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Comparative Studies of the Effects of Five Indigenous Cowpea (*Vigna unguiculata)* Varieties on Enzymes Linked to Type 2 Diabetes and their Glycemic Indices

Ganiyu. Oboh, Gbenga P. Akerele*, Bukola C. Adedayo, Adedayo O. Ademiluyi

Functional Foods and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology, Akure, P.M.B. 704, Akure, 340001, Nigeria

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ABSTRACT

Studies have supported the consumption of cowpea as a low glycemic index (GI) diet especially in managing diabetes. This study investigated the glycemic indices of five varieties of cowpea (Kano white, Sokoto white, Drum, Ife brown and Oloyin varieties) commonly consumed in Nigeria. The starch and sugar contents showed Sokoto white variety had the lowest starch and sugar (2.5 g/100 g, 9.02 g/100 g; 15.58 g/100 g, 26.69 g/100 g) content for both the raw and cooked samples, respectively, while the glycemic index ranged from 69.01 (Drum variety) to 77.08 (Oloyin variety). Furthermore, the cowpea extracts showed high antioxidant properties as revealed by their ferric reducing property and their radical scavenging abilities. Also, the cowpea varieties showed their α-amylase and α-glucosidase inhibitory potentials with Kano white variety (IC_{50} = 0.33 mg/mL), showing the highest α-amylase inhibitory potential when raw and Ife brown (IC_{50} = 0.37 mg/mL) when cooked, while Sokoto white variety (IC_{50} = 0.48 mg/mL) the lowest when raw and Oloyin variety (IC_{50} = 0.83 mg/mL) when cooked. However, Drum variety had the highest α-glucosidase inhibitory activity (IC_{50} = 0.66 mg/mL) while Oloyin had the highest (IC_{50} = 0.33 mg/mL) when cooked with Sokoto white variety (IC_{50} = 0.93 mg/mL; IC_{50} = 0.70 mg/mL) having the lowest for both the raw and cooked samples respectively. The low glycemic index of Sokoto white variety, its antioxidant properties as well as its α-glucosidase and α-amylase inhibitory activities could make it the most suitable variety as a diet for management of type-2 diabetes.

Keywords: Cowpea varieties, α-amylase, α-glucosidase, Glycemic indices.

Introduction

Cowpea (*Vigna unguiculata*) is one of the most popular grain legumes in Africa as well as in some regions of America and Asia. It is often referred to as "black-eyed pea" due to its black- or brown-ringed hylum or beans in some parts of Africa.[1] It is a very important source of protein in the diets of many populations especially in developing countries.[2] The West Africa zone is a major production zone for cowpea, mainly in the dry savannah and semi-arid agro-ecological zones. The principal countries producing cowpea in West Africa are Nigeria, Niger, Senegal, Ghana, Mali, and Burkina Faso, Nigeria being a major producer, produces 2,099,000 metric tons on 5 million hectares of land.[3] Furthermore, Nigeria is the second highest consumer of cowpea in the world.[4] Though cowpea is grown all over Nigeria, it is however largely grown in the northern part of the country because of its savanna type of vegetation and height rainfall.[5] The advent of improved varieties with an eye on producing varieties that are high yielding, resistant to major pest and disease, maturing early and having an improved taste has been a threat to the continual consumption of local varieties, hence the need to preserve the local varieties with respect to their nutritional benefits as a diet for the management of type 2 diabetes. There are different varieties of cowpea depending on size, shape, and color of seed coat.

*Corresponding author. E mail: akereleg@gmail.com
Tel: +234-7030189434

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Cowpea known locally as "ewa" in South west Nigeria is known as one of the most important legumes in Nigeria with different varieties found in Nigeria food markets.[6] It is generally consumed as boiled seeds alone or in combination with other foods.[7] The paste from cowpea flour can also be fried (Akara) or steamed (Moinmoin).[8] Cowpea has been deemed an essential food in the diet of diabetics because of its low Glycemic Index (GI).[9]

