



Comparative Study of Two Cowpea (*Vigna unguiculata* L. Walp) Varieties: Biochemical and Phytochemical Composition, Glycemic Index and Glycemic Load

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Cowpea is a nutrient-dense legume with potential relevance to diets characterised by high carbohydrate intake. The aim of this study was to conduct a comparative assessment of the nutritional value of two cowpea varieties in order to determine the Glycaemic Index (GI) and Glycaemic Load (GL) in apparently healthy subjects. This was an experimental study including 18

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adult subjects who consumed a test food each day, calculated to provide 50 g of available carbohydrate (anhydrous glucose, white and red cowpea). GI and GL were determined using the standard method. The nutritional composition of white and red cowpea in terms of protein, lipid, fibre, starch, amylopectin and amylose contents ranged from 25.37 to 24.34 g; 1.78 to 1.73 g; 6.33 to 4.78 g; 64.19% to 64.96%; 22.59 to 23.11%; and 41.07 to 42.37%, respectively. Total polyphenol, flavonoid and antioxidant activity contents ranged from 143.95 to 156.89 mg GAE/100 g DM, from 14.49 to 14.30 mg QE/100 g DM, and from 15.87 to 45.34%, respectively. Both cowpea varieties exhibited low GI and GL values, but the GI and GL of white cowpea were markedly lower than those of red cowpea. GI and GL were 25.29% and 5.72 g for white cowpea, and 29.86% and 7.17 g for red cowpea. Both cowpea varieties showed good nutritional potential together with a low glycaemic index and glycaemic load. These findings demonstrate compositional differences between the varieties while confirming their low acute glycaemic responses in the apparently healthy adults studied.

Keywords: Cowpea; *Vigna unguiculata* L. Walp; biochemical composition; phytochemical composition; glycaemic index; glycaemic load; polyphenols; antioxidant activity; postprandial glucose.

1. Introduction

Legumes produce dry seeds used for human or animal consumption (FAO, 1994). They belong to the family Leguminosae, a group of species with unique characteristics that contribute fundamentally to livelihoods, nutrition and sustainable production systems. Nutritionally, they are highly valued for their nutrient richness, particularly in their seeds, leaves and pods (Dixon & Sumner, 2003). Such legumes include chickpea, lentil, broad bean, common bean, dried pea and cowpea. The nutritional benefits of legumes are not limited to their high protein content. These plants also make a vital contribution to human nutrition by providing important sources of B-group vitamins, iron, zinc, magnesium and calcium (Messina, 1999). Phytochemical compounds and dietary fibre are likewise abundant in a legume-rich diet. Consequently, plant protein sources, which are cheaper and more readily available, are essential for human and animal nutrition (El Youssef *et al.*, 2020).

Recent studies have further demonstrated varietal differences in cowpea proximate composition, mineral profiles, and grain, cooking and other quality characteristics, supporting direct comparisons among cowpea varieties (Nimbal & Ameen, 2024; Oliveira *et al.*, 2023; Chipeta *et al.*, 2024; Oluwayomi *et al.*, 2026). Metabolite profiling of local cowpea varieties has also identified variation in amino acids, fatty acids, flavonoids, phenolic acids and other secondary metabolites, supporting the inclusion of phytochemical characterisation in varietal comparisons (Karuwal *et al.*, 2023).

Despite the significant nutritional properties and accessibility of legumes, particularly cowpea, the majority of the Ivorian population follows dietary habits rich in carbohydrates, with a greater emphasis on cereals, tubers and roots, which are responsible for numerous disorders such as metabolic diseases (diabetes, hypertension, among others). Indeed, incorporating cowpea into dietary habits, as is already the case in other West African countries, could provide the Ivorian population with a diet sufficient in both quantity and quality to reduce protein deficiencies and, above all, metabolic diseases such as diabetes, which affects 700,000 people per year (Akré *et al.*, 2021). However, this high disease prevalence is associated with inadequate healthcare provision and management across Ivorian territory (International Diabetes Federation, 2019).

