FUNCTIONAL PROPERTIES OF PROTEIN ISOLATES FROM AFRICAN OIL BEAN SEEDS (*Pentaclethra macropylla*)

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ABSTRACT

*Pentaclethramacrophylla (***African oil bean***) seed is a prominent tropical tree plant in Nigeria belonging to the family leguminosae and mimosoidaesub-family, and used to supplement starch sustenance's, and other nourishment. The present study assessed the functional properties of protein isolates from the seeds of P.macrophylla using standard methods while simple statistical analysis was used to evaluate the results. From the results, the seeds protein had in* gg^{-1}*an oil absorption capacity, emulsifying capacity and emulsion stability of 148.57±1.82, 80±0 and 44.78±3.22, respectively. The bulk and tapped density were 0.49±0.01and 0.57±0.02g/ml in that order. The effects of a temperature range of 60-90°C on swelling power and solubility index were 366.61±126.73to 280.56±5.57 g/g and 10.71±9.48to 5.73±1.03 g/g. The effects of NaCl concentration and pH value of a range of 0.1-2.0 M and 2-14 on water absorption capacity were 288.4±14.57 to 237.9±15.98 g/g and 329.8±67.60to 785.2±14.14 g/g. The least gelation concentration of protein isolates was found to be 3%, and the minimum and maximum nitrogen solubility were 8.75±2.89% at pH 6 and 131.25±0.35% at pH 2, respectively. The data generated for the effect of salt concentration and pH on water absorption capacity showed that both the salt and pH might be used to enhance the functionalities of the protein flour. The results obtained in this study compared favourably with previous studies on protein isolates from other seed plants.*

Keywords: Pentaclethramacrophylla seeds; Protein isolate; Functional properties; NaCl; pH

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1.0 Introduction

The African oil bean seed (*Pentaclethra macrophylla*: Benth) is a prominent tropical tree plant in Nigeria, with some regular names "Apara" (Yoruba), Ugba (Igbo) in the eastern piece of Nigeria. It has a place with the family leguminosae and sub-family mimosoidae and local to tropical Africa. The seeds are smooth and hard, darker in shading, endosperm white in shading and the seeds are around 6 cm long (Osagie-Eweka and Alaiya, 2013).

The Africa oil bean (seeds) is much of the time used to supplement starch sustenance's, vegetables, and different nourishments in eastern Nigeria since it contains a high extent of protein and the 20 amino acids (Osagie-Eweka and Alaiya, 2013). The oil bean seed is utilized to supply satisfactory fundamental amino acids (protein) required in diets. Not just that, the African oil bean seed contains high amount and quality protein, it additionally contains 77-78% unsaturated and 22-33% saturated fatty acid. Functional properties of any protein material are very important in food applications. Effects of fermentation and

heating on the functional properties of processed flour from African oil bean seed (*P. macrophylla* benth) have been reported Osagie and Alaiya (2013).

The expanding interest for vegetable plants protein in Nigeria, for both domestic and industrial purposes, up to date, is completely made because of the nutritious needs of the increasing population and the expanding number of businesses that require protein as their essential material. Nigeria nonetheless, being a tropical nation, has wide varieties in climatic plants that contain protein. The investigation along these lines went for uncovering the possibilities of less known seed oil, *P. macrophylla* protein, which could substitute the prominent creature protein. It therefore becomes necessary to expand the scope of usage for *P. macrophylla* for domestic and industrial purposes. This study is, however, limited to the isolation of protein from *P. macrophylla* and determines the functional properties of the protein isolates.

2.0 Materials and Methods

2.1 Sample collection

The African oil bean seed was bought in local market at Owode Yewa, Ogun State in Nigeria in August 2017. The seeds were visually inspected and defective seeds discarded. The seeds were then stored at 7^{o}C and 60% relative humidity until used.

2.2 Preparation of African oil bean seeds flour

African oil bean seeds were crushed, using a household mill (Braun, Germany), and then defatted by soaking in n-hexane for 48 hours with several changes of the solvent. The defatted flour was air-dried at room temperature (25^{o}C) and ground again to pass through a 60-mesh (British Standard Screen) sieve. Protein isolate was prepared with the flour as described by El-Adawy, Rahma, El-Bedawey & Gafar, (2001).

