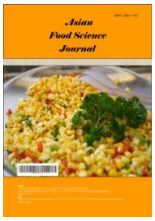

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Physico-Functional Characterization of Flour and Protein Isolates from Nigeria Cultivated Solojo Cowpea [*Vigna unguiculata* (L.) WALP]

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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, an important protein food, is used for its nutritional and functional properties. A study was carried out to determine the physico-functional characteristics of Flours and Protein Isolates from Two (2) Varieties of Nigerian Cultivated Solojo Cowpea (*Vigna Unguiculata* L. Walp), to assess their potential use in the food industry. Functional properties were analysed which include Moisture, crude protein, crude fat, crude fibre, ash, carbohydrate and dry matter which were in the range of 9.15-9.83, 26.53-29.00, 2.50-3.99, 2.95-3.22, 4.24-4.80, 50.95-53.98 and 90.17-90.85% respectively. Bulk density ranged between 0.69 and 0.80 g/dm^3. Water and oil absorption capacities ranged between 1.89 and 2.15, and 1.95 and 2.31 ml/g, respectively. Swelling power

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had values varying from 265 to 268% while foam capacity varied from 10.00 to 21.00 ml. The effect of ionic strength on foaming capacity (FC) and stability (FS) of the protein isolates using standard methods was carried out. Amino acids and molecular weight of the protein isolates were determined by amino acid analyser and sodium-dodecyl-sulphate-polyacrylamide-gel-electrophoresis. Surface morphology, functional group and thermal properties were determined for protein isolates by scanning electron microscopy, Fourier Transform Infrared (FTIR) spectrometry and differential scanning calorimetry, respectively. The results indicate that the two varieties of cowpea have great potential as functional agents in the food industry.

Keywords: Solojo cowpea; under-utilised legumes; protein isolate; functional; nutritional; DAS; BS; Vigna unguiculata L. Walp.

1. INTRODUCTION

"Solojo" belongs to the cowpea family of Vigna unguiculata, it is an underutilized legume whose growth is now limited mostly to the northern part of Oyo State and some other places like, Ilawo in Ejigbo Osun state, both in Nigeria, where it is also known as "Gbawojo". This indicates that "Solojo" may be under serious threat of extinction. "Solojo" comes in dark ash, brown and white varieties. They are not easily cooked as normal beans but are very delicious. The brown and white varieties are mostly used for moi moi (beans pudding) and akara (beans ball) because of the difficulty in cooking whole seed. It is able to withstand much water, unlike the other legumes, which is what informed the name. It is planted in rainy seasons between April and August. The pods of Solojo are tougher than normal cowpea and the leaves are bigger. Agbogidi and Egho [1] in their research sought for a variety of cowpea that will be able to withstand the weather condition of the southern Nigeria, Asaba in Delta state in particular, so that the production of cowpea will not be seasonal as is at present. Of the eight (8) varieties tested, only three (3), Ife Brown, IT848-2246-4 and TV3236, were found to be suitable. Solojo could be a solution to the issue of finding a variety of cowpea that will be able to stand the weather condition of southern Nigeria because Solojo is actuallya raining season crop. Not much has been done on this crop other than agronomical studies. If the cultivation of this crop is improved, there will be food security and provision of employment as value is being added to the crop by finding other industrial uses for it other than just food use.

Legumes perform a major function in the acceptance of uninteresting diet in various sectors of the universe. They occupy an important role as protein supplement in the daily intake of food, majorly based on cereals and millet. Not only do they provide different menu sources for human food, they are cheap sources of alternative protein [2], (Khalid and Elhardallou, 2015), [3].

All product from protein of plant origin, now perform very important part in people's diet, particularly in nations that are developing where not enough protein is consumed. Product from protein of plant origin are becoming more popular as integral part of food system, the benefit conferred on the food determines the extent of their usage as food ingredient [4,5,6], (Mao and Hua, 2014). Plant proteins as functional ingredients has attracted a lot of interest recently, especially legumes because they have 18-25% protein [4,7]. The success of the utilisation of legume proteins as food ingredient is, dependent upon their possessing applicable functionality aside from being protein of good quality and sensory character (Heng, 2005). As grain legumes utilisation is gaining prominence in food formulation as composite flour, the importance of their functional properties has increased; functional properties are those properties of protein that predicts the use to which it can be put [8,9].

2. EXPERIMENTAL

2.1 Materials and Methods

Two varieties of the underutilized cowpea (*V. unguculata*) found in South west region of Nigeria where it is called 'Solojo' were used. Seeds obtained from Bodija market in Ibadan, Western Nigeria, were screened to get rid of every irrelevant materials and unwholesome seeds. The beans were then portioned into six (6). The Solojo seeds for germination were sterilised by soaking in 0.07 % Sodium hypochlorite [10] for 30 min, then, it was rinsed thoroughly. The Solojo seeds were then immersed for 6 h in distilled water at ambient

temperature (1:10 w/v) (~25oC), then placed in a colander and germinated under subdued light in an open laboratory [11] for, 24, 36, 48 and 72 h.

2.2 Preparation of Flours

Raw flour: The grains were segregated to remove the spoilt ones; then dry dehulled with a mechanical dry dehuller (Fabricated in FIIRO), dried at 40^{o}C and later milled dry to powder then sifted using 80 µm mesh. The flour was stored in flexible bags and preserved at 4^{o}C preceding utilisation in a refrigerator freezer.

6 h Soaked flour: The seeds were segregated to remove the unwholesome ones, then immersed for 6 h in the ratio (1:10 w/v) (seed/water). The grains were then frozen to prevent germination from setting in, then the hull was removed manually, dried for 48 h at 40^{o}C later milled dry to smooth powder prior to sieving using 80 µm mesh screen. The resulting flour was packaged in plastic pack and preserved in a fridge-freezer at 4oC pending utilization.