Hyperglycemia is a condition characterized by an abnormal postprandial increase of blood glucose level. It has been linked to the onset of diabetes and associated oxidation- linked vascular complications.[10,11] Studies indicate that hyperglycemia triggers the generation of free radicals and oxidative stress in capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, and neuron cells in the peripheral nerves.[12,13] Oxidative stress can promote the development of complications of diabetes mellitus. A sudden rise in blood glucose levels, causing hyperglycemia, in diabetes patients, is a result of the hydrolysis of starch in the pancreas the enzyme α -amylase and the subsequent absorption of glucose in the small intestine by α -glucosidase. Hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood.[13] Consumption of high starch diet has been linked with increased risk of type-2 diabetes and therefore the improvement of postprandial hyperglycemic elevations could be a therapeutic approach in the management of diabetes.[14] α-amylase and α-glucosidase are two key enzymes involved in the hydrolysis of starch and can significantly decrease the postprandial rise in blood glucose if inhibited and as such it can be a therapeutic strategy of managing type-2 diabetes.[15] Glycemic indices are factors that serve as a measure in determining whether the GI of a food could be high or low; these includes starch content, soluble sugar content and amylose/amylopectin ratio. Starch, found in various parts of a plant in form of tiny granules is composed of two polymers; amylose and amylopectin. Amylose is a linear chain starch while amylopectin is a highly branched starch polymer.[16] Amylose to amylopectin ratio and dietary fiber content of foods are major factors

that determine the GI of the food.[17,18] GI, as a concept is based on the principle that the slower the rate of carbohydrate absorption, the lower the rise of blood glucose level and the lower the GI value.[19] Low-GI diet consumption has been shown to offer a number of health benefits including but not limited to lowering of blood glucose while the regular consumption of high GI foods has been linked as a risk factor for diseases such as diabetes, obesity, and cardiovascular disease; this thus makes low GI foods to be recommended for the management of such diseases.[20,21]

Cowpea which originated from Africa and was referred to as the poor man's diet is now widely accepted as a good source of protein and is consumed in various parts of the world and is recommended as a diet in the management therapy for those with degenerative conditions such as type-2 diabetes. However, there is little information on the effect of cowpea varieties on blood glucose levels and their suitability as a diet in the management of type-2 diabetes. The study investigated some of the differences in cowpea varieties with regards to their glycemic indices (GIs) and their inhibitory potentials on key enzymes linked with type-2 diabetes.

Materials and Methods

Sample collection

Five different varieties of cowpea (Kano white, Sokoto white, Drum, Ife brown and Oloyin varieties) were purchased at Oja Oba, Akure, Ondo, South West, Nigeria in May 2015. Sample authentication was done at the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure, Nigeria with voucher number FUTA 0292-0296.

Sample preparation

Raw cowpea varieties were picked, pulverized and aqueous extracts were prepared. 1 gram of the pulverized sample was soaked in 100 mL of distilled water and vigorously shaken using orbital shaker for 6 hours. Thereafter, the extracts were filtered through Whatman filter paper and further centrifuge to obtain clear supernatant. This was stored at 4^{o}C for subsequent analysis. Also, 25 g of the cowpea varieties were cooked at 120°C until acceptable softness was achieved, dried and pulverized, followed by preparation of aqueous extracts as earlier described for raw samples.

Chemicals and reagents

All chemicals and reagents used were of analytical grade and glass distilled water was used. Dinitrosalicylic acid color reagent, p-nitrophenyl-α-D glucopyranoside were sourced from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Ethanol, methanol, acetic acid, sulfuric acid, perchloric acid, sodium carbonate, sodium hydroxide, potassium acetate and phenol were procured from BDH Chemicals Ltd. (Poole, Dorset, UK).

Determination of Soluble sugars and starch

20 mg of dried and pulverized samples were weighed and placed into a 50 ml centrifuge tube; 1.0 ml of 80% ethanol and 2 ml of distilled water was added and mixed thoroughly. 10 ml hot 80% ethanol was subsequently added and thoroughly mixed. Afterwards, the samples were centrifuged at 2,000 rpm for 10 minutes and the supernatant carefully decanted into a 100 ml volumetric flask. 10 ml of hot 80% ethanol was added to the residue, then centrifuged for 5 minutes and the supernatant carefully decanted into same volumetric flask. Hot ethanol extraction was repeated and after which the reaction mixture in the flask was made up to volume with distilled water. The residue was kept for starch determination.