Given this pressing situation involving a high number of patients with diabetes, studying the Glycaemic Index and Glycaemic Load could provide appropriate data and information to predict the impact of consuming the two cowpea varieties on population health and the risk of developing diabetes. Moreover, according to Kalergis *et al.* (2005), certain parameters, such as meal frequency and the levels of lipids, proteins and dietary fibre, could be causes of diabetes onset. However, no comparative study on the nutritional value and glycaemic index of the two cowpea varieties has been conducted in Côte d'Ivoire. It is within this context that the present study on the biochemical and phytochemical characterisation and glycaemic index of these two cowpea varieties (white and red) was undertaken.

2. Materials and Methods

2.1 Plant Material

Two cowpea varieties (red and white) were used in this study. These foodstuffs were purchased from a market in the commune of Anyama (Abidjan, Côte d'Ivoire), then packaged in biodegradable bags and transported on the same day to the Laboratory of Biochemistry and Technology of Tropical Products (LBATPT) of Nangui Abrogoua University for recipe formulation and biochemical analyses.

2.2 Methods

2.2.1 Preparation of the Two Cowpea Varieties

Cowpea samples were sorted, washed and then soaked in tap water for three (3) hours to shorten cooking time. After soaking, the seeds were drained and cooked for forty-five (45) minutes using 250 g of cowpea in 450 mL of water. The flours obtained from the two cooked cowpea varieties were stored in airtight containers for biochemical and phytochemical analyses.

2.2.2 Biochemical Analyses of the Flours of the Two Cooked Cowpea Varieties

Lipid, protein, crude fibre, ash and moisture contents were determined in triplicate according to standard methods (AOAC, 1990; AOAC International, 2005).

Lipid content was determined by the Soxhlet extraction method. Protein content was determined by the Kjeldahl method, which estimates crude protein content from nitrogen content using the conversion factor 6.25.

Carbohydrate content, expressed as a percentage of dry matter, was estimated by difference (Antia *et al.*, 2006). The energy value (EV) of the different cowpea flours was calculated from the thermal coefficients of Merrill and Watt (1973), adopted by FAO (1 g of carbohydrate provides 4 kcal; 1 g of protein provides 4 kcal; and 1 g of lipid provides 9 kcal).

2.2.3 Analysis of Starch, Amylose and Amylopectin Content in White and Red Cowpea Ingredients

Starch, amylose and amylopectin levels were determined by the iodine-based method of Jarvis and Walker (1993). Briefly, a 0.1 g flour sample was taken, and 5 mL of 1 N KOH was added. The resulting solution was homogenised at room temperature (about 25°C) and then neutralised with 5 mL of 1 N HCl solution. pH paper was used to confirm that the solution was neutral. The mixture was boiled in a water bath for 15 min. The resulting supernatant was collected, filtered, and used for starch quantification. Colour intensity was read on a spectrophotometer (Thermo HELIOS OMEGA) at 580 nm for starch and 720 nm for amylose against a blank after cooling. The optical density readings were converted into the starch, amylose and amylopectin contents of the samples using the standard starch calibration curve.

2.2.4 Determination of Phytochemical Compounds in the Two Cooked Cowpea Varieties

The phytochemical screening was based on tube colouration and precipitation tests described by Khitri *et al.* (2016). Flavonoids were screened for by adding 0.5 g of each extract to 10 mL of distilled water for 10 minutes. After stirring, the extract was filtered, and 5 mL of dilute ammonia solution was added. Finally, 1 mL of aluminium chloride (AlCl₃) was added to the mixture. The appearance of a yellow colour indicated the presence of flavonoids.

Ferric chloride (FeCl₃) at 1% was used to screen for polyphenols. Briefly, 0.8 g of each extract was added to 10 mL of distilled water for 10 minutes. After stirring, the extract was filtered, and one drop of 2% alcoholic ferric chloride solution was added. The appearance of a more or less dark blue-black or green colouration indicated the presence of phenolic compounds.