Germination of seed: This was implemented by the method of Mubarak [12] with minor adjustment. The seeds for germination were disinfected by soaking in 0.07% Sodium hypochlorite [10] for 30 mins, then, it was rinsed painstakingly. The Solojo seeds were then immersed for 6 h at ambient temperature in water in the ratio (1:10 w/v) (seed/water) (~25^{o}C), then placed in a colander and germinated under subdued light in an open laboratory [11] for various h, 24, 36, 48 and 72 h. The process of germination was terminated by freezing, the seeds were manually dehulled, dried in a draught oven (Schutzart DIN EN 60529-IP 20. Memmert, Germany) at 40^{o}C for 48 h, cooled, milled and packaged in an air tight plastic bag in the refrigerator pending analysis.

2.3 General Analysis

2.3.1 Proximate analysis

The procedures of Association of Official Analytical Chemist [13] were utilised in determining the nutritional and chemical formation of the flours.

Fig. 1. Dark- ash solojo cowpea (*Vigna unguiculata*)

Fig. 2. Brown solojo cowpea (*Vigna unguiculata*)

The following analyses were carried out:

Moisture, Ash, (Fat i.e. lipids content, Protein content, Fiber) all in crude form and Carbohydrate as Nitrogen free extractive (NFE). The carbohydrate (NFE) content is calculated by difference, using the values obtained from the parameters enumerated.

$$\%\ \text{Carbohydrate} = 100 - (\text{Moisture} + \text{Crude fat} + \text{Ash} + \text{Crude Fiber} + \text{Protein})$$

2.3.2 Isolation of protein

Alkaline solubilisation and precipitation by isoelectric method were utilised for the processing of the protein isolate [5], (Wang et al., 2011); [14]. Flour of Solojo was dislodged in water, ratio (1:20 w/v), 0.5 M NaOH was used to adjust to pH 9.5, mixed thoroughly for 30 mins with magnetisable stirrer. Centrifugation of the slurry at 3,500 xg for 20 minutes using a laboratory centrifuge by Hitachii then followed. The alkaline slury, comprising of dissolvable protein, was poured out into a big beaker, 0.5 M HCl was used to adjust the pH to 4.5, then permitted to mix for 10 min. Centrifugation was used to recover the precipitated protein at 3,500 xg for 20 mins. The precipitated protein was freeze dried after neutralisation.

2.3.3 Crude protein determination

The AOAC micro Kjeldahl method was used. One (1 g) gramme of the test material was measured into a boiling tube, 10 mL of concentrated sulphuric-acid (Conc H_2SO_4) added and selenium tablet as catalyst. The mixture was boiled on a pre-heated digestion block maintained at 420^{o}C in a fume cupboard for 1 h. by which time a clear digest was obtained. A reagent blank was also carried out at the same time. The digestion flask is connected to a receiving flask by a tube. The solution in the digestion flask is then made alkaline by the addition of 50 mL of 40 % sodium hydroxide which converts the ammonium sulphate into ammonia gas the ammonia gas that was formed was steam distilled into a 250 mL conical receiving flask comprising 30 mL boric acid which traps the dissolved ammonia gas, into the boric acid is added a mixture of methyl red and bromocresol green as indicator. The low pH of the boric acid solution in the receiving flask converts the ammonia gas to ammonium ion, and simultaneously converts the boric acid to the borate ion, distillation continued till 150 mL of the distillate was collected. This ammonium borate formed was titrated against standardised 0.1 M HCl to violet end point, the titre value was recorded and used to estimate the nitrogen content.

Calculations:

Kjeldahl nitrogen % N $= \frac{Vs-Vb}{W} \times M \times 0.014$ x100

% Crude protein = % Kjeldahl N $\times F$.

In which,

N = Nitrogen, Vs= Volume of standard acid used to titrate a test sample (mL)
Vb= Volume of standardized acid used to titrate reagent blank mL), M = Molarity of standard HCl
14.01 g = Molecular weight of nitrogen N,
W = Weight (g) of test portion or standard
10 = Factor to convert mg/g to percentage and
F = Factor to convert N to protein.

2.4 Crude Fibre Determination

Two grammes of the test item was boiled in 200 mL of 1.25 % H_2SO_4 under reflux for 30 mins. The whole content was then turned into a buckner funnel fitted with muslin material and fastened with elastic tape. This was permitted to strainer and the leftover portion was then washed with boiling water till discharged of acid. The residue was placed in 200 mL 1.25 % sodium hydroxide and allowed to boil for 30 mins, then drained. This was washed two times with ethanol and the residue rinsed three times with petroleum ether. This was dried in an oven and latter ashed in a furnace at 550^{o}C. The change in measurement (loss on ignition) was reported as crude fibre and expressed as percentage of the starting weight.

Percentage crude fibre = % Crude fibre

$$= \frac{\text{Loss on ignition (Wt of dish + fibre(w1) } - \text{ Wt of dish + Ash(W3)}}{\text{Weight of sample(W1)}} \times 100 = \frac{\text{W1} - \text{W2}}{\text{W1}} \times 100$$

2.5 Fat Content Determination

A Foss tecator soxtech 2050 fat extractor machine was used for the analysis. A test portion of two grammes was loosely encased in a filter paper and inserted into a timble which is

attached by the aid of a magnetic sample holder to the Soxhlet machine. The extraction solvent, petroleum ether was then added into the aluminium extraction cup which has been previously weighed after drying. The cup is attached to the machine by the aid of a magnetic holder. The thimble was allowed to sit inside the hot extraction solvent for 1 h lifted and allowed to wash for another 1 h, after which the aluminium can was brought out, the thimble was removed and the solvent recovered. The remaining solvent was evaporated off and the can dried till constant reading. The weight difference was considered as the fat quantity and was expressed as percentage of the sample.

Calculation

$$\% \text{ Crude fat (Ether extract)} = \frac{W2 - W1}{W3} \times 100,$$

In which

W_1= Weight of can when empty, (g),
W_2 = Weight of can + residue of fat, (g),
W_3 = Weight of sample, (g)

The residual weight was calculated as percentage ash content of the initial material.

$$\text{Percentage}(\%) = \frac{\text{Weight of test sample(g)} - \text{Weight loss on ashing(g)}}{\text{Weight of test sample(g)}} \times 100 = \frac{W3}{W2} - \frac{W1}{W1} \times 100$$

2.6 Determination of Functional Properties

The physical-chemical attributes of the protein affecting its performance in food scheme through storage, preparation, processing and utilisation are collectively known as functional properties. These functional properties consist of protein solubility, bulk density, water and oil absorption capability, emulsifying activity and stability, foaming capability and stability, and the least gelation concentration (Butt and Batool, 2010), [15].

