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Production, Physico-Chemical and Organoleptic Evaluation of Protein Isolates Powder from Indigenous Legumes

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Abstract: This study is aimed at isolating and evaluating proteins from Bambara groundnut (BGN), cowpea (CP), pigeon pea (PP), African yam bean (AYB) and soybean (SB) seeds using a 1 x 5 factorial in a completely randomized design (CRD). Each legume was individually soaked for ten hours after initial wet cleaning to remove debris and unwanted matter. Soaked grains were rewashed and milled into a uniform paste and thereafter subjected to protein extraction using standard methods to obtain dried products, where Bambara groundnut had the highest percentage of protein yield. Physiochemical analysis showed that pH and colour of isolated showed a maximum of 4.40 (cowpea) and 7.67 (AYB), while acidity, crude protein, crude fat and moisture content showed 2.52 (AYB), 88.90 (SB), 8.45 (SB), and 6.55 (BGN) respectively all in percentage. Bulk density and emulsion capacity results showed maximum of 0.99gram per milliliters (PP), 47.34 (SB). Viscosity, swelling capacity and water absorption capacity showed a maximum of 30.23Newton second per meter (CP), 44.46 percent (PP) and 10.55gram per milliliters (PP) respectively. Solubility test revealed all isolates were soluble in water and 0.1N hydrochloric acid but were insoluble in chloroform. Amino acid profile of the isolates showed relatively high values for leucine 8.33 (SB) - 7.62 gram per100gram (AYB); aspartic acid 8.62 (CP) - 9.56 gram per100gram (PP); glutamic acid 13.03 (CP) - 14.34 gram per100gram (SB) and arginine 6.55 (CP) - 7.32 gram per100gram (PP) respectively. A sensory result conducted for garri enriched with the isolate indicated that the panelists scored all samples above average, thus were favourably disposed to the samples. These results will support introduction and commercialization of these isolates for more impact in terms of nutrient availability.

Keywords: *Protein Isolates, Physico-Chemical, Indigenous and Legumes.*

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I. INTRODUCTION

Plant protein production has grown interest globally due to its increasing applications in food and non-food system [23]. As their name implies, they are crude proteins extracted from specific sources in nature. Protein isolates are used widely in food products for their ability to improve the nutritional content, texture, solubility, and functionality of food products, such as in plant-based alternatives to meat and dairy [10] [8]. The growing demand for plant-based protein sources has led to an increased interest in protein isolates derived from underutilized legumes, such as soybeans, cowpeas, and other Agro crops. The global demand for plant-based proteins has risen due to factors like dietary preferences, health concerns (such as protein Energy Malnutrition), and food sustainability considerations [6]. Many African countries like Nigeria face challenges in accessing sufficient and high-quality protein sources due to some limitations in production and underutilization of commonly available legumes. In terms of utilization of these legumes, limitations may be tied to culture/tradition, hard-to-cook effect, presence of anti-nutrients, less industrialization as well as poor nutritional awareness ([13], [16], [14].

Legumes such as cowpea (*Vigna unguiculata*), Bambara groundnut (*Vigna subterranea*), African yam bean (*Sphenostylis stenocarpa*), groundnut (*Arachis hypogea*), soybean (*Glycine max*), and pigeon pea (*Cajanus cajan*) are rich in proteins and essential amino acids [37]. Utilizing protein isolates from these legumes in domestic and industrial

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food products could enhance dietary protein intake while promoting the use of underutilized local crops.

These indigenous legumes are chosen not only for their nutritional content but also for their sustainability, affordability and local availability. Unlike imported protein sources, they support local agriculture, reduce production costs, and encourage the use of native biodiversity in food innovation.

Cowpea is widely grown and consumed in Nigeria. It is rich in lysine, and essential amino acid missing in cereals like wheat. Cowpea is also a good source of dietary fiber, iron, calcium, and vitamins. It is drought-resistant, making it ideal for regions with limited rainfall. The protein isolate from cowpea enhances the amino acid profile of bread, making it more balanced for human nutrition

Soybean (*Glycine max*) is known for its high protein content (around 40 percent), and it contains all the essential amino acids, making it a complete protein source. Soy protein isolate is widely used in food fortification due to its functional properties and health benefits, including lowering cholesterol and supporting muscle mass in chronically ill patients. Soy is also rich in iso-flavones, which have antioxidant and anti-inflammatory properties.

Bambara Groundnut (*Vigna subterranea*) often referred to as a "complete food", Bambara groundnut contains a balanced proportion of carbohydrates, protein (18–24 percent), and fats. It is rich in methionine and cysteine, sulfur-containing amino acids often lacking in other legumes. It grows well in poor soils and requires minimal inputs, making it a climate-resilient crop. Its protein isolate can significantly enhance the nutritional profile of fortified bread.

