The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2017), 41(2), 31-41

ORIGINAL RESEARCH PAPER

THE EFFECT OF PROCESSING ON IN VITRO PROTEIN AND STARCH DIGESTIBILITY AND PREDICTIVE GLYCAEMIC INDEX OF FIVE *Vigna unguiculata* (COWPEA) CULTIVARS

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Received on 13th June 2017
Revised on 19th July 2017

In this study, the effects of thermal processing on in vitro starch and protein digestibility in five different *Vigna unguiculata* cultivars were evaluated along with the predicted glycaemic index of each cultivar in its raw and processed form. Starch analysis was divided into total starch (TS), rapidly digestible starch (RDS) and resistant starch (RS). Samples were also analysed from a nutritional perspective and their correlations evaluated. Results showed that thermal processing reduced nutrient contents while increasing the protein digestibility. A significant increase was found in the RDS of processed samples (6.21 - 6.75%) in comparison to the raw samples (1.89 - 2.18%). Low glycaemic indices were also obtained in the raw samples and remained within the category of a low glycaemic index food even after thermal processing, suggesting cowpea could elicit low postprandial increases in blood glucose levels subsequent to processing.

Keywords: cowpea, glycaemic index, protein digestibility, *Vigna unguiculata*

Introduction

Vigna unguiculata commonly referred to as cowpea is a widely adopted, stress tolerant grain legume (Ehlers and Hall, 1997). The use of cowpea as human food dates back to early agricultural practices for cultural, medicinal and nutritional purposes. However, it has also been utilized as fodder for animals. In developing countries, starchy legumes (pulses) such as cowpea are convenient sources of carbohydrates, protein, minerals and vitamins. Pulses are known to contain protein concentrations of approximately 20 to 30% depending on the legume and cultivar (Phillips *et al.*, 2003). According to Timko *et al.*, (2007), cowpea has a nutritional profile similar to that of other pulses e.g. chickpea with protein content two to four times greater than that of cereal and tuber crops. Cowpea is also known to be rich in the amino acids lysine and tryptophan (Afiukwa *et al.*, 2013) and have carbohydrate contents that range from 50 to 67% and fat contents of approximately

1.3%. Cowpea has also been found to contain significant amounts of vitamins such as folate, thiamin and riboflavin (Sasanam *et al.*, 2011). These are important factors to consider when choosing foods with beneficial properties for human health.

The demand for healthier foods such as those with a low glycaemic index (GI) and good quality proteins is on the rise due to the prevalence of nutrition related health diseases such as hypertension, cancer and diabetes to mention but a few. Scientists are constantly searching for new and alternative food sources to solve these health problems. Recently, there is a renewed interest in starchy legumes (pulses), specifically those that are underutilized for their slowly digestible starch and potential as low GI foods as well as for their high protein contents. Legumes in general are an important source of carbohydrates and protein because their starch is relatively more slowly digestible than those from other sources (Phillips *et al.*, 2003) and their proteins are more easily digested than those of animal origin. A diet lacking in nutrients such as carbohydrates and protein can have adverse effects on one's health. Similarly, a diet consisting only of these two nutrients can lead to malnutrition, a common problem in developing countries that lack variety in the foods they consume.

Starch digestibility as indicated by the GI, varies among starchy foods due to various factors such as botanical origin and amylose-amylopectin ratio. In terms of health, a slowly digestible starch is beneficial in controlling metabolic health disorders such as Type 2 Diabetes. Apart from metabolic diseases, legumes may also assist in maintaining or preventing other chronic health diseases such as cardiovascular disease and certain cancers (Hefnawy, 2011). Protein digestibility of legumes also plays an important role when choosing healthy foods because it is just one of the many indicators of protein quality. A highly digestible protein could indicate good quality proteins and vice versa, but carbohydrates and proteins as well as other nutritional components in legumes are affected by traditional processing.

Cooking of cowpea is usually done for improving their sensory appeal e.g. flavour and palatability but its effects on their nutritional quality are rarely considered (Deol and Bains, 2010). The method of processing legumes varies for different populations around the world due to preferences and tradition. Boiling, pressures cooking, frying or simply grinding into flour to make other products are some of the common methods of processing. This can affect the type and amount of nutrients available for consumption due to the alteration of nutritional constituents in legumes from processing, particularly those processes involving high temperatures, resulting in possible alteration of starch and protein digestibility. During heat processing, proteins are denatured whilst starch undergoes processes such as gelatinization and retrogradation, which thus alters their digestibility. Proteins and other nutritive and non-nutritive components may also influence starch digestibility (Deol and Bains, 2010). Therefore, the aim of this study was to determine the effect of processing on the *in vitro* protein and starch digestibility as well as on the predictive glycaemic index of five cowpea cultivars.