2.7 pH- Solubility Characterisation- (Protein Solubility Characterisation as Percentage Nitrogen)

The protein solubility was performed utilising the methodology of Lawal et al. [16], Adebowale et al. [17] and Ojo et al. [18]. Hundred milligrams of the flour were suspended in distilled water (20 mL) in various tubes.

The pH was adjusted using 1 M HCl or 1M NaOH to various values between 2 and 10. The obtained slurries were made to equilibrate for 1 h at 24 ^{o}C shaken vigorously before centrifuging at 3,500 xg for 20 mins. The floating portion was filtered to get a clearer filtrate. The quantity of protein in the floating part was obtained by Kjeldahl method as shown in 3.3.3, percent solvable protein was determined as percent N multiplied by 6.25 on wet basis.

$$Solubility\ (\%) = \frac{\text{Amount of nitrogen (N) in the supernant}}{\text{Amount of nitrogen(N) in the sample}}$$

2.8 Packed and Loose Bulk Density

The bulk density measurement was carried out by weighing 50 g of the sample into a hundred (mL) graduated cylinder. The cylinder was lightly hit against a soft surface about 100 times till no more change in volume occurred. The bulk density was determined as weight/ volume of the test material [4].

2.9 Water (H_2O) and Oil (Lipid) Absorption Capacity (WAC and OAC)

Two grams test material was carefully measured into the centrifuge tube, 20 mL distilled H_2O or refined vegetable oil was added and agitated thoroughly with a vortex mixer and made to remain at ambient condition (28 ± 2^{o}C) for 30 mins, then centrifugation at 3,000 xg for 20 mins was performed. The supernatant was carefully poured out, excess water/ oil made to drain off by facing the tube down carefully and the tube was then weighed again. Change in weight compared with the original weight was interpreted as the Water absorption capacity and OAC of the floury material [19,20].

$$\text{WAC/OAC} = \frac{\text{Weight of bound water or oil}}{\text{Weight of sample}}$$

2.10 Water Absorption Capacity/ Effect of Ionic Strength

Thi**s** was investigated as above, using various concentrations of NaCl with noted ionic strength (μ). Study was performed at ionic strength of between 0.1-1.0 M

2.11 Protein /Foaming Properties

The capability to foam and foam durability were executed using the technique as enumerated by Lawal et al. [16]. A measured quantity of flour / isolated protein (2-10 g) was weighed into 100 mL distilled water. The resulting suspension was well agitated energetically in a kitchen liquidizer for 2 mins at extreme velocity, turned out into a calibrated tube and the quantity reported prior of and after blending. The volume (Capacity) increment in percentage was estimated using the equation;

$$\% \text{ Volume (Capacity) of foam} = \frac{C2-C1}{C1} \times 100,$$

Where,

$C_{2=}$ Capacity (Volume) of protein blend after mixing vigorously and
C_1 = Capacity (Volume) of blend prior to mixing vigorously

2.11.1 Foaming properties /Concentration effect

This was performed by mixing vigorously 2 – 10 % w/v, of the dispersed blend as expressed above.

2.11.2 Foaming properties/ pH effect

This was performed by adjusting the pH with either 0.5 M HCl or 0.5 M NaOH, to adjust 2 % w/v blend to the required pH (2.0 to 10) followed by intense whisking as detailed above.

2.11.3 Foaming properties / Ionic strength effect

Ionic strength impact on foaming was assessed by discharging 2 g of protein flour in hundred mL NaCl blend of ionic strength (μ) ranging from 0.0, 0.1, 0.2 -to- 1.0 M accompanied by vigorous mixing as before.

The percentage volume increments directly after whisking determines foam capacity (FC), while percent volume reduction four hours after whipping determines foaming stability (FS) [4]. Three replicate analysis was conducted.

2.12 Emulsifying Activity (EA) and Stability of Emulsion (ES)

A test material of two grams (2 g), along with equal volume (20 mL) of distilled H_2O and vegetable oil, were thoroughly mixed in a calibrated centrifuge tube using a vortex mixer. The emulsion that ensued was centrifuged for 20 mins at 3,000 xg. Height of the emulsion level as a proportion of the entire height of the mixture was determined as the Emulsifying Activity (EA) as percentage. Emulsifying stability (ES) is calculated following boiling the emulsion held in a calibrated centrifuge tube at 80°C for 30 mins in a water bath, cooling for 15 mins beneath flowing water and centrifuging for 20 min at 3,000 xg [16,21,22]. The emulsion stability was calculated using the equation below;

$$\% \text{ Emulsifying Activity (EA)} = \frac{Height\ of\ emulsion\ level\ in\ the\ tube}{Height\ of\ the\ complete\ content\ in\ the\ tube} \times 100$$

$$\% \text{ EMULSION STABILITY (ES)} = \frac{\boldsymbol{Height\ of\ emulsion\ level\ after\ heating}}{\boldsymbol{Height\ of\ the\ complete\ content\ in\ he\ tube}} \times \mathbf{100}$$

2.12.1 Emulsifying properties/ Effect of pH

This was carried out on the test material as above by the adjustment of the pH of 2 g sample in 20 mL solution to the intended range, from 2.0