African Yam Bean (*Sphenostylis stenocarpa*) this underutilized legume is highly nutritious, with a protein content of about 21–29 percent. It also contains high levels of iron, zinc, and calcium. The amino acid composition is especially beneficial for children and convalescent patients. African yam bean is also known for its high dietary fiber content, which supports digestive health. Pigeon Pea (*Cajanus cajan*) is high in protein, fiber, and iron. It also contains significant amounts of potassium and magnesium. It is effective in reducing the risk of iron-deficiency anemia and supporting muscle recovery. Its protein isolate can help balance the nutrient content in fortified bread and en\ hance its functionality.

Thus, this research aims to explore the production of protein isolates as well as evaluate the physicochemical, functional, and sensory properties of protein isolate powders derived from locally available or underutilized legumes, such as Bambara groundnuts, pigeon peas, soybeans, cowpeas, and African yam beans, commonly found in the Northern region of Nigeria.

II. MATERIALS AND METHODS

➢ *Materials:*

Soybeans, Bambara groundnuts and cowpea were procured from Jalingo main Market in Jalingo, Taraba State (8.89degreeNorth and 11.38 degreeEast) while African yam bean and pigeon pea were purchased from Anyigba Market in Kogi State (7.4934 degreeNorth and 7.1736 degreeEast) and kept in a polyethylene bags on a dry place. All the chemicals used in this study (Food grade sodium hydroxide and Food grade hydrochloric acid) were of analytical grade.

➢ *Methods*

- *Preparation of Bambara Groundnuts, Pigeon Peas, Soybeans, Cowpeas, and African Yam Beans Protein Isolate*

The protein isolates were extracted according to the method used by [34] and modified. Soybeans, pigeon pea, Bambara groundnuts, African yam bean and cowpea, were sorted cleaned, washed, individually Submerged in water for 10 hours. Soaked grains were washed with clean water repeatedly until water ran clear and were milled (attrition mill) to smooth paste/slurry with intermittent minimal addition of water to facilitate grinding. The resultant slurry was homogenized with water at ratio 1:10 and was filtered manually using a cheese cloth. The pH of the suspension was adjusted to 8.5 - 9.0 using food grade 1 N NaOH. The slurry was stirred for 20 minutes by using high-speed stirrer. The protein in the extract was precipitated (isoelectric precipitation) at pH 4.5 using food grade1 N HCl. The precipitate was allowed to stand overnight to achieve total separation with the supernatant. The clear liquid was decanted off and discarded. The precipitated protein isolate was transferred into cheese cloth and pressed out to achieve a semi-dry cake. The protein isolate semi-dry cake was spread in a drying oven set to 80 degree Celsius to achieve drying of the isolates. The dried protein isolates were packaged in airtight ziplock plastic bags and stored in a cool dry environment.

- *Analytical Methods*

Proximate analysis (moisture content, protein, and fat) were determined using the standard method of [4]. The yield by weight and the protein recovery from total seed protein extraction were calculated (Equation 1 and 2).

$$\text{Yield in weight (\%)} = \frac{weight\ of\ sample}{weight\ of\ flour} x\,100 - - -\ 1$$

$$Protein\ yield\ recovery\ (\%) = \frac{Protein\ content\ of\ sample}{protein\ content\ of\ flour}\ x\,100 - 2$$

- *Functional Properties of Bambara Groundnuts Flour and Protein Isolate*

✓ *Bulk Density (BD)*

This was determined according to the method of [17]. A ten millilitres capacity graduated cylinder was weighed on a digital balance. The cylinder was gently filled with the sample. The bottom of the cylinder was tapped on the laboratory bench

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several times until no further diminution of the sample level occurred after filling to the 10 milliliters mark.

$$Bulk\ Density = \frac{weight\ of\ sample\ (g)}{Volume\ of\ sample\ (ml)} ------ 3$$

✓ *Water Absorption Capacity (WAC)*

The water absorption capacity of the flour blends was determined as described by [17]. One gram of the sample was weighed into a 20 milliliters conical graduated centrifuge tube. Using a warring whirl mixer, the sample was mixed thoroughly with 10 milliliters of distilled water in a centrifuge tube for 30 minutes. The sample was allowed to stand for 30 minutes at room temperature and centrifuged at 500 rpm for 30 minutes. The volume of free water (the supernatant) was read directly from the graduated centrifuge tube.

$$\%\ WAC = \frac{weight\ of\ water\ boumd}{weight\ of\ sample\ (dry\ basis)} X\ 100 ------ 4$$

✓ *Viscosity*

The viscosities of isolates were measured according to [7]. The apparent viscosity of the heated isolates solutions were measured using a Bohlin coaxial cylinder viscometer (Bohlin Instrument Inc., Sweden) attached to a work station loaded with software V88 viscometery programme. The system C30 was filled with the isolates solution at the measurement temperature of 20 degree Celsius. The viscosity was carried out in the up mode at shear rate ranging from 37 to 910 liter per second. The heated isolates solutions samples at 80 degree Celsius for 10 minutes were kept in refrigerator at 5-6 degree Celsius for 24 hours to examine renaturation of proteins isolates again.