To characterise tannins, 1 g of each extract was mixed with 10 mL of 90% methanol. The mixture was stirred for 15 minutes and filtered. Then, 1.5 mL of methanol was added to 1.5 mL of each filtrate. The addition of 5 mL of sulfuric vanillin reagent was used to detect the presence or absence of tannins. The resulting solution was boiled for 15 minutes in a water bath. The formation of a precipitate turning blue-black indicated the presence of gallic tannins, whereas the presence of catechic tannins was indicated by a precipitate turning brownish-green.

The antioxidant activity of the aqueous and hydroethanolic extracts was assessed using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to the method of Huang *et al.* (2011), with minor modifications. Reduction of DPPH radicals in solution by an antioxidant agent results in bleaching of the solution. Briefly, 2 mL of a methanolic DPPH solution (100 µM) was mixed with 2 mL of various dilutions of the two extracts (0-100 µg/mL). A range of vitamin C concentrations (0-100 µg/mL) was also prepared and used as a reference. The resulting mixtures were kept in the dark at room temperature for 30 minutes. Absorbance at 517 nm was measured against a blank (2 mL DPPH solution). Three (3) replicates were performed for each concentration. The absorbance values obtained were used to calculate the percentage inhibition (PI) of DPPH radicals using the following formula:

$$PI (\%) = (A_0 - A_1) \times 100 / A_0$$

2.2.5 Determination of the Glycaemic Index and Glycaemic Load of the Two Cowpea Varieties

Eighteen healthy subjects (10 men, 8 women; age: 20.64 ± 2.28 years; body mass index: 22.86 ± 3.60 kg/m²; and mean fasting blood glucose: 0.98 ± 0.08 g/L (5.44 mmol/L)) took part in the study. Volunteers were recruited based on the following inclusion criteria: absence of any known food allergy or intolerance, absence of medication likely to affect glucose tolerance, and absence of known diabetes mellitus or use of anti-hyperglycaemic medication. Subjects were also required to fast for at least 8 hours before the start of the test and to have blood pressure below 140/90 mmHg, a body mass index below 25 kg/m², and fasting blood glucose below 1.10 g/L. They were recruited from among staff and students at Nangui Abrogoua University through advertisements. All subjects involved in the study were informed beforehand about the protocol details and the risks of participation. They gave their informed, signed consent to participate in the study, in accordance with the Declaration of Helsinki on human rights. The method used to measure and calculate the glycaemic index (GI) of foods complied with the guidelines set out in the ISO 26642-2010 document and previously described by Kouamé *et al.* (2017). Briefly, on the test day, subjects consumed either the test meal (white or red cowpea varieties) or the reference meal (anhydrous glucose) dissolved in 250 mL of water. Meal portion sizes were calculated to provide 50 g of available carbohydrate. On each test day, capillary whole blood was collected by finger-prick (BD Microtainer, Contact-Activated Lancet, Poland, USA) in the fasting state (0 min) and at 15, 30, 45, 60, 90 and 120 min after meal consumption. Blood glucose concentrations were measured immediately using glucometers (Accu-Chek® Active, GC, Mannheim, Germany). GL was calculated from the GI of the tested food, taking into account the amount of available carbohydrate in the portion size consumed by the subjects (Augustin *et al.*, 2015). Table 1 presents the clinical, morphological and anthropometric characteristics of the subjects.

2.3 Statistical Analysis

Biochemical and phytochemical data were analysed using descriptive statistics, namely means and standard deviations, with XLSTAT software version 2016.02. One-way analysis of variance (one-way ANOVA) combined with Student's t-test was used to

compare means. Statistical significance was set at $p < 0.05$. Glycaemic data were analysed according to the method recommended by the ISO/FDIS 26642:2010 standard for food products with respect to GI. The area under the curve (AUC), GI and GL were calculated using XLSTAT software version 2016.02.