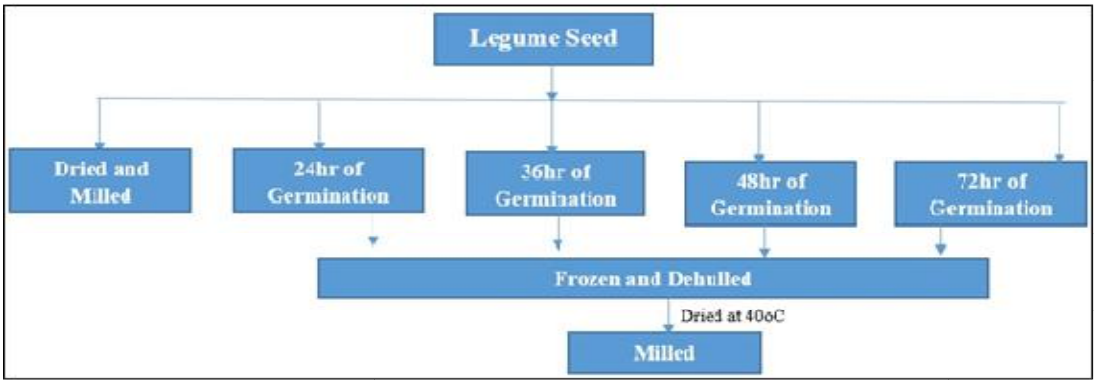

Fig. 3. Preparation of Beans Flour/Schematic representation

to 10.0, with either 0.5 M HCl or 0.5 M NaOH before adding 20 mL vegetable oil and continuing as described above

2.12.2 Emulsifying properties / Ionic Strength Effect

This was conducted on the test material as above; 2 g sample was suspended in NaCl of identified ionic strength (μ), with pH regulated to pH 7.0. Study was carried out at ionic strength range of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 M, before addition of the vegetable oil, and continuing as described above

2.12.3 Emulsifying properties/ Effect of concentration

This was performed as above with sample varying between 2 – 10 % w/v, and the inclusion of 20 mL distilled water, 20 mL vegetable oil.

2.13 Solojo Gelation Properties

Gel forming properties were performed using the technique by Lawal et al. [16]. Mixture of between 2-20 % of the samples were processed by the addition of distilled water. 1 mL of the suspension produced by each was heated in a boiling tubing placed in the water bowl at 100^{o}C for 1 h, accompanied by very quick chilling in water. Boiling tubing were extra chilled at 4^{o}C for 2 h; lowest gelling concentration was interpreted as the quantity at which the test material from the tube placed upside-down did not slide or fall-out.

2.13.1 Gelation properties/ Effect of pH

This was carried out on the test material at varying quantities by altering the pH to the required values, 2.0 to 10.0, preceding boiling, using either 0.5 M HCl or 0.5 M NaOH. Least gelation concentration was also carried out as priorly elucidated.

2.13.2 Gelation properties/ Ionic strength effect

This was examined by producing test material / solvent (2-20 % w/v) range, at varied concentration in NaCl blend of specific ionic strength (μ). Study was carried out at ionic strength range of 0.1, to 1.0 M, after, the pH has been modified to pH 7.0 each, the least gelation concentration was the carried out.

2.14 Elemental Analysis

Two grams (2 g) was measured into a well-cleaned porcelain crucible pre-ashed on a bunsen flame till it stopped smoking. Thereafter, the sample was then exposed to dry-ashing at 550^{o}C for 3 h in a carbolite furnace. Greyish-white ash that was produced was dissolved in 5 mL HNO_3/HC1 (1:1) heated on a Stuart Scientific magnetic stirrer hot plate, (Made in UK) at the boiling temperature of the solution till chocolate coloured smoke vanished. To the residue, addition of 5 mL of distilled-deionised water was done, after which heating of the mixture till a colorless solution ensued. The obtained solution which now contains the minerals was filtered using a filter paper while being conveyed into a standard flask of 100 mL capacity and the quantity adjusted to mark with water (distilled-deionised). The final analysis was accomplished with AAS- Analyst 200 by Perkinelmer USA. PFP7 Flame Photometer by Jenway USA.

2.15 Analysis of Anti-Nutrient

2.15.1 Phytate measurement

Two grams of the test material (Flour/Isolate) was measured into a test tube, containing distilled water of approximately 10 mL. Two millilitres of 0.2 M HCl (aq) was used for the extraction, of which, 0.5 mL was pipetted into a glass stopper fitting tube. 1 mL of ferric solution was then added, and covered with a clip fitted stopper. The tube was placed in a boiling water bowl for 30 mins very well capped with the bung for the initial 15 mins, chilled for 15 mins after and then brought back to ambient temperature. The content was then mixed thoroughly and swired for 30 mins at 3000 xg. Approximately 1 mL of the supernatant was taken and 2,2'-Bipyridine solution (about 1.5 mL) added. Measurement of absorbance was then carried out at 420 nm against distilled water [23].

$$\% \text{ phytate} = \frac{At}{Ar} \times \frac{C}{W} \times \frac{100}{VA} \times Ve$$

where,

At = absorbance of test sample,
W = Sample weight used
Ar = Absorbance of standard solution (Phytate reference solution),
C = concentration of standard solution
Ve = Total volume of extract,
Va = Volume of extract

Table 1. Physico-functional characteristics of nigeria cultivated solojo cowpeas

Moisture content (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Carbohydrates (%)	Dry Matter (%)	Bulk Density (g/dm^3)	Water Absorption Capacities (ml/g)	Oil Absorption Capacities (ml/g)	Swelling Power (%)	Foam Capacity (ml)
9.05-9.85	26.50-29.00	2.50-4.00	2.90-3.25	4.20-4.85	50.90-54.00	90.20-90.90	0.70-0.82	1.85-2.20	1.90-2.35	260-270	9.00-22.00

2.16 Determination of Trypsin Inhibitor

Spectrophotometric method was used as, explained by Nwosu, (2011) with slight adjustment. About (5 g) of the test material was added to 50 mL of 0.5 M NaCl solution and shaken for 30 mins at 27 ^{o}C temperature. It was later centrifuged and the upper liquid filtered using Whatman No 42-filter paper. The obtained clear solution was then assayed. Freshly prepared standard trypsin was then used to treat the substrate solution N–α–benzoyl–DL–arginine–p–nitroanilide hydrochloride (DL–BAPA). Trypsin measurement was carried out on the bases of extent of inhibition. To another tube having 2 mL of extract, was added 10 mL of the substrate (DL-BAPA). A blank was prepared by adding the remaining standard trypsin solution to 10 mL of the substrate in another test tube. The residue was made to equilibrate for 30 mins after which the absorbances of the solutions were evaluated spectrophotometrically at 410 nm wavelength. An increase of 0.01 absorbance unit at 410 nm indicates one trypsin activity unit inhibition [23].