Materials and methods

Sample Preparation

Five cowpea cultivars (Glenda, Embu buff, Makhathini, Veg Cowpea 2 and Veg Cowpea 3) were obtained from the Agricultural Research Council-Vegetable and Ornamental Plant Institute (ARC-VOPI), Pretoria, South Africa. The samples were cultivated at the Research Farm of ARC-VOPI [25.604S 28.345E], during the 2014/2015 cropping seasons at an altitude of 1168 m above sea level. This location received approximately 610 mm of rain during the growing period with a minimum and maximum recorded temperature of 9.11°C and 36.37°C respectively during the growth period. The legume samples were carefully inspected and any damaged or infected sample was discarded. Appropriate legumes were hand cleaned and stored in polyethelyene bags until further use. Legumes were soaked in distilled water in a 1:10 w/v ratio for 24 hours at room temperature (25°C) prior to processing. For boiled samples, the soaked grains were cooked in water on a stove (100°C for 30 min) at a cowpea:water ratio of 1:20 w/v. For pressure-cooked samples, sufficient water was used to cover grains in a vertical autoclave at 121°C for 15 min at a cowpea:water ratio of 1:5 w/v. Processed samples were drained of excess water, dried in a 60°C convection oven to achieve constant weight, ground to pass through a 60 mesh sieve and subsequently stored in the dark until use.

Proximate composition

Analyses of the proximate composition of samples were carried out using approved standard AOAC (2000) methods. Processed cowpea cultivars were analysed for protein (conversion factor - 6.25), fat, ash, moisture and carbohydrates, with processed samples compared to the raw flour for significance.

In vitro protein digestibility

In vitro protein digestibility was carried out according to the method of Saunders *et al.* (1973). Each cowpea sample (0.2 g) was weighed into a 50 mL centrifuge tube and 35 mL of 0.1 M sodium phosphate buffer (pH 7) containing 1.5 mg/mL of pepsin (Sigma Aldrich) was added to the tube and incubated for 3 hours at 37°C. Following incubation, the sample was neutralized with 2 mL of 2 M NaOH. The samples were centrifuged for 20 min at 4800 rpm at 4°C. After discarding the supernatant, 15 mL of a 0.1 M sodium phosphate buffer pH 7.0 was added and the sample was centrifuged under the same conditions as before. The supernatant was discarded and the sample was washed with 15 mL of the buffer and filtered using Whatman number 3 filter paper. Supernatants were then pooled and the Kjeldahl method applied to determine the nitrogen content of supernatant. The filter paper was left to dry in protein digestion tubes at 80°C for 2 hours prior to protein determination as per the Kjeldahl method for sample residue. Protein digestibility was calculated according to the following equation by Ali *et al.* (2003):

$$\text{Protein digestibility}\ (\%) = \frac{\text{Nitrogen in the supernatant}}{(\text{Nitrogen in the sample})} \times 100 \qquad (1)$$

Total starch (TS)

Total starch was determined according to the modified method of Goñi *et al.* (1997). Raw and processed samples of each cultivar were ground to pass through a 0.5 mm sieve of which, 25 - 35 mg of ground samples were dispersed in 6 mL of a 2M KOH solution and vigorously mixed for 30 min at room temperature. Thereafter, 60 µl of amyloglucosidase (Sigma Aldrich) was added to hydrolyze the solubilized starch and the samples were incubated at 60°C for 45 min in a shaking water bath. Samples underwent then centrifugation at 3000 x g for 10 min and a glucose oxidase-peroxidase kit (Sigma Aldrich) was used to measure the glucose concentration in the supernatant. Colour absorption was measured at a wavelength of 540 nm, using a factor of 0.9. The glucose concentration was converted to starch.