Table 1. Clinical, morphological and anthropometric characteristics of the subjects (n = 18)

Anthropometric parameters		Mean value (18 subjects)
Age (years)		20.64 ± 2.28
Height (m)		1.64 ± 0.09
Weight (kg)		61.54 ± 7.36
BMI (kg/m ²)		22.86 ± 3.60
FBG (g/L)		0.98 ± 0.08
BP (mmHg)	Systolic	12.30 ± 0.78
	Diastolic	7.3 ± 0.9

3. Results

3.1 Biochemical Composition of White and Red Cowpea

The biochemical composition of the two cowpea varieties, white (WC) and red (RC), is presented in Table 2. Protein (25.37 ± 0.05 g/100 g DM), lipid (1.78 ± 0.09 g/100 g DM), fibre (6.33 ± 0.02 g/100 g DM), total carbohydrate (53.42 ± 1.01 g/100 g DM) and energy value (331.18 ± 2.97 kcal/100 g DM) contents were higher in white cowpea than in red cowpea. The corresponding values for red cowpea were 24.34 ± 0.04 g/100 g DM for protein, 1.73 ± 0.07 g/100 g DM for lipid, 4.78 ± 0.01 g/100 g DM for fibre, 51.79 ± 0.22 g/100 g DM for total carbohydrate, and 320.09 ± 1.63 kcal/100 g DM for energy value.

Starch, amylose and amylopectin contents are proportionally related. Starch, amylose and amylopectin contents were higher in red cowpea than in white cowpea. These contents ranged, respectively, from 64.19 ± 0.03% to 64.96 ± 0.01% for starch, 22.59 ± 0.05 to 23.11 ± 0.04% for amylose, and 41.07 ± 0.02 to 42.37 ± 0.03% for amylopectin. For all these biochemical compounds, a significant difference was found at the 5% threshold between the two cowpea varieties analysed.

Table 2. Biochemical composition of the two cowpea varieties

Biochemical composition	White cowpea	Red cowpea
Moisture (g/100 g DM)	10.90 ± 0.91 ^b	14.50 ± 0.3 ^a
Dry matter (g/100 g DM)	89.10 ± 0.90 ^a	85.50 ± 0.30 ^b
Ash (g/100 g DM)	2.20 ± 0.3 ^b	2.86 ± 0.12 ^a
Protein (g/100 g DM)	25.37 ± 0.05 ^a	24.34 ± 0.04 ^b
Lipid (g/100 g DM)	1.78 ± 0.09 ^a	1.73 ± 0.07 ^b
Fiber (g/100 g DM)	6.33 ± 0.02 ^a	4.78 ± 0.01 ^b
Total carbohydrate (g/100 g DM)	53.42 ± 1.01 ^a	51.79 ± 0.22 ^b
Energy value (kcal/100 g DM)	331.18 ± 2.97 ^a	320.09 ± 1.63 ^b
Starch (%)	64.19 ± 0.03 ^b	64.96 ± 0.01 ^a
Amylose (%)	22.59 ± 0.05 ^b	23.11 ± 0.04 ^a
Amylopectin (%)	41.07 ± 0.02 ^b	42.37 ± 0.03 ^a

3.2 Phytochemical Composition of the Analysed White and Red Cowpea Varieties

Table 3 presents the total polyphenol, flavonoid and tannin contents and the antioxidant capacity of the two cowpea varieties studied. Statistical analysis showed a significant difference at the 5% threshold between red and white cowpea. The total polyphenol content of red cowpea (156.90 ± 1.84 mg GAE/100 g DM) was higher than that of white cowpea (143.95 ± 0.70 mg GAE/100 g DM). Flavonoid contents of the cowpea varieties ranged from 14.30 ± 0.76 mg QE/100 g DM for red cowpea to 14.49 ± 0.75 mg QE/100 g DM for white cowpea. Red cowpea recorded the highest tannin content (31.99 ± 0.56 µg CE/mg DM) compared with white cowpea (25.42 ± 0.29 µg CE/mg DM). Furthermore, the percentage inhibition (PI) was higher for the red cowpea variety (45.34 ± 0.41%) than for the white variety (15.87 ± 0.15%).