$$\text{Trypsin unit inhibited / 100 g} = \frac{Ax}{At} \times 0.01 \times \text{F}$$

Where,

Ax =Absorbance of test sample,
At = Absorbance of standard (uninhibited sample)
F = Experimental factor given as = $\frac{Ve}{Vs} \times \frac{1}{W}$

Where,

Ve = Total volume of extract,
Vs = Volume of extract analysed and
W = Weight of test material analysed

2.17 Estimation of Total Saponins

The method as enumerated by Ojinnaka and Agubolum, [24] was used with slight modification. Test material of five grams (5 g) was combined with 50 mL of 20 % ethanol solution and incubated at a temperature of 55^{o}C with constant shaking for 12 h. The combination was then filtered using No 42 grade Whatman filter paper. The remaining sample in the filter paper was extracted again with 50 mL of the ethanol solution for another 30 mins and the extracted solutions pulled as one and weighed. The volume of the joint extract was condensed to about 40 mL by evaporation and then switched to a separating funnel and 40 mL of diethyl ether was introduced to it, shaken very well and allowed to settle, the lower aqueous layer was set aside. The set aside aqueous layer was extracted again using di ethyl-ether after which pH adjustment to 4.5 was carried out using dilute NaOH solution added dropwisely. n- butanol of 60 mL and 30 mL portion respectively were used for the extraction of the Saponin from the extract. The precipitate obtained from the combined extract evaporated to dryness in a formerly weighed evaporating dish after having been washed thoroughly with 5 % NaCl solution. The obtained saponin was futher dried at 60^{o}C in the oven (to eliminate any lingering solvent) cooled and weighed till constant weight is obtained. Percentage saponin was calculated with reference to the original sample [23].

$$\% \text{ Saponin} = \frac{M2-M1}{M} \times 100$$

where,

M = weight of sample used,
M1 = weight of empty evaporation dish and
M2 = weight of dish + saponin extract

2.18 Hydrogen Cyanide (HCN) Determination

This was executed by alkaline pikrate colorimeter technique of Ojinnaka and Agubolum, [24]. Into a 250 mL conical flask was added 1 g of the sample along with 50 mL of distilled water, mixed thoroughly. Over the sample blend and the blank in their respective flasks, an alkaline pikrate paper was hung. The arrangement was incubated overnight and each of the pikrate paper was eluted into 60 mL of distilled water. Prepared also was standard cyanide solution and diluted to the requisite concentration. Spectrophotometric measurement of the absorbance was carried out at wavelength of 540 nm with the reagent blank at zero for both the eluted test material solution and that of the standard. The cyanide content was determined by the formula as described by Okwulehie et al., [25].

$$\text{HCN mg/Kg} = \frac{1000}{M} \times \frac{ax}{at} \times C \times D$$

where,

M = Weight of sample analysed,
ax = absorbance of test sample
at = absorbance of standard HCN solution,

D = Dilution factor where applicable
C = concentration of the standard in mg/Kg

2.19 Oxalate Determination

Two grams of the test material was measured and extracted three times at 50°C with agitation for an hour using 20 mL of 0.3 M HCl. The 3portions were pulled together and made up to 100 mL with distilled water and utilised for estimation of total oxalate. The evaluation of the oxalate was done by pipetting about 5 mL of the extract which has been made alkaline with 1mL of 5 M ammonium hydroxide, with three drops of phenolphthalein added to the extract and acetic acid also added dropwisely. About 1 mL of 5% CaCl (aq) was thereafter added to the mixture and allowed toequilibrate for 2 h following which it was centrifuged at 3,000 xg for 15 mins. The supernantant was discarded and the precipitate rinsed thoroughly three times with hot water, before centrifuging each time. To the washed precipitate, was then added 2 mL of 3 M H_2S0_4 and the precipitate made into solution by warming in water bath at 75°C. The dissolved solution was then titrated with newly prepared 0.01 M $KMnO_4$ at ambient temperature till the first pink colour showed all over the mixture. This was then re-warmed at 75°C and the titration carried on till the pink colour lingered [13].

$$\% \text{ Oxalate} = \frac{Vf}{Wg} \times Vem \times Titre$$

Where,

Vf = Total volume of titrate = 100,
Wg = Weight of the sample = 2g
Vem = Volume – mass equivalent (i.e.1cm^3 of 0.05 M KMnO4 is equivalent to 0.00225 g anhydrous oxalic acid)

2.20 Determination of Oligosaccharides

Sugar analysis anthrone method was used [26]. Five grams of the test sample was boiled in 20mL HCl until fully hydrolysed (negative to iodine test). The sample was then filtered through No 42 Whatman -filter paper and washed with more acid until 100 mL filtrate was obtained. Anthrone reagent of 6 mL was then mixed with a test portion (1 mL) of the filtrate in a well-corked test tube. This was boiled in a water bath for 10 mins for both the samples and standard. The samples and the standard were cooled and filtered before the absorbances were read at 620 nm with a spectrophotometer UNICO.S/No 080827. Model- 1201. USA. The result obtained for complete sugar was then multiplied by factors of 0.04 and 0.01 in order to obtain the values of the stachyose and raffinose quantity respectively.

$$\text{Hence, \% Total sugar} = \frac{100}{M} \times \frac{av}{ay} \times \frac{C}{100} \times \frac{Ve}{Va}$$

Where,

M = Weight of sample analysed,
av = Absorbance of test sample,
ay = Absorbance of standard sample,
Ve = Total volume of extract,
Va = Volume of extract analysed
C = Concentration of standard sample,
% Stachylose = % Total sugar x 0.04,
% Raffinose = % total sugar x 0.01

