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Fractionation and Some Conditions on the Extract Ability of Tropical Almond (*Terminalia catapa)* Seed Protein

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ABSTRACT

The control variables involved in the extraction of seed protein from *Terminalia catapa* seed meal have being investigated. These include extraction time, pH, solid-solvent ratio. Protein and fractionated into albumin (4.25%), glutelin (3.57%), prolamin (8.96%) and globulin (6.35%). The extraction time (min) of 15, 30, 45 and 60 were used for fractionation of the seed protein. The results showed an increase in protein yield across the fractions with increasing extraction time; highest protein yield of 10.2% was recorded in prolamin at 60 mins. The pH dependent solubility profile revealed that the region of iso-electric point (minimum solubility) was at pH 8.0, the solubility reduced as the pH increased until it reached the iso-electric point which was followed by progressive increase in solubility with further increase in pH. The solid-solvent ratio of 1:5, 1:10, 1:20, 1:30 and 1:40 were used and the result shows that there was increase across the fraction with increasing solid-solvent ratio. Highest protein yield was recorded at ratio 1:40

Key words: *Terminalia catapa*, Fractionation, Extraction, Tropical almond, Seed meal.

INTRODUCTION

Due to the exorbitant cost of protein from animal sources has led to a growing interest in industrial application of protein from plant origin to challenge the increasing demand for protein in food and non-food market. In view of this problem protein is extracted using acid, base, water solvent and ethanol. Each of this has its advantages and disadvantages on the solubilised protein quality. (Swanson, 1990). Thus, the knowledge of protein solubility will be an important factor in selecting particular vegetable proteins for possible industrial application (Lawal and Adebowale, 2006; Adebowale and Lawal, 2004; Adebowale and Lawal, 2003). Many factors have been document to affect the extractability of protein, including solvent-solid ratio, pH, centrifugal speed, extraction time, successive extraction steps and temperature during extraction (Kinsella, 1979). It is however important, to recover much of the protein during extraction so as get maximum protein content in the concentrates or isolates (Adebowale *et al*., 2008). Extractability of proteins from various legumes has been widely studied and different results were obtained due to the numerous factors involved (Govardhan *et al*., 2011; Lawal *et al*., 2004; Ragab *et al*., 2004; Badifu and Akubor, 2000).

Information is sparse on the extraction parameters of defatted seed kernel of *Terminalia catapa*). The present study aims at reporting the protein content and optimum extraction conditions of Nigerian Tropical almond (*Terminalia catapa*) seed. Since extractable protein determines the amount of protein that can be made available from a particular seed flour and non-food application, of the preliminary factor that determines whether or not a protein could be adopted for commercial exploitation is the protein extraction efficiency of such seed protein (Liu, 1997).

Therefore, the aims and objectives of the present study are to fractionate protein of defatted seed kernel of *Terminalia catapa* and to determine the influence of some extraction variables like extraction time, pH extractability protein and solid-solvent ratio on the yields of seed protein from *Terminalia catapa*. The presents study helps to determine the amount of protein present in Nigerian Tropical almond (*Terminalia catapa*) seed. Varying some conditions on the best yield of protein extraction to improve the utilization of protein and to be able to fortify other food product which are not rich in protein, such as roots, tubers and fruits which have low protein content between. Food staples with low protein must be complemented with foods with complete, quality protein content for a healthy life, particularly in children for proper development.

MATERIALS AND METHODS

Sample collection and preparation

Matured fruits of *Terminalia catapa* (wine-flesh varieties) were collected within the premises of the Federal Polytechnic Ilaro, Nigeria.

The pulp (mesocarp) of the mature and ripened fruit was manually scrapped with sharpened knife and the kernel shell was cracked to obtain the kernel. Selected whole kernels were air-dried in oven (Gallenkamp, England) oven at a temperature of 50^0 C for 5 hrs till moisture level of 5 - 8% was attained. The testa of the dried kernels were removed manually, packaged and stored in polyethylene for storage prior to further analyses and processing.