Table 3. Phytochemical composition of the two cowpea varieties (white, red)

Biochemical composition	White cowpea	Red cowpea
Total polyphenols (mg GAE/100 g DM)	143.96 ± 0.70 ^b	156.90 ± 1.84 ^a
Flavonoids (mg QE/100 g DM)	14.49 ± 0.75 ^a	14.30 ± 0.76 ^b
Tannins (µg CE/mg DM)	25.42 ± 0.29 ^b	31.99 ± 0.56 ^a
Antioxidant activity (%)	15.87 ± 0.15 ^b	45.34 ± 0.41 ^a

3.3 Glycaemic Responses of the Two Cowpea Varieties Tested

The postprandial blood glucose responses of the subjects to the two cowpea varieties and anhydrous glucose are shown in Fig. 1. This figure shows the glycaemic response to anhydrous glucose (reference food) compared with those of the two cowpea varieties

(100%) over 2 hours. Overall, the blood glucose levels induced after ingestion of the tested food portions followed a broadly similar pattern. Each of the three curves displayed two phases. The first was a rising phase, with the highest blood glucose value (9.40 ± 1.48 mmol/L) recorded for anhydrous glucose at 30 min. The red and white cowpea varieties showed glycaemic peaks of 6.62 ± 0.59 mmol/L after 45 min and 5.42 ± 0.49 mmol/L after 60 min, respectively. In the second phase, blood glucose responses to anhydrous glucose and to the two cowpea varieties declined significantly, as reflected by a downward trend in the glycaemic curves between 45 and 120 min. At the end of the experiment (120 min), following consumption of anhydrous glucose and the cowpea varieties, blood glucose responses remained above fasting blood glucose values. Analysis of this figure also showed that anhydrous glucose was absorbed to a greater extent than the cowpea varieties.