2.3 Preparation of protein isolates by iso-electric precipitation (Isolates PI)

444 g of the defatted flour sample was suspended in 2 L of distilled water and stirred for 30 minutes and the pH was adjusted to 9.0-9.5 using 1 M NaOH. The suspension was stirred for 1 h at room temperature, and then centrifuged at 3000 rpm for 30 mins. In order to obtain higher yields, the extraction and centrifugation were repeated on the residue. The extracts were combined and acidified to pH 4.5. The precipitate was recovered by centrifugation at 3000 rpm for 30 mins, then neutralized by 1.0 M NaOH to pH 7 and the residue obtained (protein) was washed twice with distilled water. The neutralised precipitate was freeze-dried (Lab Conco Freeze Dry 64312. Kansas, Missouri), then milled using a mortar and pestle and kept in an air tight container (El-Adawy et al., 2001).

2.4 Functional properties

2.4.1 Water absorption capacity

Duplicates samples (0.5 g) were suspended in 10 ml of distilled water in a centrifuge tube and mixed for 30 seconds the dispersion were centrifuged at 3000 rpm for 15 minutes. The supernatant was filtered and

the volume regained was accurately measured. Water absorption capacity (WAC) was determined according to the method described by (Deng et al., 2011). Water absorption capacity (WAC) of the protein isolate was measured at different pH values (pH 1.0-4.0 without adding NaCl) and with NaCl concentrations (0-2 M NaCl dissolved in de-ionised water).

2.4.2 Swelling power and solubility index

Swelling power and solubility of *P. macrophylla* protein were determined by heating a flour-water slurry (0.2 g flour in 10 ml of distilled water) in a water bath at 60°C for 30 mins, with constant stirring (Crosbie, 1991). The slurries were centrifuged using a Super-speed centrifuge at 168×g for 15 min, the supernatant was decanted into a weighed evaporating dish and dried at different temperature of 60, 70, 80 and 90°C for 20 mins. The difference in weight of the evaporating dish was used to calculate flour solubility. Swelling power was obtained by weighing the residue after centrifugation and dividing by original weight of flour on the dry weight basis.

$$\text{Swelling of flour} = \frac{\text{W1} - \text{W2}}{\text{weight of flour}}$$

2.4.3 Protein solubility

Solubility of protein was analysed at different pH values (pH 1-14) according to the method described by (Deng *et al.*, 2011). Duplicates sample (0.1 g) was dissolved in 10ml of distilled water and it was stirred for 30 minutes before adjusting the pH(2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, and 12.0) with 0.1M NaOH and 1M HCL. The solution was centrifuged at 4000 g for 10 mins. Protein content of supernatant was analysed using kjedahl method.

2.4.4 Bulk density

Bulk density was measured as a ratio of mass to volume. A 10 ml graduated measuring cylinder previously tarred was gently filled up to the 10 ml mark with flour. The sample was then packed by gently tapping the cylinder on the bench top from a height of 5 cm until there was no further diminution of the sample level. The weight of the filled was taken and the bulk density calculated as the weight of sample per unit volume sample (g/ml) as described by (Hamid *et al.*, 2015)

$$\text{Bulk Density} = \frac{Weight\ sample - Weight\ of\ empty\ cylinder}{volume\ occupied}$$