1.0 mL aliquot of the supernatant was pipetted into a test tube and diluted with 1.0 mL of distilled water. Then, 5% phenol was added and the reaction mixture mixed thoroughly. Thereafter, 5.0 ml concentrated sulfuric acid was directly added to the mixture, allowed to stand for 10 minutes and vortexed. The test tube was placed in the water bath for 15 minutes at 30°C and the absorbance was thereafter measured in a spectrophotometer at 490 nm.

To the residue, Perchloric acid (7.5 mL) was added and the mixture was left to hydrolyze for 1 hour; after which, it was then diluted to 25 mL with distilled water and filtered through Whatman No. 2 filter paper. An aliquot of 0.2 mL was taken from the filtrate and diluted with distilled water to 2.0 mL mark and thereafter vortexed. Then color development was as previously described for standard glucose curve preparation.[22]

Amylose and amylopectin content determination

A weight of 100 mg was weighed out of the pulverized cowpea samples into a 100-mL volumetric flask. To the sample, 1 mL of 95% ethanol and 9 mL of 1N NaOH were carefully added and the mixtures were thereafter heated for 10 minutes in a boiling water bath to gelatinize the starch. After then the mixture was cooled and made up to volume with water. Into a 100 mL volumetric flask, a 5-mL portion of the resultant starch solution was pipetted and 1 mL of 1N acetic acid (to acidify the solution) and 2 mL of iodine solution were then added. This was then made up to 100ml with distilled water. The mixture was vortexed for 20 minutes after which the absorbance was read at 620 nm in the spectrophotometer. The amylopectin content was derived from the pre-determined starch and amylose content of the samples.[23, 24]

In vitro Starch hydrolysis rate and estimated Glycemic Index (GI)

The In vitro starch hydrolysis and the estimated GI of the samples were determined according to the method described by Goni *et al*[25] with slight modifications. 1 mg of pepsin was used to incubate 50 mg of the Cowpea samples (50 mg) in 10 mL HCl-KCl buffer of pH 1.5 for 60 minutes at a temperature of 40°C in a shaking water bath. Phosphate buffer (pH 6.9) was added to the digest and diluted to 25 mL. 5 mL of α-amylase solution (0.005 g of α-amylase in 10 mL of buffer) was then added. The cowpea varieties were placed in a shaking water bath and incubated at 37°C. From each flask, 0.1 mL sample was taken at intervals of 30 minutes from 0- 3 hours and heated for 15 minutes to inactivate the enzyme. After the enzyme inactivation, Sodium acetate buffer (1mL, 0.4M, pH4.75) was added. The residual starch was digested to glucose by the addition of 30 mL amyloglucosidase and incubated for 45 minutes at 60°C. 200 µL of dinitrosalicylic acid (DNSA) colour reagent was thereafter added to determine the glucose concentration. The tubes containing the reaction mixtures were then placed in a water bath for 5 minutes at 100°C to stop every reaction and thereafter cooled to room temperature. Then, 5 mL of distilled water was added to dilute the reaction mixture and then centrifuged. The supernatant was collected and the absorbance was read on a spectrophotometer at 540 nm. The rate of starch digestion was expressed as the percentage of starch hydrolyzed per time (For the standard, a 50-mg sample of glucose was used).

Determination of total phenol content

The total phenolic content of the cowpea extracts was determined using the method described by Adedayo *et al*[26]. Dilutions of the sample extracts were oxidized with 2.5 ml of 10% Folin–Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45 °C for 40 minutes and absorbance was measured at 765 nm using the spectrophotometer. Using gallic acid as a standard, the total phenolic content was subsequently calculated.

Total phenol content = $(Abs_{sam} \times Conc._{std}) / (Abs_{std} \times Conc._{sam})$

Determination of total flavonoid content

A slightly modified method of Akomolafe *et al* [27] was used in determining the total flavonoid content of the sample extracts. Appropriate dilutions of (0-200 µl) of 0.5 mL of the samples were mixed with 0.5 ml methanol, 50 µl of 10% AlCl3, 50 µl of 1 mol·l−1 potassium acetate and 1.4 mL distilled water, and then incubated at room temperature for 30 min. Using a spectrophotometer, the absorbance was subsequently taken at 415 nm and the total flavonoid content calculated with quercetin as a standard.