- *Emulsifying Properties*

The emulsifying capacity (EC) was determined by [25] with some modifications. Aliquots (5 milliliter) of 10 milligram per milliliter protein isolates solutions at their corresponding pH values (2–11) were thoroughly mixed with 5 milliliter of soybean oil using an Ultra-Turrax homogenizer (T25, IKA WERKE, Staufen, Germany) at 950.3× gram for 1 minute. The height of the emulsified layer and the total content of the tube were measured after 24 hours. The emulsifying activity and was calculated using Equation below:

$$Emulsifying\ capacity = \frac{H2}{H1} \times 100 -------- 5$$

Where the H2 is the height of emulsified layer in the tube (millimeter) and H1 is the height of the total contents in the tube (millimeter). The emulsion stability was determined by measuring the total height after 5 to 120 minutes of the emulsion formation.

- *Color Measurement*

Color was evaluated (CIELab parameters) using a Chroma Meter CR-300C colorimeter (MINOLTA, Tokyo, Japan). Lightness (L*), equilibrium between green and red (a*), and equilibrium between yellow and blue (b*), were measured and total color difference (ΔE*) and browning index (BI), were calculated following [1] using the I6.4 as reference. Measurements were performed ten times for each sample, and the average value was reported.

$$\Delta E* = \frac{((\Delta L*)2 + (\Delta a*)2 + (\Delta b*)2)1}{2} ------ 6$$

$$BI = \frac{100}{0.17} x \left(\frac{a* + 1.75L*}{5.645L* + a* - 3.012b*} - 0.31 \right) --- 7$$

➢ *Sensory Evaluation*

Organoleptic evaluation of the enriched gari samples was carried out using 15 semi-trained panelists made up of staff and students of Taraba State Polytechnic Suntai Jalingo Campus who were familiar with soaked *gari* cosumption. Sensory evaluation of the taste, appearance, flavour, particle size, mouthfeel and overall acceptability of the *gari* samples were carried out using a 9-point hedonic scale where 9 represents like extremely and 1 represents dislike extremely [21].

➢ *Statistical Analysis*

The experiment design was a 1 x 5 factorial in a completely randomized design (CRD). All measurements apart from sensory evaluation were conducted in triplicates. Experimental data were analyzed using computer software (IBM SPSS Statistics 19.0 IBM, Chicago, Illinois, USA). One-way Analysis of Variance (ANOVA) and Duncan's multiple range tests was performed to compare mean value. Average values are considered significantly different when $p < 0.05$.

III. RESULTS AND DISCUSSIONS

➢ *Percent Yield of Protein Isolates*

The Protein Isolates percent yield of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean were high and commendable. Cowpea had the highest yield of 69.87 percent, while AYB had the least value 49.3 percent as shown in Table 1.

The cowpea protein yield obtained in this study was higher than the values (54.48 -59.68 percent) reported for cowpea as in [5]. This could be due to varietal differences.

➢ *Physicochemical Composition of Protein Isolates*

The protein isolates were characterized for their physicochemical composition, as shown in Table 2. There were significant differences ($p<0.05$) in the pH, colour, acidity, moisture, crude protein and crude fat of the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean . The moisture content of protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean were measured percent 6.55, 5.02, 5.16, 4.63 and 5.88 respectively. The crude protein content of protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean were 71.42, 76.9, 74.40, 73.62 and 88.90 percent, respectively. The crude fat content of the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean were 4.18, 4.37, 3.73, 2.20, and 8.45 percent, respectively. The pH of protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean were 3.84, 4.40, 3.72, 3.61 and 4.07,

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respectively. The colour of the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean was 7.34, 5.40, 7.12, 7.67 and 7.57 respectively. Also, the acidity of the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean was 1.37, 2.38, 2.52, 2.13 and 1.20 percent, respectively.

There were significant differences (p<0.05) in the moisture content of the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean (Table 2). The protein isolates of Cowpea, Pigeon Pea, African Yam Bean, 5.02, 5.16, 4.63 percent respectively were similar (p>0.05) in moisture content but were significantly (p<0.05).

Table 1 Protein Isolates Production and Yield

Legume	Quantity of slurry (Litre)	Initial pH/Temp. Degree Celsius ^{0}C	Quantity of 1N NaOH added (millilitres)	Quantity of 1N HCl added (millilitres)	Final pH	Initial weight (kilogram)	Final Weight (kilogram)	Yield (percent)
Soybean	30	6.39 (27.24^{0}C)	1000 (10.04 /29.3^{0}C)	2250	4.5	3.48	1.71	50.86
Cowpea	30	6.60 (28.6^{0}C)	300 (8.04 /28.9^{0}C)	900	4.5	12.68	3.82	69.87
African yam bean	30	6.40 (28.5^{0}C)	800 (8.68 /28.3^{0}C)	1520	4.54	4.58	2.32	49.3
Bambara groundnut	30	6.07 (27.2^{0}C)	850 (8.78 /27.3^{0}C)	1110	4.51	4.4	1.4	68.1
Pigeon pea	30	5.91(28.8^{0}C)	500 (8.63 /29.0^{0}C)	780	4.61	3.94	1.35	65.7

Different when compared with Bambara groundnut and Soybean, 6.55 and 5.88 percent. The moisture content of Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates obtained in this study were lower than 8.7, 8.3, 5.1 and 7.7 percent respectively, as reported by these researches [31], [32], [20] and [33], while the moisture content of Bambara groundnut protein isolates obtained was higher than 2.11 percent reported for Bambara groundnut protein isolates by [23]. This low moisture of the protein isolates powder may prevent and control microbiological spoilage, thus promoting shelf stability.

