Chemical and Functional Characterization of Baobab (*Adansonia Digitata L*.) Seed Protein Concentrate using Alcohol Extraction Method

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Abstract— This study investigated the characteristics of baobab protein concentrate prepared using alcohol extraction method. Baobab fruit and the seeds were washed out, cleaned to remove dirt, sundried for three days and finally ground in an electric mill, sieved and stored. The flour was defatted with hexane under constant magnetic stirring for 3hrs. The slurry was vacuum filtered through filter paper and the residue was used for subsequent extraction. The result obtained showed that alcohol extraction method significantly (p < 0.05) affected the chemical composition and functional properties of the baobab protein isolate. Result of functional properties shows that the alcohol extracted baobab protein concentrate displayed higher solubility index and emulsifying capacity. Baobab protein concentrate can be considered as potential functional food ingredient.

Keywords— Baobab, protein concentrate, alcohol, chemical composition, functional properties.

I. INTRODUCTION

Intense efforts are currently made in the search of cheap protein sources with good nutritional and functional properties, to attenuate the problem of protein malnutrition widely spread in developing countries [1]. Plant proteins play significant roles in human nutrition particularly in developing countries where average protein intake is less than that required. Plant protein products are gaining increased interest as ingredients in food systems throughout many parts of the world and the final success of utilizing plant proteins as additives depends greatly upon the characteristics they impart to foods.

Baobab (*Adansonia digitata L.*), locally called kuka (hausa) and luru (yoruba) is a very long lived tree with multipurpose uses [2]. Baobab is a high yielding, draught resistant and all season plant belonging to the Malvaceae family, it is widely spread throughout the hot, drier regions of tropical Africa [3]. The vernacular name for

baobab means "fruit with many seeds" [4]. The seeds are eaten raw or roasted and have a pleasant nutty flavor [5].

Murray *et al.* [6] reported that baobab seed flour is an important source of energy and protein. The nutritious seed have high values for proteins (33.7%), fats (30.6%), fibre (16.9%) and most minerals. Protein extracts have superior functional properties and are more effectively used in the formulation of foods as compared to seed flours [7].

The acceptability and optimal utilization of baobab seed as a protein source may be limited by the presence of antinutritional factors such as tannins, oxalate and phytate [8]. Nevertheless, techniques employed for extracting protein there from are known to be effective in the elimination of the above antinutrients [9].

Since oilseeds are valuable sources of lipids as well as proteins, numerous studies on protein functionality of major and minor oilseeds such as soybean [10], peanut [11], rapeseed [12], sunflower [13], almond [14], winged bean [I5], groundnut [16], have been reported. The chemical and functional characterization of baobab protein concentrate is scarce. The main objective of this present study is to investigate the properties of protein concentrate from baobab which is produced using alcohol precipitation method in order to establish the potentials application.

II. MATERIALS AND METHODS

Materials

Baobab fruits were collected from Anigbado village, Ayepe, Abeokuta, Ogun State, Nigeria.

Methods Preparation of Baobab Seed Flour

Baobab seed flour was prepared according to the method described by Nkafamiya *et al.* [17].The dried pulp was scraped from the baobab fruit and the seeds were washed out, cleaned to remove dirt, sundried for three days and

finally ground in an electric mill. It was then passed through a 40 mesh sieve and stored for use

Preparation of Defatted Baobab Flour

Defatted baobab flour was prepared according to the method described by Xiaoying and Yufei, [18]. The flour was defatted with hexane (flour/hexane ratio of 1:10 w/v) under constant magnetic stirring for 3hrs. The slurry was vacuum filtered through filter paper and the residue was used for subsequent extraction. Hexane extractions were repeated until the filtrate was clear. Residue from the last extraction and filtration step was air-dried. Defatted baobab flour (DBF) was ground to 150 meshes for further use.