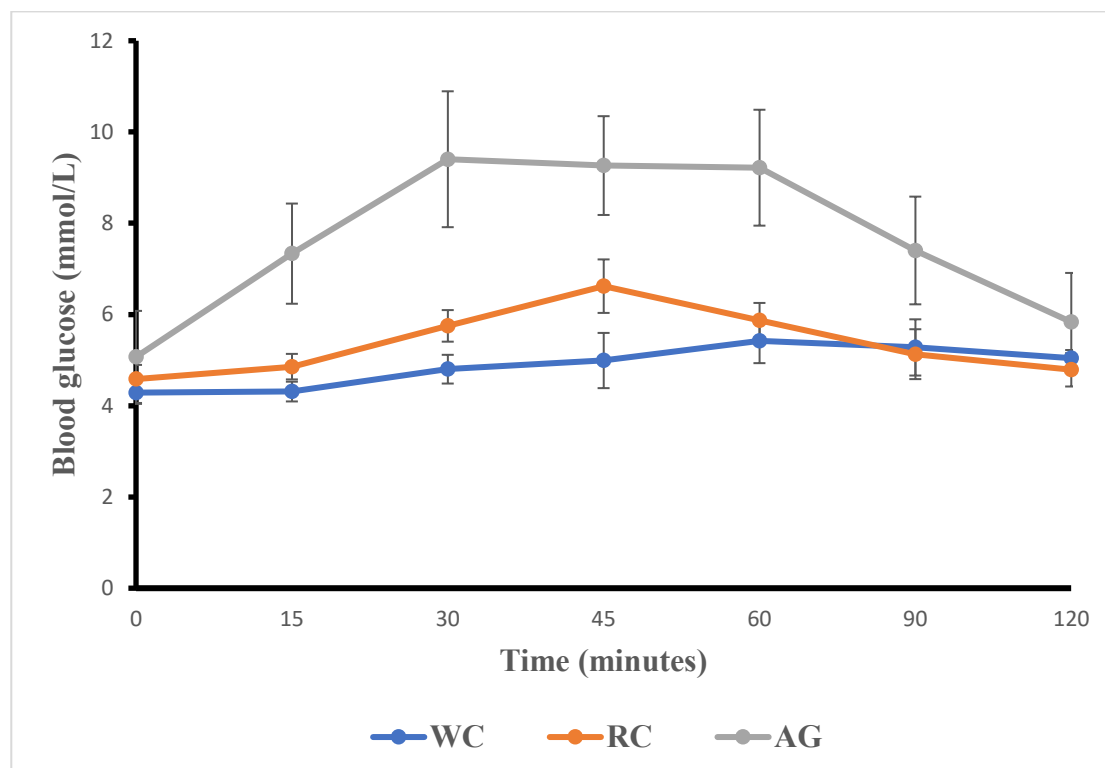


Fig. 1. Postprandial blood glucose levels of the two cowpea varieties
WC: White Cowpea; RC: Red Cowpea; AG: Anhydrous Glucose

3.4 Glycaemic Index and Glycaemic Load of the Two Cowpea Varieties Tested

The Glycaemic Index and Glycaemic Load values of the cowpea varieties and of the anhydrous glucose tested are shown in Table 4. The Glycaemic Index (GI) values of the cowpea varieties differed from one another. Both cowpea varieties (white and red) recorded the lowest GI values, at 25.29% and 29.86%, respectively. The GI values of both white and red cowpea were classified as low-GI foods. Both cowpea varieties recorded Glycaemic Load values ranging from 5.72 g for white cowpea to 7.17 g for red cowpea. All Glycaemic Load (GL) values for white and red cowpea fell within the low Glycaemic Load category, with values between 0 g and 10 g.

Table 4. Portion ingested, Glycemic Index and Glycemic Load of the cowpea varieties

Glycemic parameters	Foods tested		
	AG	White Cowpea (WC)	Red Cowpea (RC)
Portion or quantity (g)	50	248	231
AUC (mmol.min/L)	336.67	85.17	100.55
Glycemic Index (GI) (%)	ND	25.29	29.86
Classification	ND	Very low	Low
Glycemic Load (GL) (g)	ND	5.72	7.17
Classification	ND	Very low	Low

AG: Anhydrous Glucose, ND: not determined

4. Discussion

This study was undertaken to compare the chemical composition of the cowpea varieties and to determine their ability to influence blood glucose levels. The results showed high protein contents in both cowpea varieties, with white cowpea having a higher protein

content than red cowpea. This difference in protein content between the two varieties could be explained by the fact that white cowpea seeds absorb more water, and the loss of soluble constituents during soaking and cooking leads to a higher concentration of protein in the water-cooked white cowpea seeds compared with the red variety (Wang *et al.*, 2010). In general, a high protein content in cowpea is desirable for better nutrition (Lambot, 2002). The higher protein content observed in the white cowpea variety therefore suggests that it could serve as an important protein source for low-income populations.

Regarding lipid content, the results showed little variation between the two cowpea varieties. The low lipid content of both cowpea varieties (white and red) would be attributable to their botanical origin. These results are consistent with those of Khaldi *et al.* (2012), who reported a lipid content of around $2.1 \pm 0.1\%$ in a whole cowpea flour from Sudan.

The white cowpea (WC) variety had a markedly higher fibre content than red cowpea, with a statistically significant difference ($p < 0.05$). In line with the work of AFSSA (2002), the presence of fibre in foods intended for human consumption favours a reduction in postprandial blood glucose and insulin levels. Furthermore, in both cowpea varieties, fat content varied significantly from one variety to the other (Feinberg, 1996).

Moreover, the high carbohydrate content together with the energy value clearly indicates that the white cowpea seed is an energy-dense food. The energy value of approximately 331.18 ± 2.97 kcal/100 g is an indicator of this. Moreover, consumption of one kilogram of seeds can cover the daily energy requirements of an adult individual. The energy value recorded in this study was higher than that obtained by Frota *et al.* (2008), which was around 323.4 kcal/100 g.