Total flavonoid content = $(Abs_{sam} \times Conc._{std}) / (Abs_{std} \times Conc._{sam})$

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2, 20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging ability

The 2, 20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical ($ABTS^+$) scavenging ability of the cowpea varieties was determined according to the method described by Re *et al*.[28] 50 µL of the cowpea extracts were added to 2.0 mL $ABTS^+$ solution and left for 15 minutes after which the absorbance was read using a spectrophotometer at 734nm. The Trolox equivalent antioxidant capacity was subsequently calculated using Trolox as the standard.

ABTS* scavenging ability (mmol TEAC/g) = (Per_{sam} x $Conc._{std}$) / (Per_{std} x $Conc._{sam}$ x TMW)

1,1 diphenyly-2 picrlyhydrazyl free radical scavenging ability

The method as described by Gyamfi *et al* [29] was used in evaluating the free radical scavenging ability of the cowpea samples against 1,1 diphenyly-2 picrlyhydrazyl (DPPH) free radical. Cowpea aqueous extracts (0-400 mL) was mixed with 1 mL: 0.4mM methanolic solution containing DPPH radicals, and then left for 30 minutes in the dark. Thereafter, the absorbance was read at 516nm in a spectrophotmometer. The DPPH free radical scavenging ability was subsequently calculated.

Ferric reducing antioxidant property

The ability of the cowpea extracts to reduce $FeCl_3$ solution as described by Oyaizu[30] was used in determining their reducing property. An aliquot of 2.5 mL was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The reaction mixture was incubated for 20 minutes at 50^{o}C after which 2.5 mL 10% trichloroacetic acid was added. The mixture was centrifuged at 650 rpm for 10 minutes and the supernatant taken. An equal volume of distilled water was added to 5 mL of the supernatant and 1mL 0.1% ferric chloride was added. The absorbance was measured at 700 nm.

α-amylase inhibition assay

500 µL of 0.02 sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing porcine pancreatic α-amylase (EC 3.2.1.1; 0.5 mg/mL) was added to aqueous extracts (0-200 µL) of the cowpea extracts and incubated at 25^{o}C for 10 minutes. Thereafter, 500 µL of 1% starch solution in 0.02 M phosphate buffer was added to each tube. The reaction mixtures was incubated for an additional 10 minutes at room temperature and stopped with 1.0 mL of dinitrosalicylic acid color reagent; after which the mixture was incubated in a boiling water bath for 5 minutes, and cooled to room temperature. The reaction mixture was then diluted with 10 mL of distilled water, and the absorbance was measured using a spectrophotometer at 540nm. Thereafter, the α-amylase inhibitory activity was expressed as percentage inhibition.[31]

α-Glucosidase inhibition assay

Briefly, 100 µL of α-glucosidase (EC 3.2.1.20) solution in 0.1M phosphate buffer (pH 6.9) was incubated at 25°C for 10 minutes with the cowpea aqueous extracts (0-200 µL). Then, 50 µL of 5mM p-nitrophenyl-α-D-glucopyranoside solution was added. After a further incubation period of 5 minutes at room temperature, the absorbance was read at 405 nm in a spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition.[32]

Determination of IC_{50} values

Nonlinear regression analysis was used in calculating the effective concentration that causes 50% enzyme inhibition/antioxidant activity (IC_{50}) values for both enzyme inhibition assays and antioxidant assays.

