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Quality and functional characterization of baobab seed (*Adansonia digitata* L) protein concentrates using alkaline-isoelectric precipitation method

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ABSTRACT

The research investigated the proximate composition, functional properties and anti nutritional factors of Baobab protein concentrate produced through alkaline-isoelectric precipitation method. Proximate composition, functional properties and anti nutritional factors of the baobab protein concentrate were measured. Data generated were statistically analyzed. Results showed that the protein yield was 50.10%. The proximate composition of the Baobab protein concentrate recovered contain low fibre 0.26%, moisture content of 7.90% and carbohydrate content 0.30% which is an indication that the recovered protein were of high purity. The functional properties obtained, show that protein concentrate recovered by alkaline extraction method has high values of water absorption capacity 121.67%, oil absorption capacity 138.33% and emulsion stability of 56.46%. The solubility at isoelectric precipitation point was 95.80%. Emulsifying capacity and solubility were observed to increase as the pH increased from pH 4.5- pH 8. Results of anti nutritional properties shows that the alkaline method used was effective in eliminating totally the level of oxalates and reducing to an insignificant level the phytates 0.83% and tannin contents 0.66%. Conclusively, it has shown that alkaline-isoelectric precipitation method is effective in producing quality protein concentrate from Baobab seed flour. The established potentials of the Baobab protein concentrate indicate that it will find useful application in various food systems.

Keywords: Baobab seed, protein concentrates

INTRODUCTION

Adasonia digitata (L) commonly referred to Baobab belongs to the family Bombacaceae (Keay *et al.,* 1989) with large spectacular nocturnal flowers and that only one of this species exists in Africa (Baum and Oginuma, 1994). Baobab can have a lifespan of up to 6,000 years (Magaji *et al*., 2014), it is tolerant to high humidity and drought, and is widely distributed in the savannah region, Ecological zones in the arid and semi arid zones in Nigeria particularly North west and North east where it is called Kuka (Esenowo 1991; Baum 1995; Ibrahim and Otegbeye, 2014) and the southern region where it is called Ose (Keay *et al*., 1989).

The tubers, twigs, tender, root, flowers, fruits, leaves, and seeds are said to be edible since every part of the Baobab is said to be useful (Igboeli and Salami, 1997; Vazzle *et al*., 1994). Murray *et al*. (2001) reported that the seed flour is an important and nutritious flour, with protein of (33.7%), fat (30.6%) carbohydrate (4.8%), fibre (16.9) and most mineral (Glew *et al*., 1997). The seeds apart from other uses are also pressed for oil but the by-product, Baobab seed cake is underutilized (Osman 2004).

Protein concentrate is a refined form of protein containing high concentration of protein, which is more complete and contain more bioactive component (penny *et al*., 1999). Proteins utilized in food processing are in various origins, and are the animal (whey protein) and plant (soya protein) (Penny *et al*., 1999). Other examples of raw material from which protein concentrate are produced from are some animal protein source such as meat, egg, milk containing balance level of amino acid and some plant source like soybean, canola, cashew nut, pea protein which have higher content of protein in their parts (Creighton *et al*.,1993). The acceptability and optimal utilization of baobab seed as a protein source is limited by the presence of anti nutritional factors such as tannin, oxalates and phytates (Proll *et al*., 1998). Nevertheless, techniques employed for extracting protein therefore are known to be effective in eliminating the above antinutrients (Mwasru *et al*., 1999). As a plant source, baobab meal has high potential for use as protein, more so the high protein solubility at acidity and alkalinity pH suggest that the protein could be desirable food ingredient (Osman 2004).

According to Moure *et al*. (2003) proteins that are essential to growth and health are currently required more in developing countries because of the chronic problem of protein energy malnutrition, shortage and high price have caused restrictions of animal protein in the diet of these families and the concern over the demand of relatively inexpensive source of protein that can be incorporated to value-added food product worldwide.

This has then led to the investigation of alternative rich protein source (vegetable protein) for food which can be used to fully or partially replace more expensive protein and cheaper and available with great potential regarding their functional properties and also an advantage in using material previously considered to be waste (peel, skin, seed of fruit and vegetable) to recover useful protein which are either discarded or used in low commodity (Baobab seed cake). The main objective of this present study is to investigate the properties of protein concentrate from baobab which is produced using alkaline-isoelectric precipitation method in order to establish the potentials application.

