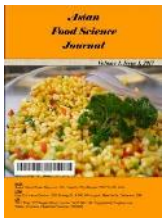

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Effect of Sprouting on the Functional Properties and Amino Acid Profile of Two Bambara Groundnut (*Vigna subterranea*) Protein Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RZA and YYM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author RZA managed the literature searches and carried out the laboratory analyses and managed the statistical analysis under the supervision of authors YYM, AAI and MKA. All authors read and approved the final manuscript.

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ABSTRACT

In this research, the effect of sprouting on the functional properties and amino acid profile of protein isolates from two Bambara groundnut landraces (Yobe black and Niger cream landraces) was studied. Proteins were isolated using alkaline extraction method. The amino acid profile and functional properties were investigated using standard analytical techniques. A significantly higher ($p<0.05$) foaming capacity was seen in sprouted samples (Niger cream 35.00; Yobe black 17.67) compared to the unprocessed samples (11.00 and 12.67 respectively). Similarly, the foaming stability of protein isolates from the sprouted samples was significantly ($p<0.05$) higher in both

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landraces studied; the foam was stable for up to 15 min. Differences were also observed between the protein isolates in terms of bulk density, swelling index, water absorption, oil absorption, emulsion capacity and emulsion stability; the differences were statistically insignificant ($p>0.05$). Aspartic acid, glutamic acid and leucine were the most abundant amino acids in the protein isolates studied. Differences were observed in the amino acid profile of Bambara groundnut proteins after sprouting. In Niger cream landrace, higher levels of all the amino acids were observed after sprouting. A similar trend was seen in Yobe black landrace after sprouting except for arginine, alanine and threonine that were slightly lower in processed compared to the unprocessed sample. The levels of total essential and non-essential amino acids of Niger cream landrace (48.27% and 50.86% respectively) and that of Yobe black landrace (47.75% and 48.45% respectively) were higher following sprouting. These findings indicate that sprouting improved the quality and properties of Bambara groundnut protein. Thus Bambara groundnut sprouting can provide a cheap protein source that can be used in alleviating malnutrition and in food industries where proteins are employed in preparations of food products.

Keywords: Bambara groundnut; protein isolate; amino acids; functional properties.

1. INTRODUCTION

The application of plant-based protein as food ingredients is rapidly growing in both developed and developing countries alike. This is due to their nutritional composition and optimisation of consumer's costs [1]. Legume protein isolates or concentrates are rich sources of amino acids. These protein concentrates may be utilised for the improvement of both nutritional and functional quality of the several food products [2]. However, the applications of protein isolates is being restricted to seeds of plants such as soybean, sunflower, peanut and cotton [3], neglecting other important species like Bambara groundnut.

Bambara groundnut (*Vigna subterranea* L. Verdc.) belongs to family Fabaceae. It is an annual herbaceous, intermediate plant with creeping stems at ground levels [4,5]. Bambara groundnut is a hardy plant that produces yields in marginal environments that may be difficult for many other plant species [6]. Among the legumes in Africa, Bambara groundnut is ranked the third most important after groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) [7]. The seed is well balanced with different nutrients and is especially rich in essential amino acids [8]. Bambara groundnut is nutritionally superior to most legumes and it is the preferred crop for many local people [9]. Though Bambara groundnut is grown extensively in [10] and represents a common staple food in the semi-arid area of Africa, it remains one of the crops less investigated and also underutilized [11]. The presence of beany flavours, processing challenges and high antinutrient content have also been suggested as a hindrance to the optimal utilisation of Bambara groundnut [12]. In view of this, this research is aimed at evaluating the effect of sprouting of the functional properties and amino acid composition of protein isolates from two landraces of Bambara groundnut.

2. MATERIALS AND METHODS

2.1 Seed samples and Sprouting Procedure

Two landraces of Bambara groundnut were used for the study. Seeds of the first landrace were obtained from Minna, Niger State, Nigeria (designated Niger cream) and the other from Damaturu, Yobe State, Nigeria (designated Yobe black). The Bambara groundnut seeds were identified at the Department of Plant Biology, Bayero University, Kano with a voucher number '0509'. For each landrace, a portion of the unprocessed seeds was used as a control in the experiments while another portion was used for sprouting.

Sprouting was carried out according to Shah et al. [13] with some modifications. The seeds were soaked by submerging in distilled water in glass containers for 4 hours (Niger cream) and 8 hours (Yobe black) at room temperature. The seeds were removed at 72 hours and 92 hours respectively for Niger cream and Yobe black landraces. The seeds were oven dried (80^{o}C) for 6 hours and then milled into flour and stored in glass containers for further analyses.