Statistical Analysis

The results of the experiments in triplicates were pooled and expressed as mean standard deviation. The Means were compared by one-way analysis of variance (ANOVA) followed by Duncan's Multiple range test and the least significant differences were carried out and accepted at $p < 0.05$[33]

Results and Discussion

The free soluble sugar contents of the raw cowpea varieties were 2.5 - 6.5 g/100 g (Table 1). Oloyin variety had the highest sugar content (6.5 g/100g) while Sokoto white (2.5 g/100 g) had the least sugar content. Similarly, the results of the free soluble sugar content of the cooked samples as presented in Table 1 also showed Oloyin variety having the highest sugar (11.46 g/100g) content while Sokoto white (9.02 g/100 g) had the lowest. The total starch content however has Ife brown variety with the highest in the raw state (29.42 g/100 g); and drum variety had the highest when cooked (43.43 g/100 g) with Sokoto white variety and Kano white variety with the lowest starch content for both the raw and cooked samples respectively. The amylose content of the cowpea varieties ranges from 8.64 - 12.96 g/100g when raw and 5.82-7.00 g/100g when cooked; with Oloyin variety having the highest amylose content (12.96 g/100 g) among the varieties when raw, while Sokoto white (8.64 g/100 g) had the lowest (Table 2). However, there is a reduction in the amylose content when cooked. The five (5) cowpea varieties had glycemic indices ranging from 44.48 to 48.31 for the raw samples and 48.31 to 53.96 when cooked. (Table 3), Kano white variety 44.48 had the lowest glycemic index and Ife brown variety 48.31had the highest when raw however after processing, Oloyin variety had the highest (53.96) and Drum variety had the lowest (48.31). Cooking was found to increase the glycemic index of the cowpea varieties.

Table 4 shows that all the samples inhibited α-amylase activity. The raw samples showed that the Kano white variety had the highest inhibitory activity on α-amylase exhibiting 50% inhibitory concentration (IC_{50}) value of (IC_{50}=0.33 mg/mL) compared to Sokoto white variety (IC_{50}=0.49 mg/mL), which had the least. However, when cooked, Ife brown variety had the highest inhibitory activity (IC_{50}=0.37 mg/mL) while Oloyin had the least (IC_{50}=0.83 mg/mL). Cooking increased the α-amylase inhibitory activity of all the other varieties except for Kano white and Oloyin variety that had their inhibitory activity on α-amylase reduced. The α-glucosidase inhibitory potential of the cowpea varieties is also shown in Table 4. Drum variety had the highest inhibitory activity on α-glucosidase (IC_{50}=0.66 mg/mL) while Sokoto white had the least (IC_{50}=0.93 mg/mL) when raw, however, cooking increased the α-glucosidase inhibitory potential of the cowpea varieties with Oloyin variety having the highest (IC_{50}=0.56 mg/mL) and Sokoto white variety the lowest (IC_{50}=0.70 mg/mL).

The total phenol and total flavonoid content of the cowpea varieties are presented in Table 5. The total phenol content of the raw varieties ranged from 4.15-5.53mg GAE/g when raw and 4.57-7.02 mg GAE/g when cooked. However, Sokoto white variety had the lowest phenolic content when raw and the highest when cooked while Kano variety had the highest phenolic content when raw and Ife brown variety had the lowest when cooked. The total flavonoid content likewise ranged from 0.52 -0.71 mg QUE/g when raw and 0.29-0.52 mg QUE/g when cooked with Sokoto white variety having the highest flavonoid content for both the raw and cooked samples respectively while Kano white variety had the lowest when raw and drum variety had the lowest when cooked. Cooking was found to increase the total phenolic content while the total flavonoid content was reduced.

The ABTS and DPPH radical-scavenging ability of the cowpea aqueous extracts are presented in Table 6 and Table 7 respectively. The results revealed that the aqueous extracts scavenged DPPH radicals in a dose-dependent manner in the range of 0.00 – 3.33 $mg \cdot ml^{-1}$. However, the aqueous extract of the cooked Sokoto white variety had a significantly higher ($P < 0.05$) free radical-scavenging ability than the rest. The cowpea varieties aqueous extracts reducing powers were assessed based on their ability to reduce Fe^{3+} to Fe^{2+} and the results are presented in Tab. 6 as ascorbic acid equivalents. The results revealed that the reducing power of Sokoto white extract was significantly higher ($P < 0.05$) than the remaining varieties. This trend is in agreement with the observed total phenolic and flavonoid contents of the cowpea varieties**.**

Consumption of cowpea as a diet has remained unpopular amidst the world population due to the perception that it is a poor man's food and that it is a food for animals hence the name "cowpea"[2] Also, there is