Preparation of raw and defatted Topical almond (*Terminalia catapa*) meal

Defatted kernel meal was prepared using the methods described by (Jitngarmkusol *et al.,* 2008) with some modifications. The seed of *Terminalia catapa* were sundried and grounded with a blender (Master Chef MC-JBL2102, Pegasus Ltd., G.P.O. Box 11819, Hongkong). The milled *Terminalia catapa* seeds were defatted. To produce partially defatted meal, the milled seeds were soaked in n-hexane for 17to 19 hours in order to obtain a residual fat content of 17.4% (from the initial 58.9%). The defatted samples were dried for 2 hours at ambient temperature in a fume hood to remove the remaining solvent. The defatted samples flour (DSF) were then ground and sifted through a 60-mesh sieve. The flours were packed in laminated bags and stored at 4 to 7 °C until use.

Protein and Moisture Content

The protein content DSF samples were determined by micro-kjeldahl technique following the method of AOAC (1990) with cupric sulphate and potassium sulphate as catalyst. The protein content of each sample was calculated by multiplying the nitrogen content with a factor of 6.25. The moisture was determined on 100mg sample by oven drying to constant weight (A.O.A.C., 2000).

Fractionation of Seed Protein

Protein fractions were isolated by successive extraction of defatted seed flour with different solvent as described by Chavan *et al.* (2001). Defatted seed flour (20g) was stir in 200ml distilled water with a magnetic stirrer for 30minute at room temperature. It was then centrifuge at 5000rpm for 15minute and the resultant supernatant was recovered by filtration with whatman paper number 41. The residue thus obtained was again extracted two more times with the same solvent. The recovered filtrates were combined with the previous filtrates and designated as water soluble fraction (Albumin). The residue was then successfully extracted with 0.5m HCl solution of pH 7.0 (Globumin), 70% (v/v) ethanol at 70°C in shaking water bath (prolamine) and finally with 0.1m NaOH solution (Glutelin) to separate the total protein in seed. The precipitate were then washed and dispersed in distilled water. Finally protein fractions were dialysed against distilled water for 72 hours at 4°C and separately lyophilised. The protein content of each fraction was determined by macro-kjeldahl procedure (AOAC, 1990).

Protein extractability

Tropical almond protein extractability was determined essentially by the procedure used by Dev *et al.* (1986) as described by Eromosele *et al.* (2008); and the conditions for optimum extractability established by considering extraction time; solid – solvent ratio; pH extractability protein. An accurately weighed (2g) of defatted sample meal was employed in each of the experiment. It was extracted at room temperature on a mechanical shaker (2000rpm). In experiment where pH was varied the temperature was adjusted to the desired pH with 0.1M HCl or

NaOH in such a manner that substantial increase in the final volume was avoided. After each extraction the suspension was centrifuge at (4000rpm) for 1 hour at room temperature. To determine the extraction efficiency, supernatant obtained from each extraction was analysed for the protein content by micro kjeldahl method of AOAC (1990). All extractions were carried out in duplicates.

Statistical analysis

Data generated from this study were subjected to Analysis of variance (ANOVA) and means were separated by the Duncan multiple range test (DMRT) using the Statistical package for Social Sciences version16.0 for windows (SPSS Inc., Illinois, USA).

RESULTS AND DISCUSSION

The chemical composition of the seed of *Terminalia catapa* and its defatted flour are reported in Table 1. Fat constituted substantial portion of the kernel weight (47.3g of fat/100g of sample). Protein content of the flour increased significantly ($p<0.05$) from 21.5 to 40.5g/100g as a result of defatting. A similar significant increase was also observed for carbohydrate, ash and crude fibre contents. Earlier studies by Ogunwolu *et al*. (2009, 2010), Alobo (2009) and; Egbetokun and Ehieze (1997) showed similar changes in cashew nut, beni seed and soybean respectively. The high-protein content of seed of *Terminalia catapa* may however suggest that, it can be supplemented with cereal and tuber flours which are not only low in protein but deficient in amino acids.

