). The RDS was positively correlated with RAG (r = 0.996, p<0.01), where RAG increased as RDS increased. SDS was positively correlated with SAG and eGI (r = 0.984 and 0.797 (p<0.01), respectively). The RS was negatively correlated with RAG, SAG and eGI (r = -0.683 (p<0.05), r = -0.895 and -0.819 (p<0.01), respectively). A higher RS content in cowpea starches was associated with lower RAG, SAG and eGI. The SAG was positively correlated with eGI (r = 0.768, p<0.01). These results were in agreement with previous studies of Sandhu and Lim[13] and Nayak *et al.*[34].

CONCLUSION

The *in vitro* starch digestibility and eGI varied widely among the five species of Indonesian cowpea. All cowpea starches had low RDS, RAG, HI and eGI and high SDS, RS and SAG. The variety KT5 had the highest RS and the lowest eGI, which are the ideal characteristics. Correlation analysis of cowpeas starch digestibility and its GI indicated a strong negative relationship between the RS content and RAG, SAG and eGI. This study suggested that because of its low GI and other characteristics, cowpea starch is strongly recommended for use in functional food formulations for the prevention of several diseases, such as obesity, diabetes, cardiovascular diseases and some types of cancer.

SIGNIFICANCE STATEMENTS

- *In vitro* starch digestibility and estimated glycemic index (eGI) of cowpea starches were evaluated
- Cowpea starch had low Rapidly Digestible Starch (RDS) and Rapidly Available Glucose (RAG) contents but high Slowly Digestible Starch (SDS), resistant starch and Slowly Available Glucose (SAG) contents, thus, it had low GI
- The low GI of cowpea starch is strongly recommended as a functional ingredient to develop a new functional food for the prevention of several degenerative diseases, such as obesity and diabetes

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REFERENCES

1. Akibode, S. and M. Maredia, 2011. Global and regional trends in production, trade and consumption of food legume crops. Michigan State University, Michigan, March 27, 2011.
2. Guzel, D. and S. Sayar, 2010. Digestion profiles and some physicochemical properties of native and modified borlotti bean, chickpea and white kidney bean starches. Food Res. Int., 43: 2132-2137.
3. Li, W., X. Xiao, S. Guo, S. Ouyang, Q. Luo, J. Zheng and G. Zhang, 2014. Proximate composition of triangular pea, white pea, spotted colored pea and small white kidney bean and their starch properties. Food Bioprocess Technol., 7: 1078-1087.
4. Guzel, D. and S. Sayar, 2012. Effect of cooking methods on selected physicochemical and nutritional properties of barlotto bean, chickpea, faba bean and white kidney bean. J. Food Sci. Technol., 49: 89-95.
5. Hoover, R., T. Hughes, H.J. Chung and Q. Liu, 2010. Composition, molecular structure, properties and modification of pulse starches: A review. Food Res. Int., 43: 399-413.
6. Huang, J., H.A. Schols, J.J.G. van Soest, Z. Jin, E. Sulmann and A.G.J. Voragen, 2007. Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. Food Chem., 101: 1338-1345.
7. Ashogbon, A.O. and E.T. Akintayo, 2013. Isolation and characterization of starches from two cowpea (*Vigna unguiculata*) cultivars. Int. Food Res. J., 20: 3093-3100.
8. Ratnaningsih, N., Suparmo, E. Harmayani and Y. Marsono, 2016. Composition, microstructure and physicochemical properties of starches from Indonesian cowpea (*Vigna unguiculata*) varieties. Int. Food Res. J., 23: 2041-2049.
9. Ambigaipalan, P., R. Hoover, E. Donner and Q. Liu, 2014. Starch chain interactions within the amorphous and crystalline domains of pulse starches during heat-moisture treatment at different temperatures and their impact on physicochemical properties. Food Chem., 143: 175-184.
10. Jenkins, A.L., 2007. The glycemic index: Looking back 25 years. Cereal Foods World, 52: 50-53.
11. Foster-Powell, K., S.H.A. Holt and J.C. Brand-Miller, 2002. International table of glycemic index and glycemic load values. Am. J. Clin. Nutr., 76: 5-56.
12. Simsek, S. and S. Nehir El, 2015. *In vitro* starch digestibility, estimated glycemic index and antioxidant potential of taro (*Colocasia esculenta* L. Schott) corm. Food Chem., 168: 257-261.
13. Sandhu, K.S. and S.T. Lim, 2008. Digestibility of legume starches as influenced by their physical and structural properties. Carbohydr. Polym., 71: 245-252.
14. Du, S.K., H. Jiang, Y. Ai and J.L. Jane, 2014. Physicochemical properties and digestibility of common bean (*Phaseolus vulgaris* L.) starches. Carbohydr. Polym., 108: 200-205.
15. Adebooye, O.C. and V. Singh, 2008. Physico-chemical properties of the flours and starches of two cowpea varieties (*Vigna unguiculata* (L.) Walp). Innovative Food Sci. Emerg. Technol., 9: 92-100.
16. Chung, H., S. Cho, J. Chung, T. Shin, H.S. Son and S.T. Lim, 1998. Physical and molecular characteristics of cowpea and acorn starches in comparison with corn and potato starches. Food Sci. Biotechnol., 7: 269-275.
17. Marsono, Y., P. Wiyono and N. Zuheid, 2002. Indeks glisemik kacang-kacangan. J. Teknol. Indust. Pangan, 13: 211-216.
18. Liu, C., S. Wang, L. Copeland and S. Wang, 2015. Physicochemical properties and *in vitro* digestibility of starches from field peas grown in China. LWT-Food Sci. Technol., 64: 829-836.
19. Englyst, H.N., S.M. Kingman and J.H. Cummings, 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr., 46: S33-S50.
20. Chung, H.J., Q. Liu and R. Hoover, 2009. Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. Carbohydr. Polym., 75: 436-447.