Significant (p<0.05) differences were also observed in the crude protein content of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates. Soybean has the highest protein value followed by cowpea, while Bambara groundnut had the least protein value as shown in Table 2. The crude protein content of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates obtained in this research were within the range 67.6 to 92.0 percent reported by these researches [31], [32], [20], [23] and [33].

There were significant differences (p<0.05) in the pH of the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean, though some of them were similar (p>0.05) to each other. The pH value of protein isolates obtained (3.6 – 4.4) in this study agreed with the standard pH of a clear whey protein (4.0) as in [30], [28], [38]. Low pH enhances solubility [29].

Also, significant differences (p<0.05) existed in the acidity of the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean, values ranging from 1.2 to 2.52. Pigeon Pea had the highest acidity while soybean recorded the least value. The acidity of a protein isolate is critical because it dictates solubility and improves emulsifying activity of protein isolates and increase solubility in beverages as reported in [38], [27], [30].

Colours of protein isolates were significantly (p<0.05) different among samples. Values ranged from 5.40 to 7.67, with the highest colour intensity recorded for African Yam Bean, while cowpea showed the least colour. This could be due to varietal differences and natural pigmentation. The colour of protein isolates of cowpea obtained agreed with the 5.13 – 10.95 reported for same by [5]. The colour of protein isolates is crucial for consumer acceptance, as it directly influences perception of flavor, freshness and purity [26]. It influences final food product colour, which is a major factor in sensory evaluation, taste expectation and overall consumer acceptability [9].

➢ *Functional Properties of Protein Isolate*

The protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean exhibited significantly different emulsion capacities (p<0.05), ranging from 41.25 to 47.34 percent (Table 3).

Soy bean protein isolate showed the highest (47.34 percent) emulsion capacity significantly outperforming the protein isolates of Bambara groundnut, Cowpea, Pigeon Pea and African Yam Bean. Although, all the protein isolates produced in this study had good emulsion capacity due to their low pH and acidity. This result supports [27] and [36] who reported that protein acidity enhances emulsification and align with [29] and [35] who found that protein isolates with greater solubility and low pH form finer emulsion. This result is also consistent with the emulsion capacity of cowpea protein isolate (2.87 to 5.4meter square per kilogram) reported by **[**39**]**, but higher than the value 1.40 percent reported for pigeon pea by [32].

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In this study, Water Absorption Capacity of protein isolates were significantly ($p<0.05$) different among samples except for Bambara groundnut and African yam bean that were similar ($p>0.05$). Values ranged from 1.55 to 3.01gram per milliliter, with cowpea having the highest value (3.01 gram per milliliter), while pigeon pea recorded the least value (Table 3). Low Water Absorption Capacity of protein isolates obtained in this study was due to the amount and nature of the hydrophilic constituents and, to some extent, on pH and the nature of the protein. The values obtain are lower than the ones (6.6 gram per gram, 52-59.86 percent, 155 percent, 1.15 milliliter per gram), and 6.0 gram per gram) published for Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates respectively [23], **[**39**]**, [32], [20]. Water Absorption Capacity reflects a proteins ability to retain moisture within a food matrix, a critical.

Table 2 Physicochemical Composition of Protein Isolates

Parameters (Percemt)	**Bambara groundnut**	**Cowpea**	**Pigeon Pea**	**AYB**	**Soy Bean**
pH	$3.84^{bc}\pm0.04$	$4.40^{a}\pm0.36$	$3.72^{c}\pm0.02$	$3.61^{c}\pm0.03$	$4.07^{b}\pm0.04$
Colour	$7.34^{c}\pm0.03$	$5.40^{e}\pm0.01$	$7.12^{d}\pm0.01$	$7.67^{a}\pm0.06$	$7.57^{b}\pm0.04$
Acidity (%)	$1.37^{d}\pm0.03$	$2.38^{b}\pm0.01$	$2.52^{a}\pm0.02$	$2.13^{c}\pm0.02$	$1.20^{e}\pm0.02$
Protein (%)	$71.42^{e}\pm0.1$	$76.9^{b}\pm0.11$	$74.40^{c}\pm0.06$	$73.62^{d}\pm0.01$	$88.90^{a}\pm0.1$
Fat (%)	$4.18^{c}\pm0.02$	$4.37^{b}\pm0.03$	$3.73^{d}\pm0.06$	$2.20^{e}\pm0.01$	$8.45^{a}\pm0.03$
Moisture (%)	$6.55^{a}\pm0.13$	$5.02^{c}\pm0.02$	$5.16^{c}\pm0.02$	$4.63^{c}\pm0.15$	$5.88^{b}\pm0.07$

Values are means of 3 replications. Means within the same row with the same superscript are not significantly different (p> 0.05) AYB =African yam bean.