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Table 1: Total starch and Soluble sugar contents of some cowpea varieties (g/100 g)

Sample	Soluble Sugar		Total Starch	
	Raw	Cooked	Raw	Cooked
Sokoto White	2.5 ± 0.71^{a}	9.02 ± 0.34^{a}	15.58 ± 2.44^{ab}	26.69 ± 0.74^{a}
Kano white	4.0 ± 1.41^{ab}	10.24 ± 0.00^{ab}	22.5 ± 2.45^{a}	26.69 ± 0.74^{a}
Oloyin	6.5 ± 2.12^{b}	11.46 ± 0.35^{b}	24.23 ± 4.90^{bc}	43.43 ± 0.74^{b}
Ife brown	4.5 ± 0.71^{ab}	9.76 ± 0.69^{a}	29.42 ± 2.45^{c}	41.86 ± 1.48^{b}
Drum	4.0 ± 0.71^{ab}	10.24 ± 0.69^{ab}	19.04 ± 2.45^{ab}	29.83 ± 2.22^{a}

Values represent mean ± standard deviation (n = 3). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

Table 2: Amylose/amylopectin contents of some Cowpea varieties (g/100 g)

Sample	Amylose		Amylopectin		Amyl/Amylp	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Sokoto White	8.64 ± 1.75^{a}	6.01 ± 0.40^{a}	9.78 ± 0.88^{a}	20.68 ± 0.40^{a}	0.88	0.29
Kano white	11.11 ± 1.75^{ab}	5.82 ± 0.00^{a}	13.86 ± 1.75^{b}	20.86 ± 0.00^{b}	0.80	0.28
Oloyin	12.96 ± 0.87^{b}	6.95 ± 0.20^{b}	13.12 ± 1.75^{b}	36.48 ± 0.40^{b}	0.98	0.30
Ife brown	8.64 ± 1.75^{a}	7.00 ± 0.19^{b}	16.46 ± 0.87^{c}	34.87 ± 0.20^{c}	0.52	0.20
Drum	9.26 ± 0.87^{ab}	6.90 ± 0.20^{b}	10.39 ± 1.75^{d}	22.93 ± 0.20^{b}	0.89	0.19

Values represent mean ± standard deviation (n = 3). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

Table 3: Estimated Glycemic index of some Cowpea varieties *(based on white bread)

SAMPLE	Raw	Cooked
Sokoto white	63.80 ± 0.86^{a}	69.88 ± 0.62^{ab}
Kano white	63.54 ± 4.17^{a}	71.78 ± 1.35^{b}
Oloyin	68.45 ± 4.75^{a}	77.08 ± 1.22^{c}
Ife Brown	69.01 ± 0.13^{a}	72.57 ± 0.50^{b}
Drum	67.45 ± 2.33^{a}	69.01 ± 0.13^{a}

Values represent mean ± standard deviation (n = 3). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

inadequate awareness and research backing the benefit of cowpea as a possible diet in managing diseases such as diabetes, obesity, and cardiovascular disease which are linked to dietary patterns. Cowpea is the largest contributor to the overall protein intake of several rural and urban families in Nigeria and it is estimated that cowpea supplies 40% of the daily protein requirement to most people in Nigeria.[3] Asumugha,[34] reported that the amino acid of cowpea seeds complements those of cereals. It has also been reported that the mineral content of cowpea; calcium and iron are higher than that of either meat, fish or egg while the iron content is equal to that found in milk.[1] Cowpea seeds have also been found to be rich in the vitamins-thiamin, riboflavin and niacin[35] and hence this makes it very useful in blood cholesterol reduction as reported by Anderson.[36] Besides its health related benefits, beans are inexpensive, considerably cheaper than any other dietary fibre type.[37] Since cowpea mixes well with other recipes it is considered to be a good item for food security.[38,39]

Table 4: IC_{50} values for the α-amylase, α -glucosidase inhibitory activities of some Cowpea varieties (mg/mL)