21. Goni, I., A. Garcia-Alonso and F. Saura-Calixto, 1997. A starch hydrolysis procedure to estimate glycemic index. Nutr. Res., 17: 427-437.
22. Sajilata, M.G., R.S. Singhal and P.R. Kulkarni, 2006. Resistant starch: A review. Compreh. Rev. Food Sci. Food Saf., 5: 1-17.
23. Chung, H.J., Q. Liu, K.P. Pauls, M.Z. Fan and R. Yada, 2008. *In vitro* starch digestibility, expected glycemic index and some physicochemical properties of starch and flour from common bean (*Phaseolus vulgaris* L.) varieties grown in Canada. Food Res. Int., 41: 869-875.
24. Kaur, M., K.S. Sandhu, R. Ahlawat and S. Sharma, 2015. *In vitro* starch digestibility, pasting and textural properties of mung bean: Effect of different processing methods. J. Food Sci. Technol., 52: 1642-1648.
25. Hughes, T., R. Hoover, Q. Liu, E. Donner, R. Chibbar and S. Jaiswal, 2009. Composition, morphology, molecular structure and physicochemical properties of starches from newly released chickpea (*Cicer arietinum* L.) cultivars grown in Canada. Food Res. Int., 42: 627-635.
26. Ambigaipalan, P., R. Hoover, E. Donner, Q. Liu and S. Jaiswal *et al*., 2011. Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. Food Res. Int., 44: 2962-2974.
27. Kaur, M., K.S. Sandhu and S.T. Limc, 2010. Microstructure, physicochemical properties and *in vitro* digestibility of starches from different Indian lentil (*Lens culinaris*) cultivars. Carbohydrate Polym., 79: 349-355.
28. Goni, I., L. Garcia-Diz, E. Manas and F. Saura-Calixto, 1996. Analysis of resistant starch: A method for foods and food products. Food Chem., 56: 445-449.
29. Dupuis, J.H., Q. Liu and R.Y. Yada, 2014. Methodologies for increasing the resistant starch content of food starches: A review. Compreh. Rev. Food Sci. Food Saf., 13: 1219-1234.
30. Kingman, S.M. and H.N. Englyst, 1994. The influence of food preparation methods on the *in vitro* digestibility of starch in potatoes. Food Chem., 49: 181-186.
31. Englyst, K.N. H.N. Englyst, G.J. Hudson, T.J. Cole and J.H. Cummings, 1999. Rapidly available glucose in foods: An *in vitro* measurement that reflects the glycemic response. Am. J. Clin. Nutr., 69: 448-454.
32. Englyst, K.N., S. Vinoy, H.N. Englyst and V. Lang, 2003. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. Br. J. Nutr., 89: 329-339.
33. Kim, H.J. and P.J. White, 2012. *In vitro* digestion rate and estimated glycemic index of oat flours from typical and high β-glucan oat lines. J. Agric. Food Chem., 60: 5237-5242.
34. Nayak, B., J.D.J. Berrios and J. Tang, 2014. Impact of food processing on the glycemic index (GI) of potato products. Food Res. Int., 56: 35-46.