Table 3 Functional Properties of Protein Isolates

Parameters	**Bambara groundnut**	**Cowpea**	**Pigeon Pea**	**AYB**	**Soy Bean**
Bulk Density(g/ml)	$0.84^{d}\pm0.00$	$0.89^{c}\pm0.00$	$0.99^{a}\pm0.00$	$0.90^{b}\pm0.00$	$0.69^{e}\pm0.00$
Emulsion Capacity (%)	$43.76^{c}\pm0.03$	$41.25^{e}\pm0.02$	$46.27^{b}\pm0.02$	$42.27^{d}\pm0.03$	$47.34^{a}\pm0.14$
Viscosity (NS/m^2)	$28.41^{c}\pm0.17$	$30.23^{a}\pm0.15$	$29.50^{b}\pm0.03$	$27.07^{d}\pm0.06$	$26.51^{e}\pm0.35$
Swelling Capacity (%)	$28.57^{c}\pm0.03$	$16.67^{d}\pm0.03$	$44.46^{a}\pm0.04$	$37.51^{b}\pm0.01$	$28.55^{c}\pm0.02$
WaterAbsorption Capacity (g/ml)	$1.81^{c}\pm0.03$	$3.01^{a}\pm0.02$	$1.55^{d}\pm0.05$	$1.76^{c}\pm0.05$	$2.01^{b}\pm0.03$

Values are means of 3 replications. Means within the same row with the same superscript are not significantly different (p> 0.05) AYB =African yam bean.

Attributes in formulating plant based foods such as sausages, custard and dough [14]. It is primarily driven by the abundance of polar amino acids residue on the protein surfaces, which facilitate hydrogen bonding with water molecules.

Bulk density of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates were studied (Table 3). Statistical analysis proved that highly significant difference ($p < 0.05$) existed for all protein isolates in bulk density. The bulking density values ranges 0.69 - 0.90 gram per milliliter, with pigeon pea having higher (0.99) values than the other protein isolate. The Bulk density results are used to evaluate the flour heaviness, handling requirement, and the type of packaging materials suitable for storage and transportation of food materials [18].

It indicates different packaging spaces and materials required for various food samples. Bulk density is a reflection of the load the samples can carry if allowed to rest directly on one another [3]. The bulk density of Bambara groundnut (0.84 gram per milliliter), Pigeon Pea (0.99 gram per milliliter) and African Yam Bean (0.9 gram per milliliter) protein isolates obtained in this study were higher than values reported for same (0.56 gram per milliliter, 0.62 gram per centimeter cube and 0.49 gram per milliliter respectively) by [34], [32] and [20]. It has been reported that bulk density is influenced by the structure of the starch polymers, and a loose structure of the starch polymers could result in low bulk density [11]. The low bulk density observed in the protein isolates is a good physical attribute in terms of transportation and storability [3]. Low bulk density is also important in infant feeding, where less bulk is desirable for their small stomach as it engenders consumption of more quantity of the lighter food items and consequently, more nutrients for the infants.

Swelling capacity is primarily influenced by the material's internal structure (cross-linking density, particle size), environmental conditions (pH, temperature, salinity), and external loading. Increased cross-linking reduces swelling, while higher pH (alkaline) and lower salinity enhance it. High initial density decreases capacity. Protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean exhibited significant ($p<0.05$) difference in swelling capacities. The values ranged from 16.67-44.46 percent, with cowpea recording the lowest swelling capacity. Swelling capacities obtained in this study were low and could be attributed to their low pH and high initial bulk density. Reference [19] reported a higher swelling capacity (2.17 gram per centimeter cube for locust bean than that of pigeon pea (1.29 gram per centimeter cube).

There were significant ($p<0.05$) differences in the viscosity of protein isolates of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean as shown in Table 3. The values ranged from 26.51 to 30.23 Newton second per meter square and were low due to their low pH values. Cowpea was more viscose than the other protein

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isolates. Reference [24] also reported low viscosity for soy isolates due to pH shift. In food systems, low-viscosity isolates with high solubility are often desired, achieved through techniques.

Table 4 Solubility Test of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean Protein Isolates

Samples	Water	HCL (0.1N)	Chloroform
Bambara groundnut	Soluble	Soluble	Insoluble
Cowpea	Soluble	Soluble	Insoluble
Pigeon Pea	Soluble	Soluble	Insoluble
AfricanYam Bean	Soluble	Soluble	Soluble
Soybean	Soluble	Soluble	Soluble

Like pH shifts and heat treatment, as shown in studies on soy protein isolates [24].