Preparation of Baobab Protein Concentrate (Alcohol extraction method)

Baobab protein concentrate was prepared according to the method described by Wolf [19] with minor modifications. Defatted baobab flour was mixed with 95% aqueous alcohol (1:10 w/v) and stirred for 1hr at ambient temperature (about 25°C). The suspension was filtered and the residues were dispersed in de-ionized water (1:10 w/v) at room temperature and stirred for 1hr. The suspension was finely filtered and the pH of the filtrate was adjusted to 4.5 by addition of 1NHCl (to precipitate out the protein). The slurry was then centrifuged (3500rpm, 15min, 36°C). The supernatants were discarded and the precipitates (protein concentrate) dried in a conventional oven. The protein concentrate was pulverized into a powdered form and stored for subsequent analysis.

Protein Recovery and Chemical Composition Analysis

The chemical composition of baobab meal protain concentrate were determined according to AOAC standard method [20]. The carbohydrate content was estimated by substracting the sum of percentage of moisture, crude fat,crude protein and ash content from 100% the protein recovery was as follows

Protein	Recovery	(%)
_weight (g)of BPC	C×protein content (%)of BPC×100	
weight (g)of D	OPC ×protein content (%)of DPC	

Determination of Funtional Properties Determination of Protein Solubility (PS)

Baobab protein concentrate solution (20 % w/v) were prepared with dispersing powdered protein into distilled water, adjusted to pH 3 to 9. The protein solutions were stirred with a magnetic stirrer at 4°C overnight, centrifuged at 2,500rpm for 30min. The protein sample was directly solubilized by 0.5m NaOH for determination of total protein. The protein content of the content of the supernatants was determined by the (Biuret method 1940) using bovine serum albumin (BSA) as a protein standard. Protein solubility was calculated as :

$Ps (\%) = 100 \times Ps / Pt$

where Ps is the protein content in the supernatant after centrifugation and filtration and PT is the total protein content present in the protein sample.

Determination of Water Holding Capacity (WHC) and Fat Absoption Capacity (FAC)

WHC and FAC of Baobab protein concentrate (BPC) were determined by the method of Gandhi and Srivastara [21].

Determination of Emulsifying activity and Emulsifying stability Index

Emulsifying activity index (EAI) and Emulsion stability index (ESI) of baobab protein flour were measured by the method described by Lopez *et al.* [22].

Determination of Foaming Capacity and Foaming Stability

Foaming capacity and foaming stability were determined by the method described by khalid *et al.* [23].

Determination of Anti nutritional Factors

The tannin content of the sample was determined by the method described by Swain [24], while Phytate and oxalate in the sample was determined by the method describe by Onwuka [25].

Statistical Analysis

Data obtained were subjected to statistical analysis. Means, Analysis of variance (ANOVA) were determined using SPSS Version 21.0 and the differences between the mean values were evaluated at $p \le 0.05$ using Duncan's multiple range test.

III. RESULT AND DISCUSSION

Result of chemical composition of alcohol extracted baobab protein concentrate is shown in Table 1. The percentage yield of baobab protein concentrate was 51.74%, this agrees with the findings of Arnold *et al.* [5] that the nutritious seeds have high values for proteins, fats (oils), fibre and most minerals. The result indicates that the protein concentrate contains 8.16% moisture, 90.36% protein, 1% crude-fibre and very low/insignificant levels of fat, ash and carbohydrate of 0.13%, 0.20% and 0.13% respectively. The protein content which is the major concern and important constituent (90.36%) was high and compared favourably with 90.05% reported for walnut protein isolate by Xiaoying and Yufei [18] and this value

is higher than 85%, 83% and 70% reported for roasted peanut protein concentrate [26], sesame protein concentrate [27] and bambara bean protein concentrate [28] respectively. Low percentages recorded for other constituents of the cellular matrix as presented in Table 1 is an indication of purity of the protein concentrate obtained and the efficiency of the alcohol extraction method adopted.

