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Proximate Composition of Two Varieties of African Yam Bean (*sphenostylis sternocarpa*) Seed Flour and Functional Properties of Their Protein Isolates

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Abstract:

Protein isolates were prepared from two varieties of African yam bean (*sphenostylis sternocarpa I* and *sphenostylis sternocarpa II*) by alkaline extraction followed by acid precipitation of the proteins at the isoelectric point (5.0). Proximate composition and functional properties of the seed flours and protein isolates were determined. Protein isolates from *S. sternocarpa I* and *S. sternocarpa II* contain 90.84 and 92.43% of protein respectively. The proximate analysis show that *S. sternocarpa II* flour and protein isolate has a higher protein content of 24.78% and 92.45%. The result of the functional properties also revealed that *S. sternocarpa II* has a better water absorption capacity, oil absorption capacity, emulsion capacity and stability. The pH dependent protein solubility profile also shows that the highest solubility in both flours and isolate in the two varieties was at pH 10 while minimum solubility was obtained at pH 5.0 which corresponds to the isoelectric point.

Keywords: *Sphenostylis sternocarpa*, Protein Isolate, Proximate Composition, Functional Properties.

1. Introduction

African yam bean (*Sphenostylis stenocarpa*) is an underutilized food legume crop in the tropics; not as popular as other major food legumes (Moyib *et al.*, 2008). It is a leguminous crop which are cultivated in tropical and subtropical region such as Cameroun, Cote d'ivoire, Nigeria and Togo. Nigeria is very significant for African yam bean production (Potter, 1992) where extensive cultivation has been reported in the Eastern (Abbey and Berezi, 1998) Western, and Southern (Saka and Ajibade, 2004) parts of Nigeria. Some of the local names in Nigeria include "Girigiri" (Hausa), "otili" (Yoruba), "Azuma" (Igbo). It is cultivated for both seeds and tubers. The seeds have protein content which ranged from 21.0 % – 29.0 % with about 50% carbohydrate mainly starch (Eromosele *et al.*, 2008). In Africa and the rest of the developing world, where malnutrition due to inadequate protein in nutrition is a prevalent problem there is an urgent need to explore the utilization of plant proteins in the formulation of new food products or in conventional food. (Khalid *et al.*, 2003). This is predicated on the fact that animal protein such as meat, milk, and eggs are expensive and relatively difficult to acquire (Chel *et al.*, 2002). The use of plant proteins in the formulation of new food products, or in conventional foods, has been the focus of much research in recent years (Chavan *et al.*, 2001). In order to develop plant proteins for use as food ingredients, their functional properties which are the physical and chemical characteristics of the specific protein influencing its behaviour in food system during processing, storage, cooking and consumption must be evaluated. Protein isolates are used as additives to improve some properties such as water binding, foaming, gelation and emulsifying capacities, viscosity and texture. These applications in food trade are almost limited to soybean seeds, whereas other vegetable proteins are less used. Among these are those from African yam bean. The objective of this work is to examine the chemical composition and functional properties of the flour and protein isolates of two varieties of African yam bean and compare the functional properties of the two varieties in relation to the possible use of their protein isolates in the food industry.

2. Materials and Methods

2.1. Source of Material and Preparation

Seeds of African yam bean (*Sphenostylis sternocarpa*) were purchased from Oja – Oba in Ado-Ekiti, Ekiti State, Nigeria. The samples were authenticated by a curator in the Department of Crop, Soil and Pest management of the Federal University of Technology, Akure, Ondo State, Nigeria. African yam bean seed were screened to remove stones, immature and bad ones, the light-brown eyed

white coat African yam bean (*Sphenostylis sternocarpa* I) and the black-eyed white coat African yam bean (*Sphenostylis sterocarpa* II) were sorted out as the two varieties used in this study. The seeds were dehulled, dried and milled into powder. The powdered samples were stored in screw-capped air-tight container and refrigerated at 4°C prior analyses.

2.2. Proximate Analysis

The two samples were defatted using n-hexane in a soxhlet apparatus. The defatted seed flours were thoroughly air-dried to remove traces of solvent. Proximate composition was carried out on the raw seed flour and protein isolates for moisture content, crude protein, crude fat, crude fibre and ash were determined according to the method of AOAC (2005). Total carbohydrate was obtained by difference.