MATERIALS AND METHODS

Materials

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

Preparation of defatted baobab flour

Defatted baobab flour was prepared according to the method of Xiaoying and Yufei (2012). Baobab flour was dried at 50°C for 1hr and was finely prior to use milled using attrition mill and was finely sieved through the use of a sieve. The baobab meal was defatted with hexane at a raahon 1:10 (w/u) under constant shaking. The hexane was changed three times and then decanted and removed in a forced air oven at 60°C for 1hr. The baobab was ground to pass through mesh and kept in vacuum containers at 4°C prior to use.

Preparation of baobab protein concentrates (alkali solution -isoelectric precipitation method)
Baobab protein concentrate (BPC) was prepared according to the method developed by Gandhi and Srivastava (2007) with minor modification. Baobab protein concentrate was mixed with water at a ratio of 1:10 (w/v). the pH of the suspended meal was adjusted to pH values ranging from 7 to 12 using 2.0m NaOH, continuously stirred with a magnetic stirrer for 1hr and centrifuged at 3,500rpm for 15min. the soluble phases were adjusted to pH 4.5 using 0.1 or 1.0M HCl, which led to precipitation of protein the suspension was centrifuged again and the supernatant poured away and the precipitates weighed and assayed for protein content by Kjeldhal method. The precipitate was dried is a conventional oven and the protein concentrate was pulverized into powder from and stored in a cool tight container for subsequent analysis.

Protein recovery and chemical composition analysis
The chemical composition of baobab meal protain concentrate were determined according to AOAC standard method (1999). 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:

$$\text{Protein Recovery (\%)} = \frac{weight\ (g) of\ BPC \times protein\ content\ (\%) of\ BPC \times 100}{weight\ (g) of\ DPC \times protein\ content\ (\%) of\ DPC}$$

Funtional Properties of baobab protein concentrate
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 2,8229 for 30 min. 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 × 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 (2007). One gram of the sample was mixed with l0ml distilled water or soy bean oil in centrifuge tubes and then allowed to stand for 30 min. Samples were centrifuged at 2,822rpm for 30 min. The supernatant was discarded and the tube was weighed. WHC (grams of water per gram of sample) was calculated using the equations.
$WHC= (W_2-W_1)/W_0$
Where W_0 was the weight of the dry sample (g)
W_1 was the weight of the test tube plus dry sample (g)
W_2 was the weight of the tube plus sediment (g)
FAC (grams of oil per gram of protein) was calculated using the equation; $FAC =(F_2-F_1)/F_0$
Where F_0 was the weight of the dry sample (g)
F_1 was the weight of the tube plus dry sample (g)
F_2 was the weight of the tube sediment (g)

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 of Lopez *et al*. (1996). BPC (4g) were suspended in distilled water (100ml). The protein solution was stired with a magnetic stirrer for 30min as the protein standard and then centifuged at 2,822rpm for 30 min. The supernatant was collected and detertmined by biuret method using bovine serum albumen (BSA). To prepare the emulsion; 60ml of protein solution and 20ml of soybean oil were homogenized at high speed for 1 min by a homogenizer. The emulsion was then dilluted 200 fold with 0.1% (W/0) containing 0.1mol/Nacl at pH 7.0. The absorbance of the dilluted emulsion was at pH 7.0. The absorbance of the dilluted emulsion was then measured at 500nm in a 1cm path lenght the cuvette at 0min (A_0) after precipitation using spectrophometer. EAI and ESI were calculated as follows:

EAI (m^2g-1)= (2x2.2303xAoxN)/(Ccx Ø)

ESI (min)= (Aox10)/(A_0-A_{10})

Where N represents a dilution factor

Ø is the oil phase volume (Ø of soybean oil=0.25)

C is the concentration of protein (mg/ml)

Determination of foaming capacity and foaming stability

Foaming capacity and foaming stability were determined by the method of khalid *et al*. (2003). 3g of Baobab protein concentration and 100ml of distilled water at pH7 were homogenized at high speed for 5 min by homogenizer and then tranferred into a measuring cylinder. The volume of foam at 30 s was calculated and the volume increase expressed as percent foam capacity. The foam stability measured the decrease in volume of foam as a function of time up to a period of 90min.