In vitro starch hydrolysis

In vitro starch digestibility was analyzed according to the procedure by Englyst *et al.* (1999) where 600 mg cowpea sample and 10 mL water were added into 50 mL centrifuge tubes. The tubes were then capped and mixed by vortexing for 5 min and placed in a boiling water bath for 30 min during which they were vortexed at 5 min intervals to prevent agglomeration. Following this, 10 mL of a 0.1 M sodium acetate buffer (pH 5.2) and five glass beads were added to each tube. The dispersion was equilibrated horizontally in a shaking water bath (160 strokes per min) at 37°C for 30 min. Pancreatin (Sigma Aldrich) and amyloglucosidase (Sigma Aldrich) in 5 mL sodium acetate buffer were added to each tube after which 1-mL aliquots were taken at 0, 20, 60 and 120 min intervals, and mixed with 10 mL of 80% ethanol. The glucose concentration was determined using a glucose oxidase peroxidase kit against white bread as the control. Using total starch (TS), the different fractions of starch i.e. rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were calculated according to the method by Güzel and Sayar (2010) using G20 (after 20 min incubation) and G120 (after 120 min incubation):

$$\mathrm{RDS} = \mathrm{G}_{20} \times 0.9 \quad (2)$$

$$\mathrm{SDS} = [\mathrm{G}_{120} - \mathrm{G}_{20}] \times 0.9 \quad (3)$$

$$\mathrm{RS} = \mathrm{TS} - [\mathrm{SDS} + \mathrm{RDS}] \quad (4)$$

Estimation of glycaemic index

The glycaemic index of the cultivars was estimated according to the method by Odenigbo *et al.* (2012), where the 60 min hydrolysis (H60) was used in the calculation of the predicted glycemic index [GI= 39.21 + 0.803 (H_{60})].

Statistical analysis

All experimental data is represented as the mean ± standard deviation (SD) of three replicates. Experimental data was analyzed using the two-way analysis of variance (ANOVA) and Tukey's multiple comparison tests of means where a level of $p \leq$

0.05 was considered significant. Statistical computations and analyses were carried out using GraphPad Prism.

Results and discussion

Legumes are processed by several techniques such as boiling, pressure cooking and frying which are known to affect nutritional components such as starch, protein, vitamins and minerals. In this study, the raw cowpea samples displayed similar nutritional compositions to those reported by Sasanam *et al.* (2011) who compared varieties of cowpea cultivars to that of red kidney bean. According to their study, the cultivars had moisture contents of between 7 to 9%, which is closely related to the moisture contents obtained from the unprocessed cowpea cultivars in this study that were approximately 9% (Table 1).

Table 1. Proximate composition of raw and processed (boiled/pressure cooked) *Vigna unguiculata* cultivars (on dry weight basis/100 g)

Cultivar	Treatment	Moisture, (%)	Ash, (%)	Fat, (%)	Protein, (%)	Carbohydrates*, (%)
Glenda	R	9.05 ± 0.07^{d}	3.10 ± 0.22^{d}	1.80 ± 0.07^{d}	24.80 ± 0.30	61.25 ± 0.00^{d}
	B	46.00 ± 0.00^{d}	0.91 ± 0.00^{d}	1.12 ± 0.07^{d}	20.10 ± 0.36^{a}	32.37 ± 0.00^{d}
	PC	46.00 ± 0.00	0.97 ± 0.00	0.99 ± 0.00	22.80 ± 0.17	29.24 ± 0.00^{d}
Embu buff	R	9.10 ± 0.00^{d}	3.72 ± 0.01^{c}	3.25 ± 0.07^{d}	26.26 ± 0.50	57.67 ± 0.00^{d}
	B	41.00 ± 0.00^{d}	0.74 ± 0.02^{d}	0.81 ± 0.00^{d}	20.36 ± 0.25^{b}	31.09 ± 0.00^{d}
	PC	45.50 ± 0.20^{d}	3.34 ± 0.00^{d}	1.14 ± 0.02^{a}	23.66 ± 0.20	26.36 ± 0.00^{d}
Makhatini	R	9.00 ± 0.00^{d}	3.55 ± 0.00^{d}	2.11 ± 0.20^{d}	26.36 ± 0.20	58.98 ± 0.00^{d}
	B	46.00 ± 0.00^{d}	0.96 ± 0.07^{d}	1.20 ±0.04^{c}	20.63 ± 0.20^{b}	31.21 ± 0.00^{d}
	PC	44.00 ± 0.00^{d}	1.06 ± 0.00	1.08 ± 0.15	20.06 ± 0.83^{a}	33.80 ± 0.00^{d}
Veg Cowpea 2	R	9.00 ± 0.00^{d}	1.25 ± 0.07	1.25 ± 0.20	26.36 ± 0.20	62.14 ± 0.00^{d}
	B	52.50 ± 0.70^{d}	1.05 ± 0.07^{a}	0.95 ± 0.04^{a}	21.46 ± 0.41^{a}	24.04 ± 0.00^{d}
	PC	46.00 ± 0.00^{d}	1.15 ± 0.03	1.37 ± 0.19^{b}	22.66 ± 0.46	28.82 ± 0.00^{d}
Veg Cowpea 3	R	9.05 ± 0.07^{d}	3.43 ± 0.00^{d}	1.51 ± 0.02	26.96 ± 0.20	59.05 ± 0.00^{d}
	B	50.00 ± 0.00^{d}	1.02 ± 0.01^{d}	1.02 ± 0.12^{c}	21.76 ± 0.15^{a}	26.20 ± 0.00^{d}
	PC	46.00 ± 0.00^{d}	1.04 ± 0.07	1.56 ± 0.04^{c}	23.23 ± 0.28	28.17 ± 0.00^{d}