- *Solubility Test of Bambara Groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean Protein Isolates*

The solubility of the Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates were also tested in different solvent as shown in Table 4. All the protein isolates were soluble in water and HCL, except in chloroform were African Yam Bean and Soybean protein isolates were soluble owning to some residual, lipophilic (fat-loving) proteins or lipids within the isolate (Table 2). Reference [22] also reported solubility of soy protein isolates in chloroform. According to [22], Soy protein isolates are generally not highly soluble in non-polar solvents like chloroform, but rather, their interaction with chloroform is often linked to the extraction of residual, lipophilic (fat-loving) proteins or lipids within the isolate. Chloroform-methanol mixtures are specifically used to remove these, as lipophilic components are more soluble in non-polar solvents than the bulk polar protein structure.

- *Amino Acids Composition of Bambara Groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean Protein Isolates*

Amino acids composition of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates are illustrated in Table 5, results show high significant ($p<0.05$) differences among protein isolates. Soy bean protein isolates was higher in leucine, isoleucine, phenylalanine, Tryptophan, Tyrosine, Glutamic acid and Glycine than the others, while Pigeon pea abound more in Lysine, Tryptophan, Valine, Proline, Arginine, Histidine, Alanine, Glycine, threonine, Serine and Aspartic acid than the other protein isolates. African Yam Bean recorded higher values in Methionine and cysteine than the others. However, the amino acids values for Bambara groundnut protein isolates are also high and could compared with the values reported for Bambara groundnut by [2] and [12].

- *The Total Essential Amino Acid (Total EAA) Content of Bambara Groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean Protein Isolates*

The total essential amino acid (Total EAA) content of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates is presented in Fig 1, values are highly significant ($p<0.05$) among protein isolates. The total EAA values ranged from 39.7 - 42.98 gram per100gram protein, indicating highest value in soybean. The total EAA values of soybean and pigeon pea protein isolates are similar and were higher than that of Bambara groundnut, Cowpea and African Yam Bean protein isolates as in Fig.1. The essential amino acid content (EAA) of Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates obtained in this study were higher in arginine, valine, phenylalanine, lysine, histidine and leucine when compared to the FAO/WHO reference pattern in [12]. More to that, their total EAA values were also higher than the provisional amino acids pattern recommended by FAO/WHO [12]. The high lysine content of the Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates is a boost to their nutritional value; this will promote their use as supplementary protein in the formulation of cereal products that are deficient in lysine.

Amino acids are organic compounds, which are precursors of proteins; therefore, they influence the quantity and quality of protein as in [2]. Amino acids are categorised as essential and non-essential and vary according to animal species and their production system. They are indispensable in the production of enzymes, immunoglobins, growth, hormones, and repair of body tissues and form the structure of red blood cells. They play an important role in the formation of glucose, function as buffer when other precursors are in limited supply. Amino acids are essential for the performance of specific functions in the body [12].

- *Sensory Evaluation of Gari Enriched with the Bambara Groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean Protein Isolates*

The result of the sensory evaluation of gari enriched with 10 percent each of Bambara groundnut (BG), Cowpea (CG), Pigeon Pea (PG), African Yam Bean (AG) and Soybean (SG) protein isolates is shown in Fig 2.

There was no significant ($p>0.05$) difference in appearance between the 10 percent Bambara groundnut protein isolate enriched gari (BG) and the control (100 percent cassava gari [GA]), while the other enrichment differ ($p<0.05$) significantly from the control as shown in Fig 2. Gari enriched with Cowpea, African Yam Bean and Soybean protein isolates were similar ($p>0.05$) in appearance. Gari enriched with Pigeon Pea protein isolates differ ($p<0.05$) significantly from others in appearance. This is owned to the redish brown colour of the pigeon pea seed coat. Enrichment of gari with Bambara groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean protein isolates did not alter ($p>0.05$) the sensory attributes of the gari in terms of particle size, flavor, taste and mouthfeel, except in overall acceptability, where the control gari sample differs ($p<0.05$).

Table 5 Amino Acids Profile of Bambara Groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean Protein Isolates (g/100gProtein)