Anti-nutritional factors baobab protein concentrate

Determination of Tannin

The tannin content of the sample was determined by folindenis colour, metric method of Itarborne (1993). 5g sample was mixed with distilled water in the ratio of 1:10. The mixture was shaken for 30 min at room temperature and filtered to obtain the extract . A standard tannin acid solution was prepared and 2ml of the solution and equal volume of distilled water were dispersed into a seperated 50ml volumetric flask to serve as standard and reagent blank respectively. The 2ml of each of the sample extract was put in their respective flask and labelled. The content of each flask was mixed with 35ml distilled water and 1ml of folin-Denis reagent was added to each, followed by 2.5ml of saturated Na_2CO_3 solution. Then each flask was dilluted to the 50ml mark with distilled water and incubated for 90min at 100°C. The absorbance was measured at 760nm in a colorimeter with the reagent blank at zero. The tannin was calculated as shown below

% tannin =100/w x au/as x c/1000 x vt/va

W = weight of sample

au= absorbance of test sample

as= absorbance of standard tannin solution

c=concentration of standard tannin solution

vt= total volume of extact

va= volume of extract analysed

Determination of Phytate

Phytate in the sample is determined using the bipyrimidine colorimeter method describe by Onwuka (2005). 2g of the sample was soaked in 50ml of 0.2 NHCL solution and shake for 30 min in a shaker. It was filtered to obtain the extract. A portion of the extract(0.5mls) was dispersed into a test tube and 1ml of acidified ferrous ammonia sulphite solution was added to it. The tube was stoppered and boiled in water bath for 30 min. It was then cooled in ice water for 15 min and allowed to reach room temprerature. The mixture was centrifuged at 3000 rpm for 5 min and the supernatant was collected for analysis. 1ml of the supernatant was mixed with 1.5 ml of 2.2 bioyxine solution. Meanwhile, a standard solution of phytate was prepared and dilluted to a chosen concentration. 1ml of the standard solution was treated same way as the sample were read in a spectrophotometer at a wavelenth and the sample were read in a spectrophotometer at a wave length of 519mm.

% phytate = 100 × au × c × vt

Where au = Absorbance of sample

c = concentration of the standard

vt = Total volume of extract

va = volume of extract analyzed

Determination of Oxalate

Oxalate was determined according to the method of Onwuka (2005). 5g of the sample was weighed into a 100ml beaker, 20ml of 0.3N HCl was added and armed from 40 – 50°C, using magnetic hot plate and stirred for one hour. It was extracted three times with 20ml of 0.3N HCl and filtered into a 100ml volumetric flask. The combined extract was diluted to100ml mark of the volumetric flask. The oxalate was estimated by pipetting 5ml of the extract into a conical flask and made alkaline with 1.0m of 5N aminonium hydroxide. A little indicator paper was placed in the conical flask to enable us know the alkaline region. It was also made to phenolpthalein (2 or 3 drop of this indicator added, excess acid decolourize solution) by drop wise of glacial acetic acid. 1.0ml of 5% CaC_{12} was then added and the mixture allowed to stand for 3h after which it was then centrifuged at 3000rpm for 15min. The supernatants were discarded and the precipitates washed 3 times with hot water with thorough mixing and centrifuging each time. Two millliters of 3N H_2SO_4 was added to each table and the precipitates dissolved by warming in a water bath (70 – 80°C). The content of all the tubes was carefully poured into a clean conical flask and titrated with freshly prepared 0.05 m $Kmno_4$ at room temperature until the first pink colour appeared and the solution became colourless. The solution was then warmed to 70 – 80°C and titrated until a permanent pink colour that persisted for at least 30 seconds is obtained.

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 $p \leq 0.05$ using Duncan's multiple range test.

RESULT AND DISCUSSION

Chemical composition of baobab protein concentrates

The result of chemical composition of Baobab protein concentrates obtained through Alkaline-isoelectric precipitation method is presented in Table 1. The result indicates that Baobab protein concentrate contains 90.90% of protein, 0.2% fat, 7.0% moisture, 0.05% fibre and 0.30 carbohydrates. Similar results were recorded for baobab protein concentrate as reported by Inyang and Iduh (1996) on moisture, fibre and carbohydrate of sesame protein concentrate. Fat and protein content of baobab protein concentrate is also similar to cashew nut (89.99%) and pea protein concentrates (93.54%) respectively (Nandi, 1990). The low percentage recorded for other constituents of the baobab protein concentrates in this study is an indication of purity of the protein concentrate extracted.