2.21 Total Amino Acids. Determination of Amino Acid Profile

Obtaining the amino acid profile in the test material was carried out using the procedure described by Omoyeni et al. [27]. Defatting and hydrolysis of the dried weighed sample was carried out, after which evaporation in a rotary evaporator and subsequent loading of the ensued sample into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM). About 4 g of the sample was defatted in a soxhlet extractor for 15 h using a mixture of chloroform and methanol (2: 1 ratio) [13]. 7 mL of 6 MHCl was added to the defatted sample of known weight in an ampoule, nitrogen was passed into the ampoule in order to eliminate oxygen which could cause the oxidation of some amino acids such as methionine and cystine during hydrolysis. Then the glass ampoule was sealed with fire from a burner flame then put in an oven set at 105oC± 5oC for 22 h. The ampoule was cooled, cracked open at the tip and the content sieved to remove the humins. Evaporation to dryness of the filtrate was achieved in a hot air oven. The residual portion obtained after dissolving in 5 mL of acetate buffer (pH 2.0) was cold stored in specimen bottles (plastic). About 5 to 10 µL of the hydrolysate was then measured into the holder of the analyzer and loaded into the TSM (The Technicon Sequential Multi-Sample Amino Acid Analyser). The separation and analysis of the free acidic, neutral and basic amino acids of the hydrolysate were carried out bythe TSM analyser USA. The analysis was carried out for 76 mins [27] (Tryptophan is

destroyed by 6 M HCL during hydrolysis, so this method is not used for tryptophan)

2.22 Method of Calculating Amino Acid Values from Chromatogram Peaks

The peak area proportional to the concentration of each of the amino acids was calculated by an integrator fastened to the analyser. The approximate peak area for each peak was then obtained by multiplying the height with the width at half-height. Each amino acid noreleucine equivalent (NE) of the standard mixture was calculated using the formula.

NE= (Area of Noreleucine Peak)/(Area of each amino acid).

Each amino acid in the standard mixture also had a constant S calculated,

Where

Sstd = NEstd x Molecular weight x μMAAstd

The amount of each amino acid present in the sample was calculated in g/16gM or g/100 g protein using the following formula: Concentration (g/100g protein) = NH ×W@NH/2 ×Sstd ×C

Where

C= (Dilution ×16)/(Sample Wt (g)×N% ×10 ×Vol.loaded) ÷NH ×W(nleu)

Where:

NH = Net height, W = Width at half height, and nleu = Noreleucine

2.23 SDS- PAGE Electrophoresis

2.23.1 Sample Treatment

Flour/protein isolates (20 mg) were mixed in 1.5 mL of borate buffer, vigorously vortexed to make a homogenous solution which was then incubated at 37°C for 2 hours acompanied by centrifugation at 4,000 xg for 15 mins. The clear supernatant was then transferred to a sterile 1.5 mL microcentrifuge tube and stored at 4°C overnight. For sample treatment, 30 μL protein isolate extracts were mixed with 70 μL of Laemmli sample buffer while 50 μL defatted flour extracts were mixed with 50 μL of Laemmli sample buffer. The resulting mixture was vortexed, incubated at 95°C for 15 minutes and centrifuged at 13000 rpm for 1 min. The clear supernatant (10 μL) was analyzed using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis [28].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out using the Cleaver Scientific VS10WCBS OmniPAGE unit. The unit which consists of glass plate sandwich, a casting frame, a casting base and an electrophoresis tank was assembled according to manufacturer's instructions. The glass plates were fixed with a spacing of 1 mm, slid into the casting frame and tightly clamped into the casting base with the two clamp levers of the casting base.

Resolving gel (10 %) contained distilled water (2 mL), 1.5 M Tris-HCl, pH 8.8 (1.625 mL), 40% Acrylamide/Bisacrylamide Solution (1.25 mL), 20 % SDS (125 μL), 30 % Ammonium Per Sulphate (12.5 μL) and Tetramethylethylanediamine (5 μL). The resolving gel was carefully casted by pipetting into the 1 mm spacing of the glass sandwich. A layer water was added on top of the resolving gel to avoid drying and prevent air (oxygen), an inhibitor of polymerization. The unit stood for 30 -40 mins for polymerization of gel. The layer of water was drained out and water droplets on the surface of the gel removed with a filter paper.

Stacking gel (4%) contained distilled water (1.95 mL), 1.0 M Tris-HCl, pH 6.8 (250 μL), 40% Acrylamide/Bisacrylamide Solution (250 μL), 20 % SDS (50 μL), 30 % Ammonium Per Sulphate (8 μL) and Tetramethylethylanediamine (4 μL). The stacking gel mixture was carefully casted by pipetting, the combs inserted and stood for 30 mins to polymerize. Upon polymerization, the casting apparatus was disassembled, and casting frame inserted into the electrophoresis tank. The running buffer was then poured onto the apparatus filling the lower portion of the casting frame and glass cassette and fully immersing the inner chamber to cover the comb, the comb was then gently removed leaving perfectly casted wells.

Treated samples (10 μL) were carefully loaded onto each well without spilling into neighbouring wells. Pre-stained 200 kDa New England Biolabs protein marker (5 μL) was run as a standard. The electrophoresis was run on the Cleaver Scientific power Pro -300, at a constant current of 100

volts for 20 minutes for stacking and 160 volts for 1 h 15 mins for separation. Afterwards the casting chamber was taken out of the electrophoresis tank, and the gel cassette was gently disassembled. The stacking gel was cut-off using a spatula and the separating gel was immersed in the staining solution. Staining was done using Coomassie brilliant blue G-250 for 2 hours on an orbital shaker, thereafter the stain was decanted off, and the gel was immersed in the de-staining solution of 25% methanol on the shaker. Destaining was done until the bands were visible. The stained gel image was viewed and documented on Cleaver Scientific Omni-Doc system (Rapala Kozik et al., 2007); [29].

3. RESULTS AND DISCUSSION

Those physico-chemical properties that dictates the reaction in foods of protein at the time of preparation, storage and consumption are known as functional properties. These properties of proteins are capable of being grouped into three major categories according to the mechanism of their action: (i) hydration relating properties (solubility, water and oil absorption) (ii) protein make up and rheological attribute associated properties (gelation, viscosity, elasticity), and (iii) protein surface characteristics (foaming, emulsifying). Food quality, processing, applications, and acceptance are influenced by the reaction of protein with other functional components directly or indirectly. The final product quality and usefulness in any food system is affected by solubility, water imbibition, gelation, surface activity, swelling, and viscosity.