All data is expressed as mean± SEM (n=3) data with different superscripts are significantly different at $p < 0.05$. * Carbohydrate content calculated by difference [R - raw untreated sample; B - boiled and PC - pressure cooked]

The ash content was slightly lower than 4% obtained in literature, but was nevertheless around the expected 3% ash content usually found in cowpea (Sasanam *et al.*, 2011). This may be attributed to the fact that cowpea is significantly richer in minerals such potassium, calcium and sodium (Khalid and Elharadallou, 2016). Depending on botanical source and cultivar, ash contents have been known to have slight variations. In a study by Sasanam *et al.* (2011), fat contents of up to 4% were recorded. These were slightly different to those obtained in the five cultivars studied where fat content ranged between 1.5 and 3.3%. The low-fat content offers the advantage of not having to remove the fat for processes requiring the use of defatted cowpea flour. Protein and carbohydrate contents are known as two important variables that govern the nutritive and processing quality of cowpea (Henshaw, 2008). The protein component of each of the five cultivars closely resembled the cultivars studied by Henshaw (2008). Of the 28 varieties screened by Henshaw (2008), each one produced protein contents above 20% with the highest protein content being 27%. In this study, protein contents in all five cultivars were above 24%, which is in alignment with other studies, which stated that cowpea has comparable if not higher protein contents than the more common legumes. In a study by Atuobi *et al.* (2011) it was also stated that cowpea is rich in essential amino acids such as lysine and tryptophan. Cowpea being known for its beneficial starchy components exhibited carbohydrate contents around 61% which was in agreement to those found in black, white and red cowpea varieties as reported by Sasanam *et al.* (2011) that had carbohydrates contents of up to 69%.

As previously mentioned, slight variations were expected to occur as a result of the difference in cultivars under investigation. In this study, no significant difference was found between the cultivars, which displayed similar nutritional compositions with respect to each other, and those previously studied. Both processing treatments resulted in an increase in moisture but a decrease in the other nutritional parameters.

The ash content in this study was seen to decrease after processing with a similar trend observed by Adegunwa *et al.* (2012). A possible reason for this decrease may be the leaching out of water-soluble minerals and vitamins into the processing medium (Deol and Bains, 2010). Higher values were obtained from pressure-cooking showing that this could be the preferred technique for processing legumes compared to boiling in order to retain important nutrients such as vitamins and minerals to a greater extent than boiling. This is in agreement with a study by Adegunwa *et al.* (2012) that found processing methods such as boiling to cause the leaching of soluble nutrients into water used in the process that decreased ash content and other nutritive parameters. Taiwo (1998) also noted the depletion of the vitamins riboflavin and thiamine as well as oligosaccharides when cowpea is thermally processed. Hefnawy (2011) compared the effects of different processing techniques on common legumes and found that boiling and pressure-cooking as opposed to the use of a microwave witnessed significant mineral losses. Other nutritional parameters such as fat and carbohydrates were also found to decrease after processing. The carbohydrate content is comparable to those in literature of approximately 50%, as observed by Rehman and Shah (2005). Processing of

cowpea resulted in an increase in the moisture content from 9% in raw samples to approximately 40%. This sharp increase is similar to that of Oboh *et al.* (2010) where the average moisture content of 40-51% was obtained after boiling legumes under similar conditions as the methods used in this study. This result was also correlated to a study by Taiwo (1998), where the moisture content increased upon moist heat processing to 50 %. This may be attributed to the fact that processing involves the use of substantial quantities of water, which is partially absorbed by the seeds. Seed coat texture is said to play an important role in water absorption with smooth textured seed coats absorbing less water than those that are wrinkled. The destruction of the seed coat may also explain the increase in moisture content after processing (Taiwo, 1998).