2.2 Preparation of Protein Isolate

Protein isolate was obtained from the defatted extract using the method described by Nkosi et

al. [14], with slight modifications. For each dry defatted extract, 100 g was resuspended in 1500 mL distilled water and adjusted to pH 10. The resultant suspension was filtered to remove debris and the filtrate adjusted to pH5 with 1N HCl followed by centrifugation at 7650×g for 15 min. The supernatant was discarded while the pellet containing the protein isolate was retained and oven-dried to yield a brown extract. The dried extract was kept in a sealed container prior to analysis.

2.3 Protein Yield

The yield of protein concentration was determined as the dry weight of protein concentrate after precipitation, per weight of the defatted flour as shown below [2].

$$\text{Yield } (\%) = \frac{\text{Protein content of concentrate}}{\text{Protein content of defatted fl}} \times 100$$

2.4 Amino Acid Profile

The Amino Acid profile was determined as described by Benitez [15]. The defatted sample was weighed into a glass ampoule and 7 ml of 6NHCl added; hydrogen was expelled by passing nitrogen into the ampoule (to avoid possible oxidation of some amino acids during hydrolysis e.g., methionine and cystine). The glass ampoule was then sealed with Bunsen flame and placed in an oven preset at 105°C ± 5°C for 22 hours. After cooling, the ampoule is broken open at the tip and the content filtered to remove humins. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in plastic bottles in the freezer. The amount loaded was 60 µl. This was dispensed into the cartridge of an amino acid analyzer (120A PTH, Applied Biosystems, USA). An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids.

2.5 Functional Properties

Water and oil absorption capacity: Water absorption capacity was determined using the method of Sathe and Salunkhe [16]. Ten (10) mL of distilled water was added to 1.0 g of the sample in a beaker. The suspension was stirred for 5 min. The suspension obtained was thereafter centrifuged at 4000×g for 30 min and the supernatant measured in a 10 mL graduated cylinder. The density of water was taken as 1 g/cm^3. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant. The same procedure was repeated for oil absorption except sunflower oil was used instead of water.

Foaming properties: The foaming characteristics of the protein isolates were investigated using the methods of Coffmann and Garcia [17] and Vani and Zayas [18]. The procedure involved blending of 50mL of a protein suspension (1g/100g, adjusted to pH 7) for 1min. The volume of foam present above the surface of the liquid in a glass cylinder of 100mL was then determined. Foam expansion and foaming stability were then obtained from the relations:

$$\text{Foam expansion } (\%) = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100$$

$$\text{Foaming stability } (\%) = \frac{\text{Foaming volume after time } t}{\text{Initial foam volume}} \times 100$$

Emulsifying activity and stability: Emulsifying activity and stability were determined using the method described by Lawal et al. [19]. A portion of protein solution (5 mL) was homogenised with 5mL sunflower oil and centrifuged at 1100×g for 5min. The height of the emulsified layer and that of the total contents of the tube was measured. The emulsifying capacity was calculated using the expression:

$$\text{Emulsifying capacity } (\%) = \frac{\text{Height of e layer in the tube}}{\text{Height of the total content in the tube}} \times 100$$

Emulsion stability was determined by heating the emulsion at 80°C for 30 min after which it was centrifuged at 1100×g for 5 min.

$$\text{Emulsion stability } (\%) = \frac{\text{Height of e n layer after heating}}{\text{Height of e layer before heating}} \times 100$$

Bulk density: The base of a 10 mL measuring cylinder filled with 2 g of sample was gently tapped on the laboratory table severally to a constant volume. Bulk density was calculated from the relation below [20].

$$\text{Bulk density } (\text{g/mL}) = \frac{\text{Weight of samples}}{\text{Volume of sample after tapping}}$$

Swelling index: Swelling index was evaluated using the method described by Akinyele et al. [21]. One gram (1 g) of the sample was weighed and dispersed into a test tube, levelled, and the height noted. Distilled water (10 mL) was then added and allowed to stand for 1h. Swelling index was calculated as follows:

$$\text{Swelling index} = \frac{\text{Initial height(H1)}}{\text{Final height(H2)}}$$

2.6 Statistical Analysis

All data are presented as the mean ± standard deviation of three replicate analyses. For multiple comparisons, the data were analysed using independent sample T-test using SPSS (Version 20). Values with $p<0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

Sprouting of Bambara groundnut led to increasing in emulsion properties, water absorption properties, swelling index and bulk densities in the two landraces studied. However, the increase in all cases was found to be statistically insignificant ($p>0.05$). In both landraces studied, foaming capacity significantly ($p<0.05$) increased following sprouting (Table 1). Both emulsion activity and emulsion stability were seen to slightly increase the following sprouting in both landraces studied.