Ability to dissolve is the most crucial functional property of any protein, in order to be useful in food systems. Apart from this, some other abilities like emulsification, foaming, and gelation are solubility-dependent. Solubility of protein is an expression of the thermodynamic demonstration of the balance occurring among protein-solvent as well as protein and protein. It can also be expressed as having balance existing amidst hydrophilic (water-loving) and hydrophobic (oil-loving) reactions.

The distribution of amino acid residue on the covering of proteins influences to a considerable magnitude the solubility of protein in aqueous buffer. The hydrophilic (polar) and hydrophobic (non-polar) composition. Hydrophobic (oil loving) residuals are majorly obtained in the protein globular core, but could also be found in bits on the covering. Proteins with high hydrophobic (oil-loving) amino acid composition on the surface possess low dissolving power in water. Increased solubility is often brought about by the interaction of charged and hydrophilic surface residues having other ionic assemblage in the solvent. Protein solubility is also influenced by pH, its lowest at the isoelectric point, which makes surrounding pH a very crucial component when discussing the extent of protein solvation. Other parameters that control protein solubility in food are, freezing, temperature, heating, ionic-strength, drying as well as shearing. Proteins that are insoluble are not suitable for food utilization, therefore, it is of uttermost importance that denaturation is controlled especially that of heating, so that protein solubility is not influenced negatively.

The denseness of any flour sample or protein isolate, which is affected by the size of the particles and its compactness constitute the bulk density. Extremely crucial in deciding the packing specification, substance management and functions affecting wet treatment in industries producing. Upsurge in carbohydrate composition brings about a correspondent increase in density and it provides a clue of the approximate type of packaging material necessary. In the preparation of nourishment for recuperating children and older adults, high bulk density is generally preferred. This is to facilitate better dissolution, minimizing the density of the paste. The bulk density of flours also signifies their application as good thickeners in food products

Lipid imbibation is connected to the visible enmeshment of fat, as well as the quantity of non-polar branches on the proteins that fastens hydro-carbon link on the fatty acids. Oil absorption capability is influenced by various components, such as the protein quantity, hydrophobicity, circumference, the charge, shape, the viscidity of the oil and procedure deployed. Oil absorption is a crucial characteristic in food formulation, reason being that, fat enhances the retention of flavour and savor of foods in the mouth, noticed that proteins that are hydrophobic demonstrate better fastening of phospholipids, implying that the side chains of amino acid which is hydrophobic fasten better to the hydrocarbon chains of oil. Oil imbibition is determined by the availability on the exterior of oil loving (lipophilic) amino acids; therefore, rise in lipid imbibition capability may be connected to an upsurge in hydrophobic amino acids by reason of germination

The most important chemical constituent that influence oil assimilation capacity is protein, which contains the polar and oil-loving portion both. Hydrophobic amino acid branches can bring about through hydrocarbon groups of fats, hydrophobic interactions. Flour that have good lipid absorption capacity are conceivably beneficial in constituent synergy in food, particularly in flavor keeping, advancement of tastefulness and elongation of the useful life, especially in confectionery and victual produce in which lipid imbibition is

The interaction between water and protein are very germane to both the structure of the protein and the presentation in food systems. Water absorption capacity is the capability of the total nitrogenous compound's structure to sponge up, keep enclosed fluid, and physically trap water against gravitational pull (Narayanasamy and Pushpa, 2012); [30]. Singh [8] defines it as the capability of a produce to connect with water under circumstances in which water is restricted [31]. Kaur and Singh, [4], is of the opinion that, finely milled grain having greater water imbibing ability, posses' additional polar components in the likes of carbohydrates [32].

Water imbibing is crucial for many products' characterisation, such as the succulence of the commodity, gelatinized starch realignment on cooling which could result in product flaking [33], (Amon et al., 2011). Ability to absorb water is an essential handy attribute in grubs, such as highly seasoned minced meat, custard and dough. In the above-mentioned edibles, protein imbibes water, without liquefying as a result of inadequate water [34], as a consequence, swelling occurs, conferring character like body setting and viscidity. Water fastening capacity is a valuable indicator of if the flour or isolate maybe integrated into a liquid edible formulation particularly as relating to dough management [35]. High WAC values are desirable because it assures the maintenance of moistness of the products. Moisture depletion negatively impact the outcome of the product [36].

Total nitrogenous material and carbohydrate perform significant function in water fastening. This is because protein subunits have additional aqua binding positions, thereby intensifying WAC [37]. Transformed total nitrogenous material in legumes posses' greater water-assimilating capability than natural proteins, due to the unravelling of the denatured total nitrogenous material, which uncovers more water fastening site [38]. According to Ma et al. (2011) constituents influencing WAC of flours of protein seeds comprise of, amino acid configuration, protein structure, surface hydrophilicity/ nonpolarity, concentration of ion, ion type, hydrogen ion concentration and temperature (Sandra et al., 2012). Other components like lipid and carbohydrates also affect WAC. Water absorption capability of protein is extremely crucial as it influences the texture, succulence, as well as the taste of food formulations, especially the useful life span of confectionery [39].

Foaming is a surface-active quality of protein [15]. Foam (Suds) is a double-phase arrangement comprising of air globule divided by a tinny unbroken fluid film known as the lamellar layer (Nicorescu et al., 2011). Foams (Suds) which are made up of a vapour layer, a fluid layer and foaming agent (e.g. proteins) which on agitation produce foams. Foam formation develops, when air is whipped into a liquid vigorously [40] but because of the instability of this foam, a surfactant is critically required to rapidly reduce the free energy (interfacial tension) to provide the needed stability for food product [41]. Foams are formed as a result of uncoiling and incorporation of the total nitrogenous material at the air-water border, along with a thin layer creation round the air globule. The ability to produce and stabilize foams is connected to various physical characteristics of the proteins. The surfactant gravitates naturally to the intersection, where the free energy of the molecule is smaller than at either of the two phases. Food foams are complicated systems, which includes a combination of liquids, gases, surfactants, and solids. The size of air globule and its distribution in suds affects the foam products characteristics and consistency; suds with a homogenous dispersion of small air globule, impart bulk, sleekness and fluffiness to the foods (Nicorescu et al., 2011); [42]. Proteins have been found to be better than smaller molar mass surfactants in performing like macromolecular surfactant in foam-like preparation [43].