Functional properties of baobab protein concentrate

The result of functional properties of baobab protein concentrate obtained through alkaline-isoelectric precipitation method is presented in table 2. Emulsifying capacity of baobab protein concentrate was 13.20%. However, 5.95% had been previously reported for soybean concentrate and 49.70% for sesame protein concentrate (Lopez *et al*., 2003). The Emulsion stability of baobab protein concentrate recorded was 56.46% as compared to that of soy protein 63.5%. The stability of the protein film formed at interface of the emulsion is dependent on the interactions of the protein in oil and aqueous phases (Damodaran, 1996). The foaming capacity of baobab protein concentrate obtained in this present study was 65.83% which was higher than 38.78% obtained for walnut protein concentrate (Gandhi and Philips, 1976). Foaming stability of baobab protein concentrate 46.43% compared to that of walnut protein concentrate 28.18% and this shows that baobab has a more flexible protein structure in aqueous solutions and interacted strongly with the air-water interface to form more stable foams. Such high foaming capacity and stability can make baobab protein concentrate find useful applications in whipped toppings and cakes (Kinsella, 1982). The water absorption capacity of the extracted protein concentrate was 121.67%. However Ogunwolu *et al*. (2009) reported 610.20% for cashew nut protein concentrate. Aletor *et al*. (2002) reported that water absorption capacity of the range of values from 149 - 472.5 are considered critical in viscous, this suggest that the baobab protein concentrate can be of good use in food products requiring high water retention (Adeyemi and Aye, 1998). The oil absorption capacity for baobab protein concentrate was 138.33% which was higher than 102.29% reported for bambara groundnut protein isolate (Elteyeb *et al*., 2011). Elnasri and Eltmay (2007) reported that high protein content shows high oil absorption capacity. This high value of baobab protein concentrate makes it suitable for use in meat, sausages and doughnuts production. (Kinsella, 1982). Protein solubility is a useful indicator for the performance of protein isolate /concentrate when incorporated in food system and to determine the extent of protein denaturation because of heat or chemical treatment at different pH (Horax *et al.*, 2004). The solubility of alkaline extracted baobab protein concentrate at the isoelectric point was 95.36%, it compares favourably with 95.0% reported for cashew nut protein concentrate (Moure *et al*., 2006). This solubility ensures the usefulness of the baobab protein concentrate in beverages (Kinsella 1982).

Figure 1 shows the protein solubility and emulsifying capacity of the alkaline extracted baobab protein concentrate as affected by different pH (pH5, pH6, pH7, pH8). The result indicates that the solubility index and emulsifying capacity was pH dependent as their value increase with

increasing pH. The result shows that solubility kept on increasing as the pH was shifting from around isoelectric point to the alkaline region.

Anti-nutritional properties of baobab protein concentrate

The result of the anti nutritional properties monitored shows that the alkaline-isoelectric method adopted is effective in eliminating the oxalates and reducing the phytates and tannin content to a very insignificant level of 0.83% and 0.66% respectively.

CONCLUSION

Results of proximate composition of baobab protein concentrate extracted using alkaline-isoelectric precipitation method shows that protein of high yield (50.10%) was obtained and also signifies high purity of the protein concentrate considering the low values of other component of other components of the cellular matrix. The water and oil absorption capacity were high, the emulsion stability and solubility of the alkaline extracted baobab protein concentrate were remarkably high. The alkaline method was effective in reducing the antinutrients to a very low insignificant level. This potentials show that it will find useful applications in various food systems where applicable. The functional properties and proximate data obtained indicate that the alkaline method adopted is substantial for extracting protein concentrate.