2.3. Preparation of Isolates

In preparation of the protein isolate, the flour, was dispersed in distilled water at a ratio 1:20 w/v (flour/water) and the pH adjusted to the pH at which the protein in the flour is most soluble (pre-determined for each sample). The solution was stirred for 2 hours at 30 ± 2°C using a Gallenamp magnetic stirrer to enhance high degree of protein solubility. The pH of the supernatant obtained after centrifuging at 10,000xg for 30 min at 5°C was adjusted to the pH at which the protein in the flour is least soluble (the isoelectric point which has been predetermined) with 0.5M HCl to precipitate the protein. The isolate was recovered by centrifugation for 30 minutes after which it was neutralized, dialyzed and freeze dried.

2.4. Functional Properties

The method of Beuchat, 1977 was employed for the water and oil absorption capacity determination. One gram of sample was mixed with 10ml distilled water or king's vegetable oil for 30 sec. the samples were then allowed to stand at room temperature (30 ± 2°C) for 30 min after which they were centrifuged at 5000rpm for 30mins. The volume of the supernatant was noted by difference.

Foaming Capacity and Stability was determined by the method of Coffman and Gracia 1977. A weighed amount of sample was dispersed in 100 ml distilled water, after which the suspension was whipped vigorously for 2 min using Philip kitchen blender set at speed 2. Volumes were recorded before and after whipping. The percentage volume increase was calculated according to the following equation:

$$\% \text{ volume increase} = (V_1 - V_2) / V_1 \times 100,$$

Where V_2 is the volume of protein after whipping and V_1 is the original volume of protein solution

Foam stability was determined as the volume of foam that remained after 2h expressed as a percentage of the initial foam volume.

Emulsifying properties: Emulsion capacity and stability was determined using the method of Beuchat, 1977 as described by Fagbemi, 1999. Two gram of sample was dispersed in 40 ml distilled water using a magnetic stirrer at 500 rpm for 30 min. 10 ml of king's vegetable oil was added over a period of ten minutes at 1ml per minute with continuous stirring. The mixture was transferred into a calibrated centrifuge tube and water bathed at 85°C for 15 min. The tubes were removed, cooled to 25°C and centrifuge at 4500 rpm until the volume of oil separated from the emulsion was constant. Results are expressed as the percentage of oil emulsified per gram of the flour used. The emulsion formed were allowed to stand at room temperature and percentage stability after two hours was calculated as the height of remaining emulsified layer to that of the original emulsified layer. The least gelation concentration of the seed flours was determined using the method of Coffman and Gracia, 1977 with slight modification. Sample suspension of 2 – 20 % m/v were prepared in 5ml distilled water. The test tubes containing these suspensions were then heated for one hour in a boiling water bath following by rapid cooling under running cold water and further cooling to 4°C for 2 h. The LGC was determined as the concentration when the sample from the inverted test tube did not slip or fall.

Protein solubility of the seed flours was determined using the method described by Ige *et al.*, 1984. The effect of pH on protein solubility of the seed flours was determined by adjusting the pH as described using either 0.1M HCL or 0.1M NaOH solution. The slurry was centrifuged and the protein content of the supernatant was determined using the Kjeldahl method. The pH was from 2.0 to 11.0. The result was expressed as percentage protein solubility.

2.5. Statistical Analysis

Data obtained were statistically analyzed using one-way Analysis of Variance (ANOVA) and the result expressed as mean ± Standard deviation (SD) of three replicates.

3. Results and Discussion

Parameters	<i>S. Sternocarpa I</i>	<i>S. Sternocarpa II</i>
Moisture Content	5.50 ± 0.27	4.36 ± 0.09
Fat	1.97 ± 0.13	2.55 ± 0.08
Crude Protein	20.73 ± 0.12	24.78 ± 0.92
Ash	1.97 ± 0.05	3.83 ± 0.05
Crude Fibre	2.57 ± 0.02	2.84 ± 0.05
Carbohydrate	67.26 ± 0.16	61.64 ± 0.93

Table 1: Proximate Composition (%) of the seed flours

Parameters (%)	<i>S. Sternocarpa I</i>	<i>S. Sternocarpa II</i>
Moisture Content	4.21± 0.05	2.55± 0.01
Fat	1.07±0.02	1.15±0.01
Crude Protein	90.84± 0.45	92.43± 0.02
Ash	2.17± 0.08	1.65± 0.01
Crude Fibre	1.67± 0.38	2.27± 0.01
Carbohydrate	ND	ND

Table 2: proximate composition (%) of the protein isolates from the legume seed flours