3.0 Results and Discussion

3.1 Results

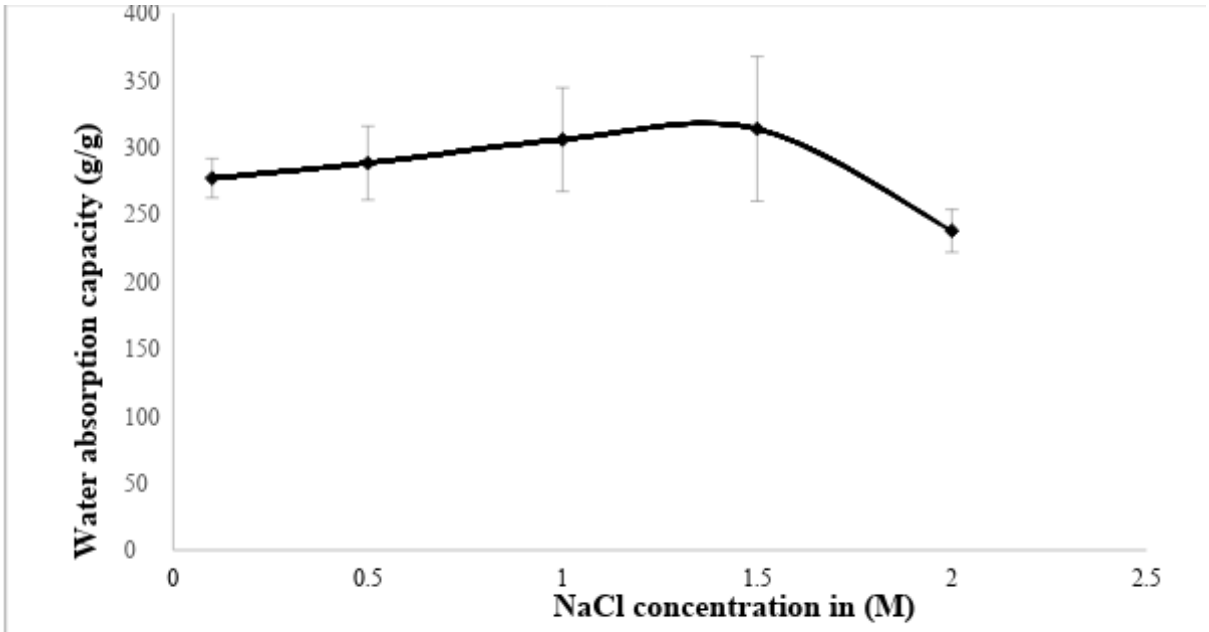

Fig. 3.1.1 Effects of NaCl concentration on water absorption capacityo

Fig 3.1.1 shows the effect of NaCl concentration on the water absorption capacity of *P. macrophylla*; Values ranged from 277.1 - 313.9 g/g. The results shows that the water absorption capacity (WAC) increased as the concentration increased from a range of 0.1-1.5 M (277.1, 288.4, 305.9 and 313.9 g/g) and decreased as the salt concentration increased to 2.0 M.

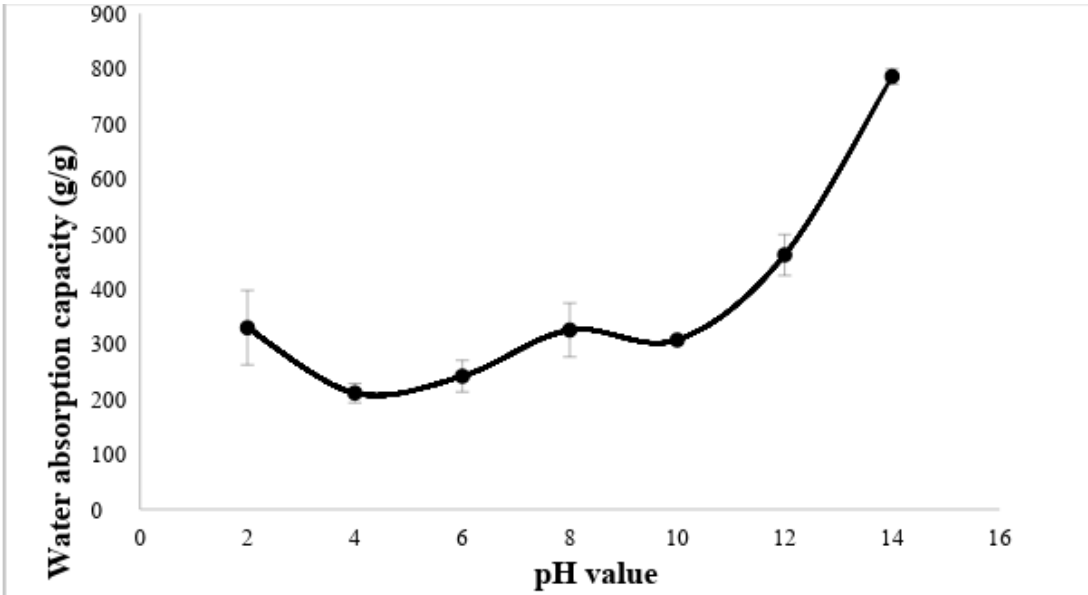

Fig. 3.1.2.Effects of pH value on water absorption capacity of *P. macrophylla*

Fig 3.1.2, shows the effect of pH value on the water absorption capacity (WAC) of *P. macrophylla;* value decreased as the pH increased from 2-4 (329.8 g/g-211 g/g) and later increased as the pH increased from pH 4-14 (211 g/g-785.2 g/g). The decrease in pH from 2-4, shows that the iso-electric point of the protein is 4 and which indicated that the protein sample is acidic in nature.