On the other hand, foam stability (Table 2) was observed to be higher in sprouted Niger cream landrace where the foam was stable for a period of 20 mins compared to the raw samples. Conversely, in Yobe black landrace, foam stability by 20 mins was higher in the raw compared to the sprouted samples. The observed differences were statistically insignificant ($p>0.05$).

Table 3 presents the amino acid composition of the two landraces. Increase in the amount of all the amino acids was observed in both landraces following sprouting except for arginine, alanine and threonine which slightly decreased in Yobe black landrace. Sprouting also resulted in an increase in the total amino acids (TAA), total essential amino acids (TEAA) and total non-essential amino acid (TNEAA) in both landraces. Glutamic acid, aspartic acid and leucine were the most abundant amino acids in the studied samples.

3.2 Discussion

The amino acid analysis shows that glutamic acid, aspartic acid and leucine were the most abundant amino acids in all the samples as expected in legumes. The levels of the various amino acids were found to be higher after sprouting of the Bambara groundnut seeds. This observation could likely be due to increase in protein content as a result of metabolic activities that occur during germination. However, the levels of arginine, alanine and threonine in sprouted Yobe black landrace were slightly lower than in the unsprouted sample. The observed decrease could be due to complex metabolic processes that occur during germination. Feireria et al. [23] and Ziegler [24] reported that during germination and fermentation, the lipids, carbohydrates and storage proteins within the seeds are broken down in order to obtain the energy and amino acids necessary for the developmental processes in the plant. The

Table 1. Functional properties of raw and sprouted Bambara groundnut landraces

Parameter	Raw Niger cream	Sprouted Niger cream	Raw Yobe black	Sprouted Yobe black
Yeild (%)	19.6%	17.3%	22.37%	16.7%
Swelling index (mL)	0.59± 0.12	0.52 ±0.12	0.52±0.06	0.48± 0.02
Bulk density (g/mL)	0.67± 0.04	0.72±0.05	0.64 ±0.03	0.17± 0.03
Emulsion capacity(%)	38.17 ±0.81	39.30± 0.22	37.61± 2.56	38.34± 1.88
Emulsionstability(%)	38.94± 0.74	43.61± 0.99	39.45± 0.35	41.35± 3.14
Water absorption (%)	6.43± 0.30	6.66 ±0.12	6.20± 0.10	6.50± 0.26
Oil absorption (%)	3.05± 0.11	3.51 ±0.01	2.97 ±0.59	3.42± 0.71
Foaming capacity(%)	11.00±0.00[a]	35.00 ±1.00[b]	12.67 ±0.58[a]	17.67± 1.15[c]

Data were expressed as mean ± standard deviation of triplicate determinations. Means bearing dissimilar superscript along a row are significantly different ($p<0.05$).

Table 2. Foaming stability (%) of raw and processed Bambara groundnut landraces at different time intervals

Time(min)	Niger cream (%)		Yobe black (%)	
	Raw	Sprouted	Raw	Sprouted
5	1.33±1.05[a]	6.00±0.00[b]	2.00±0.00[a]	8.33±0.58[c]
10	7.67±0.58	5.67±0.58	6.00±0.0	4.67±1.15
15	1.67±0.37	2.67±1.15	2.00±0.0	1.33±0.94
20	-	2.00±0.00	2.00±0.0	-
25	-	-	-	-
30	-	-	-	-

Data are expressed as mean ±standard deviation of triplicate determinations. Means bearing dissimilar superscript along a row are significantly different ($p < 0.05$).