Sample	A-Amylase (mg/mL)		A-Glucosidase (mg/mL)	
	Raw	Cooked	Raw	Cooked
Sokoto white	0.49 ± 0.01^{d}	0.38 ± 0.01^{ab}	0.93 ± 0.00^{d}	0.70 ± 0.01^{e}
Kano white	0.33 ± 0.01^{a}	0.50 ± 0.04^{b}	0.76 ± 0.03^{bc}	0.67 ± 0.01^{d}
Oloyin	0.41 ± 0.01^{c}	0.83 ± 0.09^{c}	0.85 ± 0.04^{cd}	0.56 ± 0.00^{a}
Ife Brown	0.38 ± 0.01^{b}	0.37 ± 0.01^{a}	0.73 ± 0.05^{ab}	0.61 ± 0.01^{b}
Drum	0.42 ± 0.01^{c}	0.40 ± 0.03^{ab}	0.66 ± 0.04^{a}	0.65 ± 0.00^{c}

Values represent mean ± standard deviation (n = 3). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

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Table 5: Total Phenol, Total Flavonoid Content of the Aqueous Extracts of both Raw and Cooked Samples of Some Cowpea Varieties

	Total Phenol (mg GAE/g)		Total Flavonoid (mg QUE/g)	
Sample	**Raw**	**Cooked**	**Raw**	**Cooked**
Sokoto white	4.15 ± 0.15^{a}	7.02 ± 0.30^{c}	0.71 ± 0.67^{d}	0.52 ± 0.67^{d}
Kano white	5.53 ± 0.30^{C}	5.74 ± 0.30^{b}	0.52 ± 0.67^{a}	0.43 ± 0.67^{c}
Oloyin	5.11 ± 0.30^{C}	5.43 ± 0.15^{b}	0.61 ± 0.67^{b}	0.47 ± 0.00^{c}
Ife Brown	5.11 ± 0.30^{c}	4.57 ± 0.15^{a}	0.66 ± 0.00^{bc}	0.33 ± 0.67^{b}
Drum	4.89 ± 0.30^{b}	5.00 ± 0.45^{ab}	0.62 ± 0.67^{b}	0.29 ± 0.00^{a}

Values represent mean ± standard deviation (n = 3). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

Table 6: Ferric Reducing Antioxidant Property (FRAP) and ABTS* Scavenging Ability of the Aqueous Extracts of Both Raw and Cooked Samples of Some Cowpea Varieties

	FRAP (µg AAE/g)		ABTS (µmol TEAC/g)	
Sample	**Raw**	**Cooked**	**Raw**	**Cooked**
Sokoto white	3.24 ± 0.40^{a}	12.06 ± 0.39^{b}	17.97 ± 1.33^{a}	88.91 ± 2.65^{b}
Kano white	2.5 ± 0.21^{b}	4.56 ± 0.21^{e}	6.62 ± 1.34^{c}	18.92 ± 0.00^{e}
Oloyin	3.97 ± 0.20^{c}	6.32 ± 0.20^{d}	23.65 ± 1.33^{d}	32.16 ± 2.68^{d}
Ife Brown	2.5 ± 0.21^{a}	2.79 ± 0.19^{a}	12.30 ± 1.32^{b}	6.62 ± 4.01^{a}
Drum	3.38 ± 0.20^{bc}	4.26 ± 0.21^{c}	12.30 ± 1.32^{b}	25.54 ± 1.34^{c}

Values represent mean ± standard deviation (n = 3). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different

Table 7: IC_{50} for DPPH* Scavenging Ability of the Aqueous Extracts of Five Cowpea Varieties (mg/mL)

	IC_{50} (mg/mL)	
Sample	**Raw**	**Cooked**
Sokoto White	3.24 ± 0.04^{b}	2.20 ± 0.01^{a}
Kano white	4.12 ± 0.10^{d}	2.54 ± 0.05^{b}
Oloyin	3.66 ± 0.09^{c}	2.68 ± 0.04^{c}
Ife Brown	3.36 ± 0.04^{b}	2.97 ± 0.08^{d}
Drum	2.94 ± 0.09^{a}	2.85 ± 0.04^{d}

Values represent mean ± standard deviation (n = 3). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