Amino Acids	Bambara groundnut	Cowpea	Pigeon Pea	AYB	Soybean
Leucine	7.62^{d}±0.02	8.15^{b} ±0.01	7.83^{c}±0.02	7.51^{e}±0.02	8.33^{a}±0.03
Lysine	4.36^{d}±0.01	3.85^{e}±0.02	4.96^{a} ±0.02	4.53^{c}±0.02	4.74^{b}±0.02
Isoleucine	3.31^{e}±0.01	3.67^{b}±0.02	3.51^{d}±0.01	3.60^{c}±0.01	4.13^{a}±0.02
Phenylalamine	4.98^{c}±0.01	4.35^{e}±0.02	4.89^{d}±0.01	5.32^{b}±0.02	5.50^{a}±0.01
Trytophan	1.21^{c}±0.01	1.26^{b}±0.01	1.34^{a}±0.01	1.23^{c}±0.03	1.36^{a}±0.01
Valine	4.12^{d}±0.01	3.95^{e}±0.02	4.59^{a}±0.01	4.34^{b}±0.01	4.21^{c}±0.02
Methionine	1.31^{d}±0.02	1.40^{c}±0.01	1.43^{b}±0.01	1.51^{a}±0.02	1.21^{e}±0.02
Proline	5.39^{c}±0.01	4.77^{e}±0.01	6.09^{a}±0.02	5.49^{b}±0.01	4.88^{d}±0.02
Arginine	7.14^{b}±0.01	6.55^{d}±0.01	7.32^{a}±0.01	6.89^{c}±0.01	7.13^{b}±0.01
Tyrosine	3.44^{c}±0.02	3.61^{b}±0.01	3.62^{b}±0.01	3.61^{b}±0.02	3.79^{a}±0.01
Histidine	3.13^{c}±0.01	3.26^{b}±0.02	3.40^{a}±0.01	3.26^{b}±0.03	3.06^{d}±0.01
Cystine	1.34^{d}±0.01	1.39^{c}±0.01	1.51^{b}±0.02	1.63^{a}±0.02	1.39^{c}±0.01
Alanine	4.11^{d}±0.01	4.36^{c}±0.01	4.59^{a}±0.01	4.51^{b}±0.02	4.55^{b}±0.04
Glutamic Acid	13.48^{d}±0.01	13.03^{e}±0.02	14.09^{b}±0.01	13.71^{c}±0.02	14.34^{a} ±0.03
Glycine	3.71^{c}±0.02	3.61^{d}±0.02	4.16^{a}±0.01	3.92^{b}±0.02	4.15^{a}±0.01
Threonine	3.59^{b}±0.01	3.26^{e}±0.01	3.67^{a}±0.01	3.41^{c}±0.01	3.31^{d}±0.01
Serine	4.21^{c}±0.01	3.76^{e}±0.01	4.51^{a}±0.01	4.33^{b}±0.01	3.83^{d}±0.02
Aspartic Acid	9.31^{c}±0.01	8.62^{e}±0.01	9.56^{a}±0.01	9.40^{b}±0.02	8.89^{d}±0.02

Values are means of 3 replications. Means within the same row with the same superscript are not significantly different (p> 0.05) AYB =African yam bean

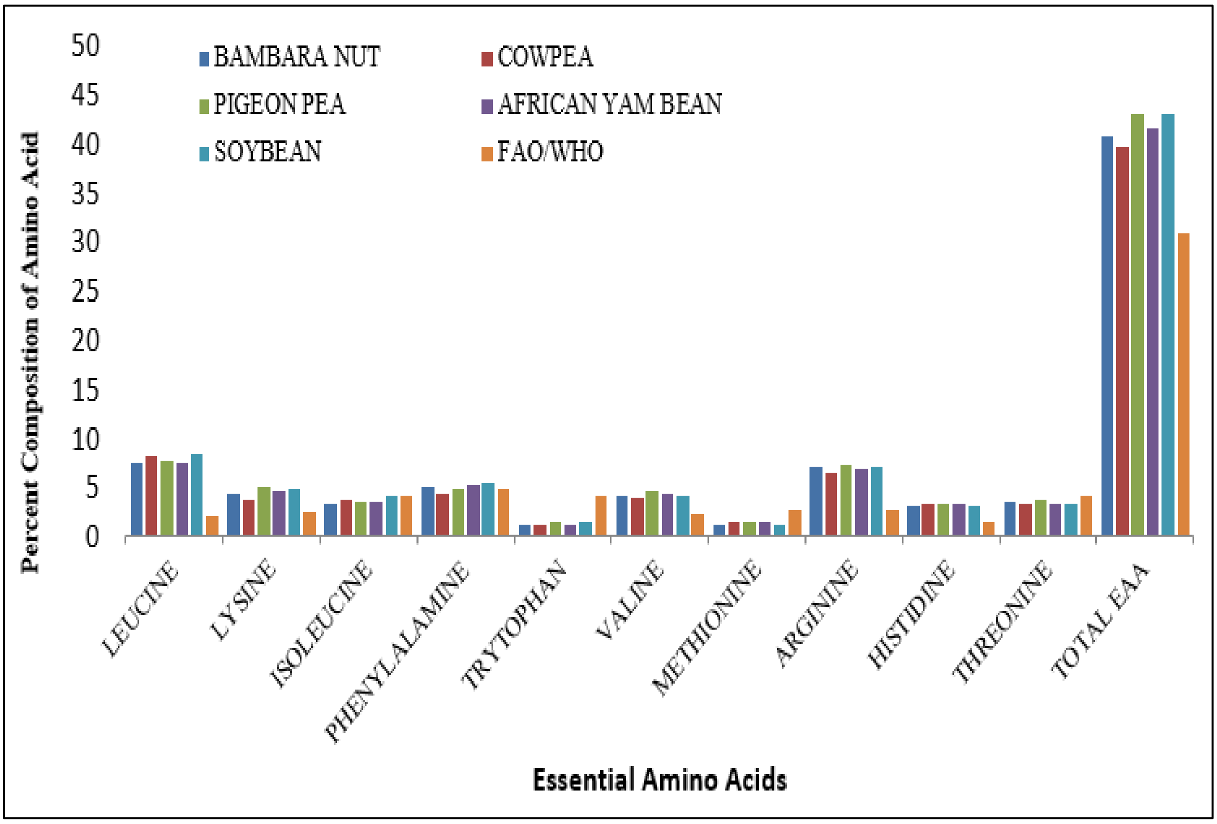

Fig 1 Essential Amino Acids (EAA) Profile of Bambara Groundnut, Cowpea, Pigeon Pea, African Yam Bean and Soybean Protein Isolates Protein Isolates
*Provisional Amino Acids Pattern Recommended by FAO/WHO [12].