3.1. Proximate Composition

The results of the proximate analyses of the raw seed flours and protein isolates are presented in tables 1 and 2. Isolation significantly affected the proximate composition of the seed flours (tables 1 and 2). The moisture content of the raw flour and protein isolates of the seed flours are 5.50% (*S. sternocarpa I*), 4.36% (*S. sternocarpa II*) and 4.21 %, *S. sternocarpa I*, 2.55 % (*S. sternocarpa II*) respectively. Generally, the moisture content of these seed flours are lower than for similar legumes like *Larthyrum martinus*, 9.7 % (Chavan *et al.*, 1999). The moisture content obtained for both varieties are lower than the 10 % recommended for storage stability of flours and this might be advantageous in terms of prolonging the shelf-life and retaining the qualities of the flours. However, the moisture content decreases significantly on isolation. The low crude fat in the seed flour suggests that it is not an oil seed. Crude protein in the seed flour is higher in *S. sternocarpa II* (24.78 %) than *S. sternocarpa I* (20.3%). However, the values compared favourably with value obtained for *plukenetia conophora* 23.65 %, *A. breviflorus* seed 28.25% (Akintayo and Baeyer 2000 respectively). Their protein also increased significantly on isolation ranging from 90.84% to 92.43%. The values were higher than those reported for similar legumes; Mung beans isolate, 87.9% (Rahman *et al.*, 2000), chickpea isolate 88.1% (Sanchez *et al.* 1999), *Canarvalia einsformis*, 73.3%, (Chel-guerrero *et al.*, 2002). The variations in protein contents are attributed to genetic make-up of legumes along with some environmental factors (Kaur, 2007). The high protein content obtained in the protein isolates suggests that they may be a better protein supplement than the full-fat seed flour and also contribute significantly to alleviating the problem of protein malnutrition in the third world and developing countries. The total ash content of the raw flour and protein isolates are 1.97 % (*S. sternocarpa I*) 3.83 % (*S. sternocarpa II*) and 2.17 % (*S. sternocarpa I*), 1.65 % (*S. sternocarpa II*) respectively. These values are comparable with some reported works (Oshodi, 1992, Fokou *et al.*, 2004, Aremu *et al.*, 2006).

Parameters	<i>S. sternocarpa I</i>	<i>S. sternocarpa II</i>
Water absorption capacity (ml/g)	2.21 ± 0.01	2.27± 0.06
Oil absorption capacity (ml/g)	1.87 ± 0.04	2.00 ± 0.01
Foaming capacity (%)	7.39±0.15	18.18±0.07
Foaming stability (%)	60.00 ±0.07	40.25 ±0.04
Emulsion capacity (%)	60.07± 0.12	52.88 ± 0.01
Emulsion stability (%)	40.75±0.25	52.38±0.29
Least gelation concentration (%)	10.00	16.00

Table 3: Functional Properties of the Raw Seed Flours

Parameters	<i>S. sternocarpa I</i>	<i>S. sternocarpa II</i>
Water absorption capacity (ml/g)	2.60± 0.03	2.80 ± 0.01
Oil absorption capacity (ml/g)	2.00± 0.02	2.40± 0.03
Foaming capacity (%)	32.20±0.13	20.00±0.12
Foaming stability (%)	56.40 ±0.02	71.43 ±0.09
Emulsion capacity (%)	52.41± 0.12	54.11 ± 0.01
Emulsion stability (%)	45.10±0.16	50.00±0.06
Least gelation concentration (%)	8.00	8.00