The *in vitro* protein digestibility of cowpea was found to significantly increase after pressure cooking and boiling (Table 2). Glenda had the lowest digestibility of 52.10% in the raw state. However, after boiling, Embu buff had the lowest protein digestibility of 57.85%. Pressure-cooked cultivars were found to have a higher *in vitro* protein digestibility value than those obtained from boiling. Vegetable cowpea 3 produced the highest protein digestibility (71.00%) after pressure-cooking. The seed coat of cowpea is known to contain antinutrient components which, when reduced upon processing, were found to increase protein digestibility and quality (Deol and Bains, 2010). All of the five cultivars in the raw form had a lower protein digestibility as opposed to processed samples. Since the samples were not subjected to thermal heat processing, the presence of antinutrients resulted in low protein digestibility for each cultivar in its raw form. In a study by Tuan and Phillips (1991), it was observed that processing of cowpea increased their protein digestibility as compared to raw flour that was used as reference with a similar trend observed in both processing techniques in this study. Although processing improved protein digestibility, boiling showed lower protein digestibility values than pressure-cooked cowpea. Soaking the cowpea seed prior to boiling and pressure-cooking is often conducted to aid in preparation and digestibility of cowpea. It is also known to further promote the loss of antinutrient factors into the soaking medium, which in turn improves the legume digestibility (Taiwo, 1998). This can also explain why the protein digestibility of boiled and pressure-cooked cowpea improved as soaking of the grains prior to processing was conducted. The protein digestibility of raw cowpea ranged from 52.1 (Glenda) to 60.8 (Vegetable cowpea 3). Boiling and pressure-cooking increased the digestibility of proteins in all five cultivars by reducing the content of antinutrients present in the seed coat. The increases in protein digestibility after processing may be attributed to the leaching out of antinutrients, namely tannins which is water-soluble polyphenol as reported by Khalid and Elharadallou (2016) and is mainly found in the seed coat or testa. The quality of cowpea protein is lower than that of animal origin due to the limiting amino acids composition. Both boiling and pressure-cooking increased their digestibility, which is expected to subsequently improve quality. Protein quality increases to a maximum, primarily due to the loss of antinutrients (Deol and Bains, 2010).

Table 2. *In vitro* protein digestibility of raw and processed *Vigna unguiculata* cultivars (on dry weight basis)

Treatment	Glenda	Embu buff	Makhatini	Vegetable Cowpea 2	Vegetable Cowpea 3
R	52.10 ± 0.35^{d}	52.70 ± 1.41^{d}	54.50 ± 0.35^{d}	52.80 ± 0.00^{d}	60.80 ± 0.77^{d}
B	62.12 ± 0.77^{d}	57.85 ± 0.94^{d}	59.16 ± 0.80^{d}	66.43 ± 0.01^{d}	67.93 ± 0.36^{d}
PC	67.60 ± 0.72^{d}	66.94 ± 0.53^{d}	69.41 ± 0.83^{d}	70.00 ± 0.00^{c}	71.00 ± 0.00^{c}

All data is expressed as mean± SEM (n=3), data with different superscripts are significantly different at $p < 0.05$. [R - raw untreated sample; B - boiled and PC - pressure cooked]

An *in vitro* method that estimates the potential rate of starch digestion and glucose absorption in the small intestine was used to determine the GI value of the cowpea samples (Lai *et al.*, 2016). As depicted in Figure 1., the curves exhibit different kinetics of starch hydrolysis between the various cowpea cultivars. The percentage of starch hydrolysis at 120 min in the white bread sample, which was used as reference, reached 76.3%. This result is similar to findings in studies by Goñi *et al.* (1997) and Germaine *et al.* (2008) who looked at starch digestibility and GI of various grain foods. Although Figure 1 shows a linear model with starch hydrolysis, critical analysis indicates that while the white bread exhibited a monophasic digestogram, biphasic digestograms were obtained from the cowpea samples which may be attributed to interactions between the starch and non-starch components that are well-known in legumes (cf. hard-to-cook phenomenon) (Liu and Sopade, 2011).