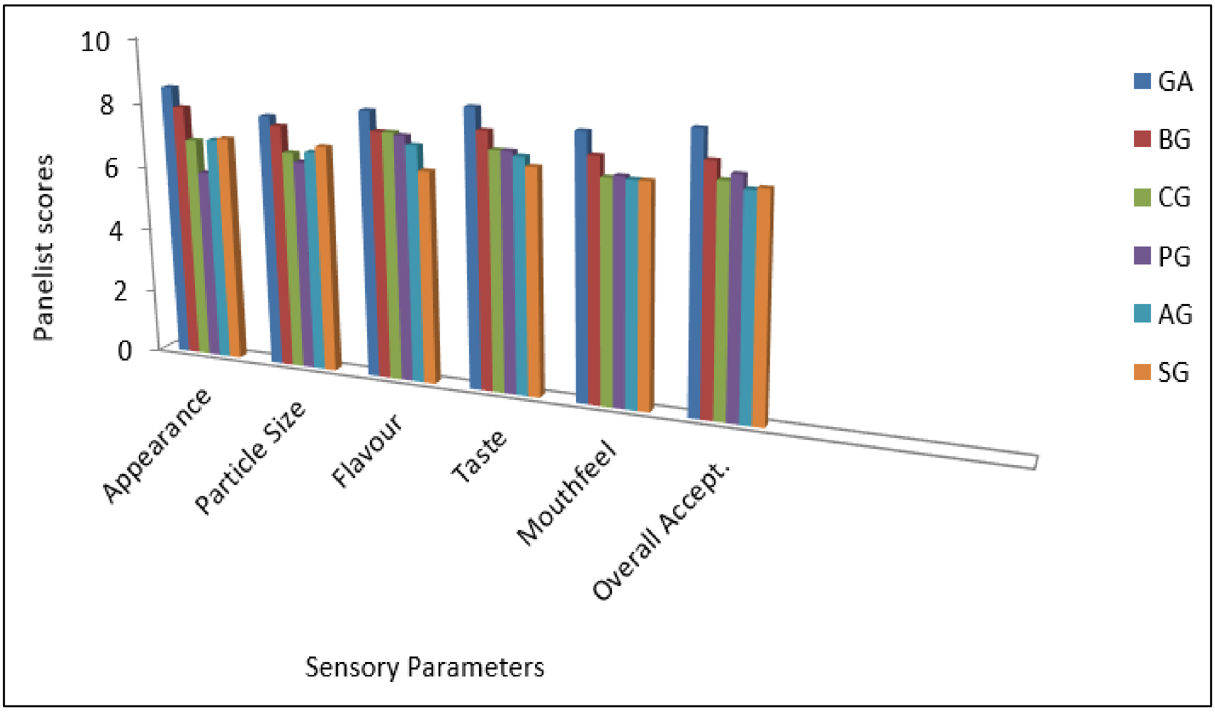

Fig 2 The Result of the Sensory Evaluation of Gari Enriched with the Bambara Groundnut (BG), Cowpea (CG), Pigeon Pea (PG), African Yam Bean (AG) and Soybean (SG) Protein Isolates

Significantly from other gari enriched samples. The values for the acceptability ranged from 6.8 -7.5, indicating that the enrichment was accepted.

IV. CONCLUSION AND RECOMMENDATIONS

This study has extensively amplified the possibilities and benefits associated with the production as well as chemical, physiochemical and sensory properties of protein isolate powders obtained from four tropical legumes. All samples showed low moisture content and high acidity, which will support storage stability. Similarly, in terms of crude protein content, soybean is found to be the richest although each isolate contained above 70 percent proteins. The physiochemical parameters like bulk density, viscosity, emulsion and water absorption capacities all showed positive and interesting data, portraying the suitability of these isolates in other food systems. All isolates were found soluble in water and acids but not in chloroform, and exceeded the FAO standards in terms of amino acids. Values for glutamic acid, aspartic acid, proline, leucine among others, were highest in soybean, which also ranked highest in total essential amino acids content. In terms of sensory evaluation, the panelists were favourably disposed towards gari samples enriched with the isolates, by scoring all above average, although pigeon pea isolate enriched gari had lowest score due to its appearance.

It is therefore recommended that protein isolates from soybeans, bambara groundnuts, pigeon pea and cowpea should be commercialized as well as embraced and incorporated to daily meals of all individuals in standard recommended concentrations, especially for low protein foods for nutrient enrichment. Further research is encouraged to ascertain the shelf life of the isolates and optimized production procedures for more yields of the products. Subsequent research is also encouraged towards the utilization of the waste or by products generated this study for higher impact.

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