Table 3. Amino acids composition (mg/100g) of raw and sprouted Bambara groundnut

Amino acid	Raw Yobe black	Sprouted Yobe black	Raw Niger cream	Sprouted Niger cream	FAO/WHO (children)
Leucine	1.23	1.37	1.52	1.58	0.74
Lysine	5.09	5.65	5.94	6.44	5.20
Isoleusine	3.96	4.32	4.71	5.24	3.10
Phenylalanine	4.52	5.14	4.91	4.08	------
Tryptophan	1.23	1.37	1.52	1.58	0.74
Valine	4.33	4.85	5.03	5.00	4.20
Methionine	2.19	2.99	2.56	2.97	-----
Threonine	4.50	5.33	5.00	5.62	2.70
Arginine	5.16	5.94	6.54	6.28	----
Histidine	2.20	2.36	2.30	2.62	1.80
TEAA	39.78	47.75	46.42	48.27	
Proline	3.86	4.06	4.47	5.08	
Tyrosine	3.79	4.99	4.13	4.47	
Cystine	1.76	2.36	2.00	2.24	
Alanine	4.02	5.01	4.55	4.32	
Glutamic acid	12.64	13.32	13.78	14.23	
Glycine	3.52	3.99	4.01	4.66	
Serine	5.00	5.70	4.94	6.40	
Aspartic acid	8.62	8.99	9.30	9.46	
TNEAA	43.21	48.45	47.18	50.86	
TAA	82.99	96.20	93.60	99.13	
TSAA	3.95	5.35	4.56	5.21	
TAAA	8.31	10.13	9.04	8.55	

TEAA: Total essential amino acids, TNEAA: Total non-essential amino acids, TAA: Total amino acids, TSAA: Total sulphur containing amino acids, TAAA: Total aromatic amino acids.
** FAO/WHO children [22].*

nutritive value of a protein depends primarily on its capacity to satisfy the needs for nitrogen and essential amino acids [25,26]. In the present study, the levels of total amino acids in the protein isolates were within the FAO/WHO recommendations [22]. Sprouting led to higher total amino acids, total essential amino acids and total non-essential amino acid. Similarly, the sulphur containing amino acids in both landraces following sprouting were seen to be higher than in unsprouted samples.

Functional properties are important characteristics in nutrients and have been reported to be modified by processing methods like sprouting, cooking and soaking [27-29]. Findings revealed that sprouting of Bambara groundnut led to protein isolates with improved foaming capacity and stability. The observed higher foaming capacity might be associated with higher concentration of soluble and flexible protein molecules that enable the reduction of surface tension and rapid adsorption at the air/water interface [30]. In addition, the proteins might be utilised to form stable foam by unfolding of the polypeptide residues into air in liquid phase thereby providing stable foam [16,29,31]. On the other hand, lower foamability seen in unsprouted samples may be related to highly ordered proteins which resist surface denaturation. Similar observations were reported by Yusuf et al. [32] in Bambara groundnut and Nwosu et al. [33] in malted *Vigna sesquipedalis*. Based on observations from the present study, Bambara groundnut can be used as an aerating agent in whipped toppings, frozen desserts and sponge cakes in view of the good foaming properties.

The observed increase in emulsion capacity in the sprouted samples studied may be due to the effect of sprouting in modifying the protein in foods. Increase in emulsion activities of the sprouted samples could also be as a result of higher protein contents [34]. Proteins possess both polar and nonpolar amino acids which increase the interactions between oil and water molecules in food system [29,34]. Similar observations have been reported in other plant species [33,35]. The emulsion capacity and stability observed in this study points towards the potential of the isolate as an ingredient in many food formulation industries.

A higher water absorption capacity was observed in the sprouted samples of both Bambara groundnut landraces. The higher water absorption of sprouts isolates is attributable to the water binding sites on the side chain groups of protein units [36,37]. The increased water absorption in sprouted samples suggests their applicability in aqueous food formulations especially those involving dough formation like bread and cookies [26,38]. Similarly, oil absorption capacity was higher following sprouting. Although the mechanism of oil absorption is yet to be well understood, surface area, macromolecule sizes and charge and the hydrophobic nature of a protein have been shown to influence the oil absorption [26,39]. Also, alteration in conformation caused during protein isolation may result in more oil binding sites in protein structure. From the results of this study, sprouted Bambara groundnut isolates are potentially useful in improvement of palatability, flavour retention and increasing the shelf life of meat products.

Swelling index determines the amount of water absorbed and the degree of swelling within a stipulated time. A general decrease in swelling index was observed in all the sprouted samples which may be due to decreased total carbohydrate or starch content caused by sprouting [33,40]. Swelling index is influenced by many factors including water availability, temperature and carbohydrate and protein contents [41,42]. Results also indicated higher bulk density following sprouting in both landraces of Bambara groundnut studied. Bulk density is the packing capacity of food material; thus low bulk density ensures more economic packing. On a nutritional point of view, low bulk density is of high nutritional value as it is related to ease of digestion of the food material [29]. The low bulk density obtained in the present study would be an advantage in the formulation of complementary foods [1,43].

4. CONCLUSION

In the present study, Bambara groundnut protein isolates have been found to be rich in essential amino acids and sprouting generally improved the amino acid profile and functional properties. This indicates its usefulness as a supplementary protein source and also as a raw material in the food industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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