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Table 1. Promimate Composition of Baobab meal protein concentrate

Constituents (%)	Baobab Concentrate
Protein yield	50.10 ± 0.00^{a}
Moisture	7.90 ± 0.10^{a}
Fat	6.20 ± 0.10^{c}
Protein	90.90 ± 0.10^{b}
Fibre	0.26 ± 0.05^{b}
Ash	0.20 ± 0.10^{a}
Carbohydrate	0.30 ± 0.10^{c}

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

Table 2. Functional Properties of the Baobab Protein Concentrate

Functional properties (%)	Baobab Concentrate
Emulsion Capacity	13.20 ± 0.20^{a}
Emulsion Stability	56.46 ± 0.20^{c}
Foaming capacity	65.83 ± 0.40^{b}
Foaming stability	46.43 ± 0.10^{a}
Water absorption capacity	121.67 ± 2.88^{a}
Oil absorption capacity	138.33 ± 2.88^{a}
Solubility	95.36 ± 0.15^{b}

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

Table 3. Antinutritional properties of baobab protein concentrate

Antinutritional factors	Baobab concentrate
Oxalate	ND
Phytate	0.83 ± 0.01^{a}
Tannins	0.66 ± 0.00^{b}

Mean values with different superscripts within the same column are significantly different ($p < 0.05$); ND= Not detected

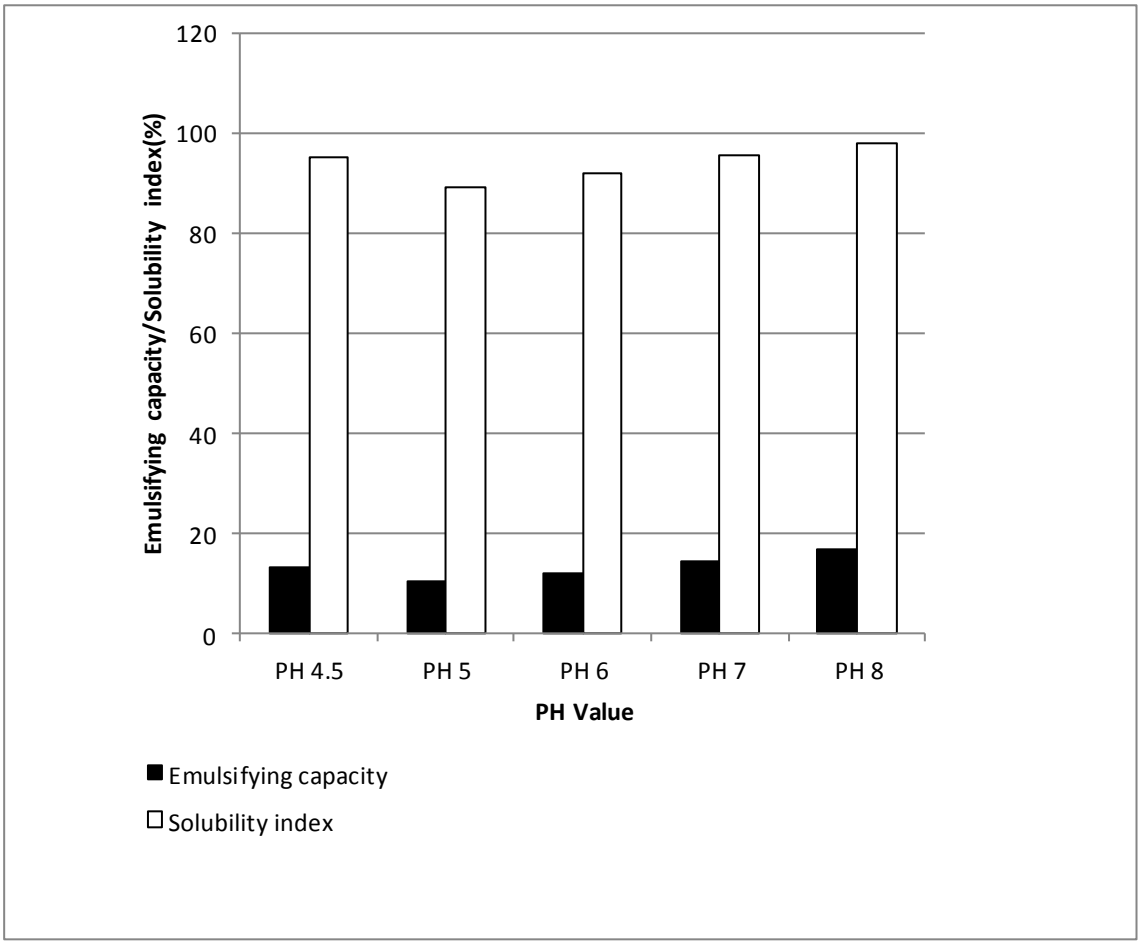

Figure 1. Emulsifying capacity and solubility Index of baobab protein concentrate using alkaline-isoelectric precipitation method