The surface activeness of proteins makes them to have the ability to relate with both air and water. The hydrophobic part of protein interacts better with air while the hydrophilic parts favour polar solvent such as water. Proteins are excellent foaming agent since they can rapidly disperse into the air-water intersection and produce a strong cohesive and elastic film by

incomplete unfolding [44]. The characteristics of foam formation by proteins is basically connected to the ability of producing film at the water and air intersection [45]. Protein which can open and promptly adsorb, give superior foaming properties than the ones that are sluggishly adsorbed with more challenging to open structures at the interface. Good foam is obtained when the protein is properly unfolded, which brings about the uncovering of the concealed hydrophobic groups and the creation of a strong cohesive network around the air pockets with the hydrophobic group sticking inside the air surface and polar into the water area (surface denaturation). This alteration in arrangement brings about loss of solubility and thereby reducing surface tension which brings about the creation of new interface and more foams. This moderately uncoiled compound combines to create a stabilizing coating round the foam that is crucial to ensure the foam balance [46].

High foaming properties of protein is dependent on it having significant solubility in the fluid layer along with the capability of rapidly creating a coating round the air beads in the grub system. Some external component which influence foaming characteristics are, temperature, pH and ionic strength. Capacity to create and stabilize foams of proteins is likewise of great significance. For protein to create durable foams the intersurface film must be inflexible so as not to allow the enmeshed air leak (i.e. nearly impenetrable). Dispersed total nitrogenous material causes the lowering of the superficial pressure at water-air border, thereby initiating foaming ability [47]. Foaming capacity measures the boost in bulk upon whipping the protein solution or dispersion; therefore, the more the lessening of the peripheral pressure the greater the foam-forming capability; Whereas, foam durability is connected with the capacity of suds to maintain its increased volume for a period of time. This is generally ascertained by the frequency of seepage of fluid from the froth [41]. Foam durability is affected more by unfolding of the protein instead of the surface change [47]. Foods comprising of foams include, ice creams, angel cakes, chiffon desserts and whipped toppings. Among these are solid foams, such as cakes and other confectionaries. Protein ought to possesses the capability of creating strong linkage such as hydrophobic interactions and hydrogen bonding, the ability to unfold minimally at the surface is also very important, so as to keep viscidity and firmness. Oil seed proteins have of recent found expanded utilisation as airing medium in sponge cakes, angel food, whipped toppings, and frozen desserts (Kathiresan and Manivannan, 2006); [48].

Least Gelation Concentration (LGC) is a quality specification which indicates the lowest quantity of protein at which gel stayed in the inverted tube without gliding [49]. The smaller or the least gelling concentration of the protein, the more excellent is the power to form gels.

Gelation denote the enlargement of protein and starch content on heating of flour matrix. The gel-forming capability of flours is found to be connected to unfolding, collection and breakdown of starch and protein thermally. For the preparedness and receiving of several edible produce as well as plant product, protein gelling ability is found to be crucial. Gel presentation and mechanism are basically regulated by the stability amongst magnetic hydrophobic interplay and repellent electrostatic interactions. The uncovering of varied characteristic clusters by the thermal unravelling of protein is responsible for the repeling powers. Protein gel creation is the product of a process which involves two-step, the fractional unfolding of distinctive proteins so as to permit greater accessibility to responsive clusters inside the protein unit; followed by the collection of those proteins through their responsive side clusters to a three-geometrical structural framework competent to hold back substantial water quantity. The consumer acceptability of various edible produce has been observed to be greatly affected by the rheological and textural properties conferred by the gelation mechanism [34,50]. The process of gelation may be represented thus:

Native protein→ Protein denaturation → Gel formation

Emulsion formation, as well as stability, is great significance in arrangement of food like salad dressing [44]. This is because, emulsions are thermodynamically not-stable blend of immiscible liquids usually (water and oil). Inserting protein to the mixture of lipid and water leads to native protein structure unfolding. This unfolding bares the oil loving portion of the total nitrogenous material to the oil and the polar section to aqua, thereby lowering the boundary pressure in between the lipid and aqua. The structure as well as flexibility of protein are essential components that establish the performance of the protein in this process [51,52]. Emulsions are highly

unstable systems, but protein has been found to fulfill an essential role at the time of emulsification, particularly in emulsion stability [9]. Emulsifying activity indicates the capacity and capability of protein to assist during the creation of a blend (emulsion) which is connected to protein's capacity to integrate at the surface part of lipid and water in a blend (emulsion). The capability of the protein to impact firmness to the blend for protection against pressure and fluctuations is emulsion stability. It usually indicates and is connected to the homogeneity of the outward area over a duration of time [53]. A good emulsifier is identified based on its capability to sustain through processing the emulsion especially in the process of cooking and canning, which makes emulsion stability of great importance [9]**.** Emulsion stability of products that consist of protein such as legume flours and isolates may occur from both soluble and insoluble protein including polysaccharides. Protein emulsifies and stabilizes the emulsion by reducing interfacial pressure on the lipid globule and producing electrostatic repelling force on the surface of the droplet. Emulsion can also be heightened through the help of Polysaccharides which increases the viscidity of the system. Similarly, the isoelectric point is the region of smallest emulsion capacity (EC) and stability (ES). Emulsifying stability and activity have also been found to have a good direct connection with surface non-polarity. The better hydrophobic the outward part of the protein the greater the emulsification property which also increases with denaturation (Nishinari et al., 2014).

4. CONCLUSION

This research work shows that biochemical modification (Germination/Malting/ Sprouting) had an enormous impact on the nutritional composition, functional properties, mineral bioavailability, anti-nutrient content and amino assay of Solojo bean, thus, it could be used as protein supplement in infant, young children and geriatric foods.

Efforts should be increased to promote the cultivation, encourage the consumption and industrial application of this under-utilized legume by the Government, especially in the south-western region where it can survive the rain fall level. Large scale production of this legume which is gradually going into extinction should be encouraged in order to fight the menace of malnutrition in developing countries where animal protein price is exorbitant; This will ensure food security and also creation of jobs because people can engage in different aspects of the production process and thereby reducing the rate of unemployment.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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