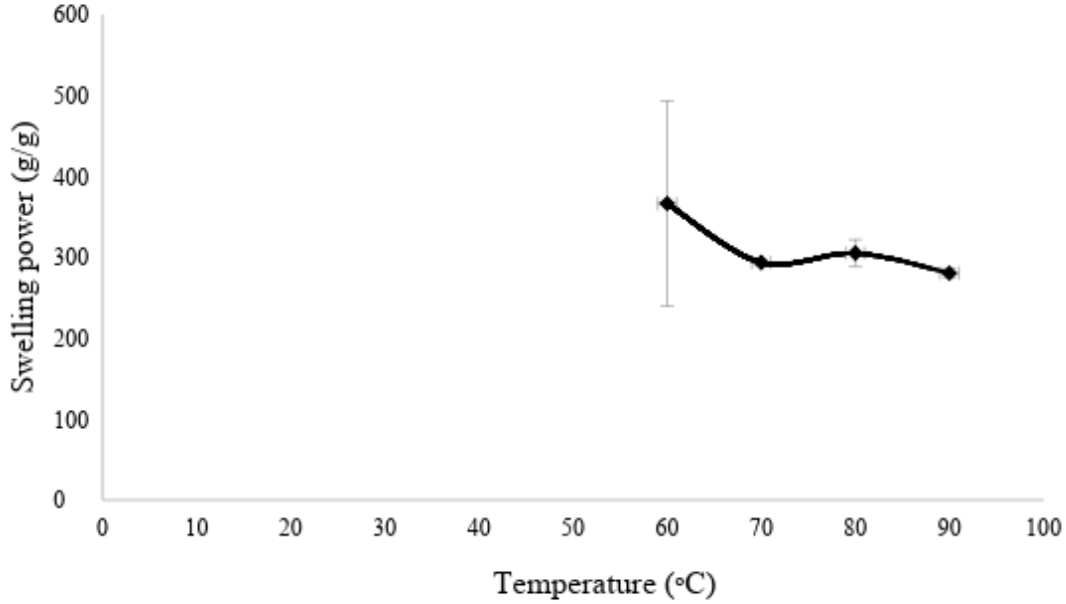

Fig. 3.1.3. Effect of temperature on swelling power of *P. n*

Fig 3.1.3 shows the effect of temperature on swelling power of *P. macrophylla;* which decreased as the temperature increased from 60-90°C (366.61-280.51 g/g).

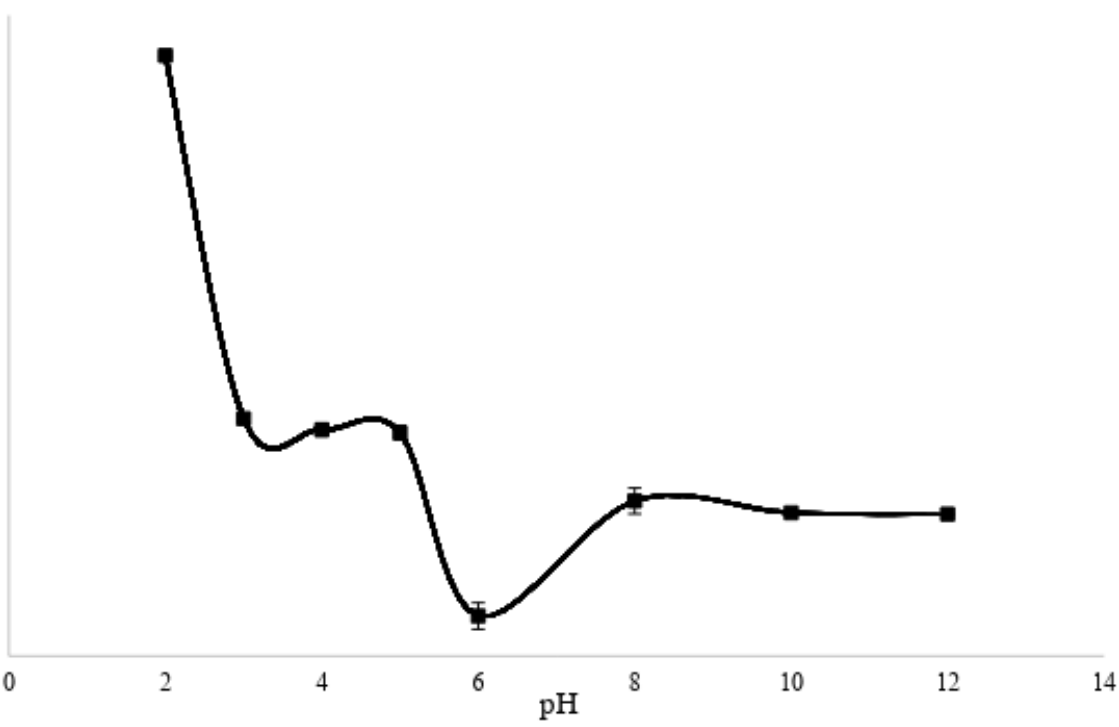

Fig.3.1.5 Solubility of *P. macropylla* protein isolates as a function of pH

Fig 3.1.5 shows the effect of pH on solubility of *P. macophylla* protein which showed the minimum solubility at pH 6 (8.75%) and the maximum solubility at pH 2 (131.25%).