Table 4: Functional Properties of the Protein Isolates

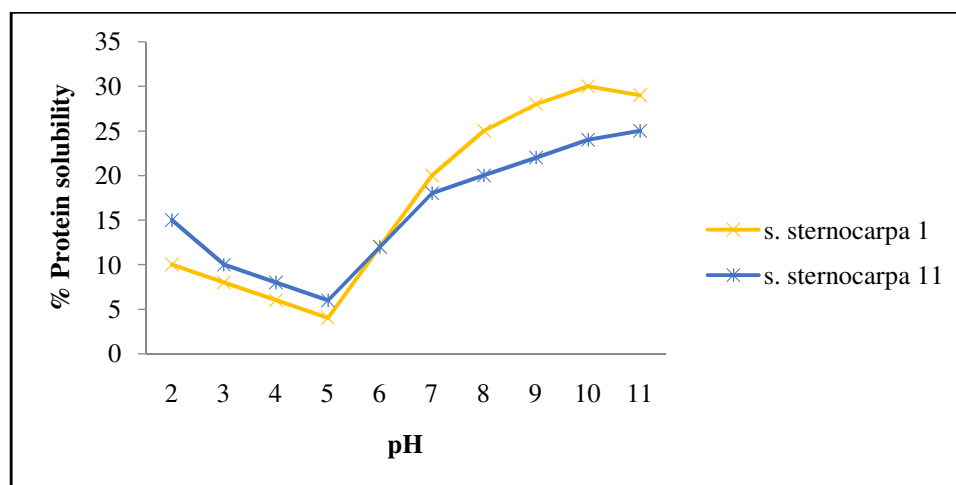


Figure 1: Effect of pH on Protein Solubility of Raw Seed Flour

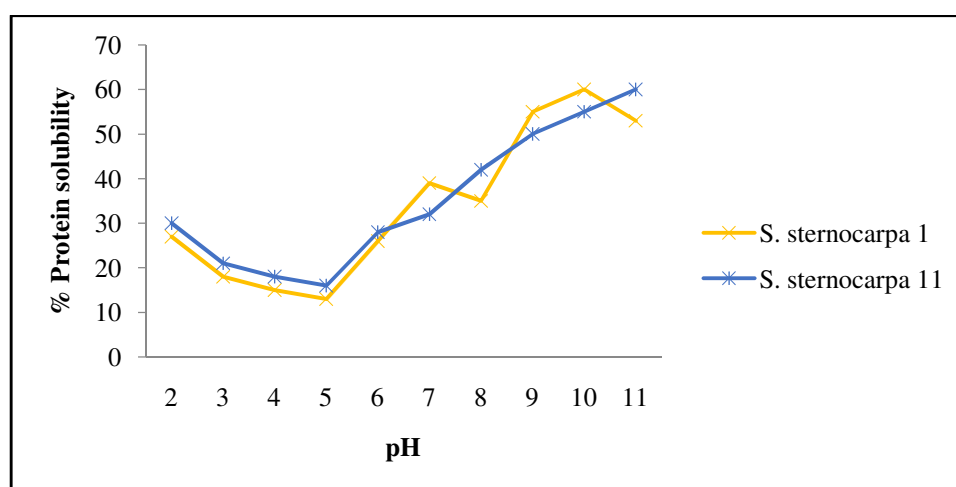


Figure 2: Effect of pH on Protein Solubility of Protein Isolate

3.2. Functional Properties

Tables 3 and 4 Present the functional properties of the seed flour and the protein isolates. It was observed that the WAC increased with the level of protein content as the isolates have the higher value. The values for the raw flour are comparably higher than the value of *triticum durum* flour (140.63 %) (Adeyeye and Aye 2005), oil seed flour (70 - 120 %) (Olaofe *et al.*, 1994) and quinoa flour (147 %) reported by Ogundele *et al.*, 2010. The WAC of the protein isolate were higher/lower than the values reported for commercially available protein isolate including soybean isolate (338 %), sodium caseinate (60 %), dried white egg (190 %) and solubilized wheat protein isolate (166 %) (Mohammed *et al.*, 1999). However, *S. sternocarpa II* has a better WAC. Water absorption of protein is a function of several parameters, including size, shape, steric factors, conformational characteristics, hydrophilic – hydrophobic balance of amino acids in the protein molecules. So there is a relationship between protein concentration and WAC which means that the isolate being richer in protein content have more hydrophilic groups exposed to water than the seed flours. Therefore, the isolates would be more useful in enhancing the water-binding capacity of food products like dough and sausages. The OAC of the protein isolates was better than for the raw seed flours. The value obtained for the OAC in the raw seed flour (1.87ml/g and 2.00 ml/g) is consistent with value of bambara groundnut (1.37 ml/g), Mucuna bean flour (1.67 ml/g) and jack bean flour (1.7 ml/g) reported by Adebawale and Lawal, 2004. Considering the protein isolate, the OAC increased significantly. This may be attributed to the high concentration of proteins in the flours. The OAC of protein isolates considered in this study is however, higher than for the corresponding flours. The values ranged between (2.00 - 2.40 ml/g). The result is higher than for beach pea protein isolate (64 – 82 ml/100g) as reported by Chavan *et al.*, 2001, Woodstone pea protein isolate (90.1 - 94.5 %) Nack *et al.*, 1986. Oil absorption capacity is the binding of by non-polar side chain of proteins and it is a useful indication whether the protein material will perform well as meat extender or analogues (Okezie and Bello, 1988). The result obtained from the current investigation shows that the flour and more importantly the isolates will be potentially useful in flavor retention, improvement of palatability. The least gelation concentration of the raw flours is 10 % for *S. sternocarpa I* and 16% for *S. sternocarpa II*. The result obtained for the raw flours compares well with those reported for cowpea (10 %), lupin seed 14 % but higher than those reported for breadnut flour (6 %), unripe plantain 8 % reported by (Oshodi *et al.*, 1999, Fagbemi 1999, Oshodi and Ekperijin 1989, Sathe *et al.*, 1982). It is also consistent with LGC of two varieties of bambara groundnut, kershington groundnut and two varieties of cowpea which ranges from 12 % to 16 % as reported by Aremu and Akintayo, 2007. Variation in the values obtained may be linked to the relative ratio of different constituents-