Table.1: Chemical properties of baobab protein concentrate

Chemical properties (%)	Composition
Protein yield	51.74±0.12
Moisture	8.16±0.15
Protein	90.36±0.00
Fat	0.13±0.05
Ash	0.20±0.10
Crude Fibre	1.00±0.10
Carbohydrate	0.13±0.05

Mean values with different superscripts within the same column are significantly different (p < 0.05)

Result of functional properties of alcohol extracted baobab protein concentrate is presented in Table 2. The foaming capacity of baobab protein concentrate obtained in the present study was 67.36% and it compared favourably with 59% in sesame protein concentrate [27] and higher than 38% in walnut protein concentrate [18]. The foaming capacity was enhanced by high protein concentration, as high protein concentration increases the formation of a multilayer, cohesive protein film at the interface [29]. The high foaming stability in this study (44.86%) shows that baobab has enough flexible protein structure in aqueous solutions and interacted strongly with the air-water interface to form more stable foam. The water absorption capacity of the alcohol extracted baobab protein concentrate recorded was 130%. However, 220% and 610% were reported for the water absorption capacity of cashew nut protein concentrate [30] and sesame protein concentrate [27] respectively. Aletor et al. [31] reported that water absorption capacity of the range of values from 149% to 472% is considered critical in viscous food. The oil absorption capacity was 150% and this compared better than 102% reported for bambara groundnut protein isolate [32], but lower than 294% and 306% in sesame protein concentrate [33] and lupin protein isolate [34] respectively. Elnasri and Eltmay [35] reported that high protein content shows high fat absorption capacity. Kinsella [36] also reported that the ability of protein to bind fat is very important for such applications as meat replacement and extenders principally because it enhances flavor retention and improve mouthfeel. Therefore baobab protein concentrate may be used as thickener and binder in food system. The solubility of the

alcohol extracted baobab protein concentrate at the isoelectric point of the protein was 97.26%. The emulsifying capacity of the alcohol extracted baobab protein concentrate was 14.66%. This value compared favourably with 14% reported for sesame protein concentrate [27] but higher than 5% reported for soy protein isolate [27] but lower than 27% reported for walnut protein concentrate [18].

Table.2: Functional properties of baobab protein concentrate obtained through alcohol extraction method

Functional properties (%)	Composition
Foaming capacity	67.36±0.25
Foam stability	44.86±0.05
Water absorption capacity	130.0±5.00
Oil absorption capacity	150.0±5.00
Solubility	97.26±0.15
Emulsifying capacity	14.66±0.15

Mean values with different superscripts within the same column are significantly different (p < 0.05)

Table 3 shows the protein solubility and emulsifying capacity of the alcohol extracted baobab protein concentrate as affected by different pH as shown in Table 3. The mean values for the solubility index at pH₅, pH₆, pH₇ and pH₈ are 90.46%, 93%, 97.73% and 98.63% respectively. These solubility index compared significantly (p≤0.05) higher than 83% and 47% reported for sesame protein concentrate [27] and walnut protein concentrate [18]. These solubility indexes ensure the usefulness of the baobab protein concentrate in applications such as in beverages. It was observed that as the pH increases, the solubility index and emulsifying capacity were also increasing. This indicates that both solubility and emulsification are pH dependent. Similar observation was reported for Bambara groundnut protein concentrate [32].

Table.3: Solubility and emulsifying capacity of baobab protein concentrate obtain at different pH

protein concentrate obtain at different pH			
Sample pH	Solubility	Emulsifying	
		capacity	
pH ₅	90.46±0.15	10.80±0.30	
pH_6	93.00±0.26	12.33±0.15	
pH ₇	97.73±0.11	14.76±0.15	
pH_8	98.63±0.15	17.53±0.15	

Mean values with different superscripts within the same column are significantly different (p < 0.05)

The result of the antinutritional properties is presented in Table 4. This shows that the oxalate, phytate and tannin were undetectable in the baobab protein concentrate. This may be due to the earlier report of Mwasaru *et al.* [9] that

the techniques employed for extracting protein from baobab seeds are known to be effective in the elimination of the above antinutrients.

Table.4: Antinutritional properties of baobab protein		
concentrate		

Antinutritional factors	Baobab concentrate
Oxalate	ND
Phytate	ND
Tannins	ND
$\mathbf{ND} = \mathbf{N} + 1 + 1 + 1$	

ND= Not detected.

IV. CONCLUSION

The result obtained from this present study indicates the efficiency of the alcohol extraction method adopted as high purity of the protein concentrate was obtained and the oxalate, phytate and tannin content of the protein concentrate were at undetectable level. The functional properties of the protein concentrate compared favourably with other legumes especially with significantly higher solubility index and emulsifying capacity at different pH. This suggested that the baobab protein concentrate can find useful application in food systems.

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