Analysis of the results showed that the starch, amylose and amylopectin contents of the red cowpea variety were significantly ($p < 0.05$) different from those of the white cowpea variety. This difference could be related to the size and structure of the starch granules, their botanical origin, and even their content (Massaux *et al.*, 2006). Amylose and amylopectin, the main constituents of starch, represent 98 to 99% of the dry weight of the granules (Copeland *et al.*, 2009).

The two cowpea varieties also showed significantly different total polyphenol, flavonoid, tannin and antioxidant contents. Parikh and Patel (2018) noted that polyphenols and flavonoids are abundant in legumes, with contents reaching up to 500 mg/100 g DM. The presence of polyphenols in the cowpea varieties studied would therefore be an advantage for consumers, as they protect the body against the harmful effects of free radicals likely to accelerate cellular ageing (Deghal & Derradji, 2018). Daily consumption of these two cowpea varieties would therefore be beneficial to the consumer.

A feature common to both cowpea varieties (white and red) in the present study is their moderate average carbohydrate content, representing on average 52.60% of energy intake. This carbohydrate profile enabled the foods studied to induce low postprandial glycaemic responses. Furthermore, the GI values of white and red cowpea were below the reference threshold (55%) according to Robet *et al.* (2020). These two cowpea varieties should be encouraged, as their low GI could have beneficial effects by improving glycaemic control and insulin sensitivity (Brand-Miller *et al.*, 2003).

The lower glycaemic peaks observed for the two cowpea varieties compared with anhydrous glucose could be explained by the fact that anhydrous glucose is a simple sugar. Indeed, the early and pronounced glycaemic peak obtained with pure anhydrous glucose (a simple sugar) would result from its rapid absorption. In addition, the dietary fibre, protein, antioxidants and phenolic compounds (flavonoids, tannins, etc.) present in legumes could exert positive effects on the glycaemic response (Koutouan *et al.*, 2019).

The low GI and GL values of white and red cowpea would be attributable to their soluble dietary fibre, protein and phytochemical compound contents. Furthermore, white and red cowpea could help patients better manage their diabetes and achieve lower postprandial glycaemic excursions. Based on this quality indicator, these two cowpea varieties would therefore lead to reduced glucose absorption (Wolever, 2013; Bhupathiraju *et al.*, 2014). A low-glycaemic-index diet has a positive impact on blood glucose, blood pressure, cholesterol and triglycerides, and on the risk of certain cancers (Sieri *et al.*, 2007; Thomas & Elliott, 2010).

5. Conclusion

The white and red cowpea varieties differed significantly in several biochemical and phytochemical characteristics. White cowpea showed higher protein, lipid, fibre, total carbohydrate and energy values, whereas red cowpea contained higher starch, amylose, amylopectin, total polyphenols and tannins and exhibited greater antioxidant activity. Both varieties produced lower postprandial glucose responses than anhydrous glucose in the apparently healthy adults studied. Their GI values were 25.29% and 29.86% for white and red cowpea, respectively, while GL values were 5.72 and 7.17 g. These findings indicate that both varieties can be classified as low-glycaemic foods under the conditions of the present experiment. Further studies are required before extending these findings to long-term glycaemic management or disease prevention in clinical populations.

6. Limitation

This study evaluated two cowpea varieties in 18 apparently healthy adults using an acute 2-hour postprandial protocol. Its findings therefore describe responses under the specific preparation and testing conditions used and may not represent other cowpea varieties, preparation methods, population groups or longer-term glycaemic outcomes. Future work should expand this study to include a diabetic population and conduct a long-term study of the glycaemic index to better understand the impact of the formulated recipes on blood glucose levels.

Consent

All Participants involved in the study were informed beforehand about the protocol details and the risks of participation. They gave their informed, signed consent to participate in the study, in accordance with the Declaration of Helsinki on human rights.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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