proteins, carbohydrates and lipids as the interaction between such compounds may affect functional properties. The LGC of protein isolate was the lower with both varieties having 8 %. These values were higher than those reported for mung bean protein isolate 6 %, lupin protein isolates 12 % and (Akintayo *et al.*, 1998, Iqari *et al.*, 2002 and respectively). Since the smaller the value of LGC, the better the gelation property of the protein ingredient, the isolate may be a great asset for the formulation of curd or an additive to other gel forming materials in food products. The result of the emulsion capacity of the raw seed flour are 60.07 % and 52.88 % respectively and 52.41 % and 54.11 % in protein isolates. The values for the flour are comparable to benniseed 63.0 % (Oshodi *et al.*, 1991), but higher than the values reported for *A. hybrid* (47.5 %) and *T. occidentalis* (48.7 %) (Adeyeye *et al.*, 2011). The study of the emulsion stability shows the flours from the two varieties and their isolates have comparable stabilities. The ability of flour and isolate to emulsify oil and protein suspension into a mixture of fine globule dispersion can be attributed to the soluble proteins. Soluble proteins are inherently surface active due to their amphiphilic nature and tendency to adsorb at oil-water interphases. The above result shows that both flour and protein isolate would be a potential ingredient in many food formulations such as salad dressing, sausages, mayonnaise etc. The values of the FC obtained for the seed flours are 7.89 % and 18.18 % for *S. sternocarpa I and II* respectively. While a higher value was obtained for protein isolates 32.20 % (*S. sternocarpa I*), 20.00 % (*S. sternocarpa II*). The higher value obtained for the protein isolates may be due to the low oil content as well as increase in the protein concentration which encourages foaming (Altschul and Wilcke, 1985). The above values compare favourably with benniseed (18.0 %), pearl millet (11.3 %) and guinea (9.0 %) as reported by Oshodi *et al.*, 1998 and bilphia sapida pulp (26.62 %) and seed flour (8.2 %) reported by Akintayo *et al.*, 2000. There is no significant difference in the foam stability of the raw flour and the protein isolate. However, it should be noted that FC depends on several factors like pH, type of protein, processing method, temperature, whippability, presence or absence of calcium ion (Fernema 1996, Townsend & Nakini, 1983). The result for variation of protein solubility with pH for the raw flour and protein isolates of the legumes are shown in fig1 and 2. It was found that the isoelectric point of the proteins was 5.0. The isolates had the higher solubility. Generally, the solubility reduced as the pH increases until it reaches the isoelectric point. This was followed by progressive increase in solubility with further increase in pH. Similar observation was reported for winged bean and chickpea (Sathe *et al* 1982; Sanchez *et al.*, 1999). The higher solubility of the protein isolates over the raw seed flour could be due to the elimination of some interfering substances that may alter the ionic changes of the flour protein, thereby modifying their solubility behaviour near isoelectric point. The profile also indicated highest solubility each at alkaline region for both varieties as observed by Oshodi (1981) for *Adenopus* benth seed, Oshodi and Adeladun, 1993, for lima bean. This implies that the legumes could best be extracted by alkaline extraction followed by precipitation by acid at their isoelectric pH. The more soluble the protein, the better will be its functionality in food systems. The high solubility of the proteins both in the alkaline and acid medium indicate that they could have promising food applications.

4. Conclusion

The results of proximate composition of the flours and protein isolates reveals that *S. sternocarpa II* has the higher percentage of protein than that of *S. sternocarpa I*. The high protein content of the two varieties show that they could be used as protein supplement in cereal- based diet. Functional properties of the flours and isolates also indicated that the protein isolate had a better functional property. The good water absorption capacity of the isolate makes it of great use in bakery products. The increased solubility of the isolates at both acidic and alkaline pH shows that it could be incorporated into liquid food and beverage. It could also be used in toppings and ice- cream products due to its foaming properties.

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