Allen *et al*,[40] have shown cooking to increase sugar content of food. This may be due to the breakdown of starch during cooking. Cooking also cause hydrogen-bonding sites involved in intermolecular bonds of starch molecules to engage more water, releasing individual molecules unlike the starch in raw food which is stored in compact granules that are difficult to digest,[41,42] this explains the rise in the starch composition of the cooked cowpea varieties. Foods rich in amylose have been reported to induce low blood glucose and insulin responses when compared with those rich in amylopectin. This study shows the amylopectin content of the cowpea samples being higher than the amylose content, yet, they displayed a good potential in hyperglycemic response. This might be linked to the other contents such as fiber and phenolic constituents of the cowpea samples which have been confirmed to lower blood glucose in previous studies.[43,44] However, the raw samples with higher amylose content when compared to the cooked samples had a lower glycemic response. From the study, there is a significant decrease in the amylose content of the cowpea varieties after cooking. As previously reported;[45] consequents upon cooking, starch gelatinization takes place, which causes an increased randomness in the starch granule structure thereby causing swelling and leading to loss of soluble amylose molecules and hence a decrease in the amylose content and a seeming increase in the amylopectin content.

The glycemic index (GI) of a food is characteristic of how the food influences postprandial level of blood glucose.[46, 47] Dietary changes are important in the management of type 2 diabetes. Therefore, in order to help diabetic patients in their choice of diet, the concept of GI became imperative.[48] Foods are classified as having low (0-55), medium (55-69), or high (>70) GI when Glucose is used as the reference food. However, when white bread is used as a reference food, there are altogether a different set of GI values (with glucose=100 and white bread = 140).[49] The low GI of the cowpea varieties as shown in Table 3 could provide the basis for the recommendation of their consumption for prevention/management of diabetes. The observed low GI could be as a result of the starch composition and/or fiber content. Viscous, soluble fibers transform intestinal contents into gel-like matter that slows down enzymatic activity on starch, which may result in low GI,[46] Previous reports have suggested that diets of a low GI improves glycemic control in individuals with impaired glucose tolerance and type-2 diabetes by lowering fasting blood glucose and glycated proteins, and improving insulin sensitivity.[50] Previous works have also established that differences in the moisture content and in the cooking time can result in differences in the degree of starch gelatinization and consequently, the GI values of different varieties of a particular food.[51] Cooking makes food more susceptible to the actions of digestive enzymes thereby increasing the glycemic index of foods.[52] This explains the difference in the GI values of the different varieties.

The cowpea samples also demonstrated their ability to inhibit α-amylase and α-glucosidase activities in vitro. α-amylase and α-glucosidase are key enzymes of dietary carbohydrate digestion and inhibitors of these enzymes may be effective in retarding glucose absorption.[53] The inhibition of the enzyme α-glucosidase slows down

the breakdown of disaccharide to simple glucose and by so doing reduces the amount of glucose absorbed in the blood thus influencing the GI. Jenkins *et al*[54] affirmed that in addition to consuming low GI foods, the use of glycoside inhibitors, are also very important in the management of diabetes.

The cowpea samples also demonstrated strong free radical scavenging activities as shown by their scavenging activity of moderately stable ABTS and DPPH radicals. The result followed the pattern observed with the phenolic contents and reducing activities, where Sokoto white variety with higher phenolic content had significantly higher antioxidant activity. This finding agreed with earlier reports that have indicated a correlation between plant antioxidant properties (free radical scavenging ability) and their phenolic content[55]. The mechanism of action of phenolics on free radicals is majorly based on the redox properties of their hydroxyl groups attached to their chemical structure.[55] Reducing power is an antioxidation defence mechanism that is affected by either electron transfer or by hydrogen atom transfer.[56] The redox activities of phenolics enables them exert their antioxidant activities and thus allow them act as reducing agents.[56]

Conclusion

The cowpea varieties used in the study exhibited their antioxidant capabilities by their radicals scavenging abilities. The low glycemic indices of the cowpea generally, most especially in drum and Sokoto white variety combined with their inhibition of α-amylase and α-glucosidase activities could be the biochemical justification for the recommendation of their consumption for the prevention/management of diabetes. Nevertheless, further *in vivo* studies and clinical trials are recommended.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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