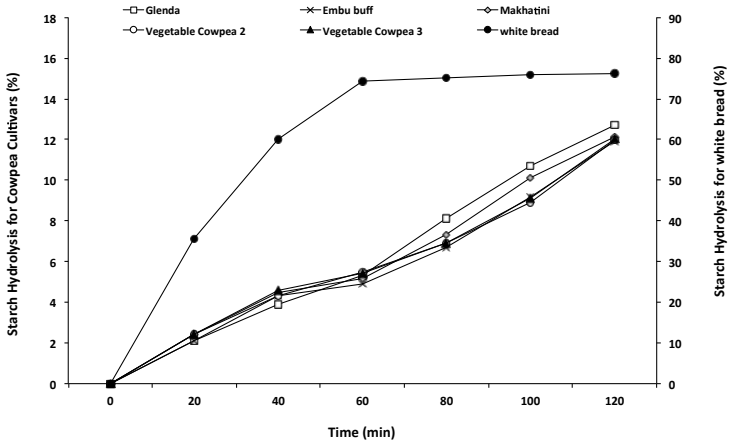

Figure 1. *In vitro* starch hydrolysis curve for five *Vigna unguiculata* cultivars with white bread as positive control

Processing by both methods resulted in the decrease of total starch as outlined in Table 3 showing initial starch concentrations ranging from 21.00 – 23.21% decreasing to 18.22 – 19.63%, which may be attributed to the solubilisation of

soluble starch into the processing medium. The differences in the percentage of starch hydrolyzed amongst cultivars may be as a result of the variation in granule size, which in turn gave rise to different degrees of degradation of starch during the cooking process. The heat treatment from the two processing methods caused starch granules to rupture, which reduces polyphenols, and amylase inhibitors thereby facilitating starch hydrolysis (Isiosio *et al.*, 2015). Rapidly digestible starch (RDS) was lower than the resistant starch (RS) contents in all five cultivars. The total starch contents decreased in all five cultivars whilst RDS increased and RS decreased respectively. The predicted glycaemic index (GI) of raw cowpea cultivars ranged from 43.14% (Embu buff) to 43.62% (Vegetable cowpea 2). Both processing techniques increased the glycaemic index of all the cultivars. Pressure-cooking had lower GI values than boiling, however Embu buff had the same estimated glycaemic index after both processing techniques.

Table 3. Total starch (TS), rapidly digestible starch (RDS), resistant starch (RS) and the predicted glycaemic index for raw and processed *Vigna unguiculata* cultivars **(**on dry weight basis)

Cultivar	Treatment	TS (%)	RDS (%)	RS (%)	Predicted Glycemic Index
Glenda	R	23.21	1.89	9.64	43.60
	B	18.22	6.39	3.26	50.45
	PC	19.31	6.21	5.47	49.88
Embu buff	R	21.00	1.89	9.25	43.14
	B	18.50	6.57	3.20	50.29
	PC	19.23	6.23	5.07	50.29
Makhatini	R	23.19	2.18	11.06	43.36
	B	18.32	7.11	3.87	50.54
	PC	19.10	6.30	4.70	50.39
Veg Cowpea 2	R	22.12	2.16	11.29	43.62
	B	18.87	7.29	4.74	51.41
	PC	19.54	6.75	5.44	49.62
Veg Cowpea 3	R	23.00	2.17	10.98	43.57
	B	18.90	7.47	3.96	51.25
	PC	19.63	6.58	5.54	49.78

[R - raw untreated sample; B - boiled and PC - pressure cooked] means, duration of digestibility for starch in samples was 45 min

The glycaemic index of raw cowpea classifies cowpea as a low GI legume as they were all below 50%. Although both processing methods resulted in an increase in each cowpea glycaemic index, they still remained within the range of a low GI food of below 55%. Pressure-cooking had lower glycaemic indices than boiled with the exception of pressure-cooked Embu buff, which had the same estimated GI as when boiled. Processing as indicated by Rehman and Shah (2005) increases the percentage of rapidly digestible starch, which is responsible for the increase in the GI of legumes.

Conclusions

Time and temperature of processing are important factors that play a role in the rate and extent of starch degradation. This should therefore be controlled to yield maximum positive effects in order to obtain a low GI even after processing (Taiwo, 1998). The present *in vitro* method can be applied to predict the glycaemic response of legumes such as cowpea before and after processing.

Acknowledgments

The authors acknowledge the Agricultural Research Council-Vegetable and Ornamental Plant Institute (ARC-VOPI), Pretoria, South Africa for providing the cowpea samples and the National Research Foundation [grant number 93988] and ARC Consortium: Broadening the Food Base for support.

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