Table 1: Showed results for oil absorption capacity (OAC), emulsifying capacity (EC), emulsion stability (ES), bulk density (BD) & tapped density (TD) of *P. macrophylla* protein isolates (g/g)

Analyses	Result
OAC	148.57±1.82
EC	80±0
ES	44.78±3.22
BD	0.49±0.01
TD	0.57±0.02

3.2 Discussion

The results show that the water absorption capacities of *P. macrophylla* increased as the sodium chloride concentration increased from (0.1 M-1.5 M) (288.4 g/g, 305.9 g/g, 313.9 g/g) and later falls as the salt concentration increases to 2 M. while the water absorption capacity showed similar curves at different pH values (Fig.3.1.2). WAC of *P. macrophylla* protein decreased with an increase of pH values in the range 2-4. The minimum WAC value of *P. macrophylla* was observed at pH 4 (211 g/g) while the maximum WAC was at pH 14 (785.2 g/g). The result from this study is similar to that of Ginkgo biloba protein isolates, in which the WAC values decreased with an increase of pH values in the range 1-4 (1.51 g/ml) (Deng et al., 2011). The results showed that the iso-electric point was in the range of 4-4.5 for *P. macrophylla* protein, which indicated that African oil bean seeds are acidic, this was confirmed by the highest protein content and yield obtained from the iso-electric precipitation method for *P. macrophylla* seed protein production. According to (Deng et al., 2011)., it was stated that when pH values were near the iso-electric point, proteins aggregated with strong intermolecular interactions, resulting in less interaction with water, which led to a reduced water absorption capacity.

The bulking properties of powder are dependent upon the preparation, treatment and storage of the sample. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density.

The swelling power and solubility decreased as the temperatures increased from 60-70^{o}C. An increase in temperature shows increase in swelling power and solubility from the range of 80-90^{o}C, which has a lower results compare to that of red and black cowpea (4.13 g/ml-6.93 g/ml) (3.88 g/ml-8.03 g/ml) for swelling power and (0.31 g/ml-0.37 g/ml) (0.34 g/ml-0.45 g/ml) for solubility over a temperature range of (60-90^{o}C), (Hamid et al., 2015). Swelling power and solubility can be used to measure the extent of interaction between starch chains, within the amorphous and crystalline domains of the starch granule (Sindhu, 2016). Solubility is the amount of protein in a sample that dissolves into solution. Protein is recommended as food additives and can be wholly or partly soluble or completely insoluble in water. Fig.3.1.5 shows the effect of pH on the solubility of *P. macrophylla* protein. The graph shows decreased in solubility as the pH increased from pH 2-12 and the maximum solubility was obtained at pH 2(131.25%) while the minimum solubility was at pH 6 (8.75%). For *P. macrophylla* protein compared to that of beach pea protein a gradual increase in solubility above and below their iso-electric points and the minimum solubility was at pH 4.5 (Chavan, McKenzie & Shahidi, 2001). The minimum solubility of bitter lupin seed flour proteins was quite sharp at pH 4.5 with (17.6%), and sweet lupin seed proteins exhibited a broad range of minimum solubility at a pH range of 4.3–4.9 with (15.1%) (El-Adawy et al., 2001). Solubility is an essential property for the design of new drugs in the pharmaceutical industry; it also plays a vital role in foods and cosmetics industry (Agrawal & Noble, 2005). The effect of pH on the solubility of P. macrophylla showed that it could be useful in many industries, such as foods, cosmetics and pharmaceutical industry.

4.0 Conclusion and recommendation

4.1 Conclusion

The functional properties of protein isolates from P. macrophylla were investigated. The results obtained showed that the P. macrophylla protein isolates had desired physicochemical properties. As compared with other vegetable protein, *P. macrophylla* protein exhibited better solubility, water absorption capacity, oil absorption capacity, bulk and tapped density, swelling power and solubility index. Therefore, these results suggest a possible use of African oil bean protein isolates as nutrient supplements and as functional agents in many food systems.

4.2 Recommendation

It is recommended that the following study is carried out on the functional properties of protein isolates from African oil bean seeds. The information gathered on the functional properties serves as a useful tool in the isolation of protein from African oil bean seeds for industrial uses.

However, further studies are still needed on other relevant of functional properties for the design and processing technique of reducing the negative effects.

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