Table. 1: Proximate composition of raw and defatted meal from *Terminalia catapa*

Composition (g/100g)	Raw seed	Defatted meal
Moisture	9.9 ± 0.01[a]	9.1± 0.01[b]
Protein	21. 5 ± 0.02[b]	40.5 ± 0.02[a]
Fat	47.3 ± 0.01[a]	7.2 ± 0.03[b]
Ash	3.7 ± 0.04[b]	8.3 ± 0.15[a]
Crude fibre	3.8 ± 0.01[a]	5.2 ± 0.05[b]
*Carbohydrate	13.8 ± 0.07[b]	29.7 ± 0.03[a]

Values are means of duplicate determinations ± standard deviations; Means with different superscript on the same row are significantly different (pd" 0.05).

Table. 2: Effect of extraction time on the yield (percentage extractability) of protein fractions from *Terminalia catapa* meal

Protein fractions (%)	Extraction time (min)			
	15	30	45	60
Albumin	3.32	4.25	4.70	4.93
Glutelin	2.55	3.57	3.97	4.20
Prolamin	7.65	8.96	9.98	10.20
Globulin	5.27	6.35	6.40	6.63

Values are means of triplicate determinations

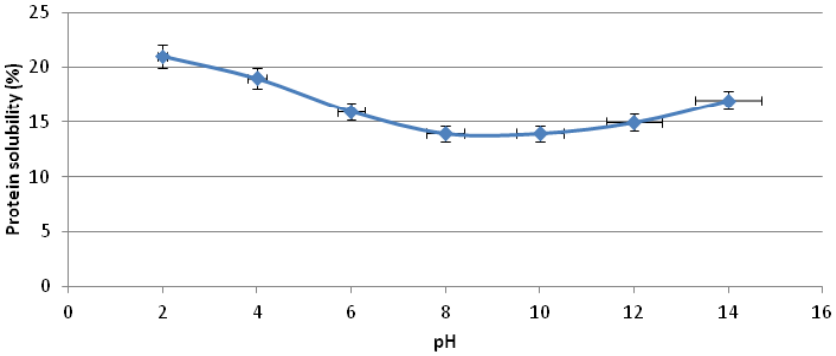

Fig. 1: Protein solubility profile of *Terminalia catapa* defatted meal

Table 3 Effect of solid-solvent ration on the extraction of proteins from *Terminalia catapa* meal

Protein fractions (%)	Solid-Solvent ratio				
	1:5	1:10	1:20	1:30	1:40
Albumin	4.11	4.23	4.26	4.27	4.31
Glutelin	3.51	3.55	3.58	3.59	4.12
Prolamin	8.75	8.94	8.96	8.96	9.10
Globulin	6.31	6.33	6.33	6.33	6.40

The effect of extraction time on protein extractability from defatted seed of *Terminalia catapa* using varying extraction times (15, 30, 45 and 60 min) were shown in Table 2. This result showed that, protein yield increased gradually with increasing extraction time. Highest yield was recorded at 10.2% prolamin at 60 min while lowest was 2.25% glutelin at 15 min.

DISCUSSIONS

Protein solubility

The pH-dependent protein solubility profile is presented for the meal in Figure 1. It was found that, the isoelectric point of the proteins was at pH 8.0. Conversely, the solubility reduced as the pH increased until it reached the isoelectric point; this was however followed by gradual increase in solubility with further increase in pH. This is somehow similar to the observations reported for mucuna bean flour (Adebowale, 2008) and chickpea (Sanchez-Vioque *et al.,* 1999). Adebowale (2008) opined that, the solubility profile of a protein provides some insight into the extent of denaturation or irreversible aggregation and precipitation that might have taken place in the course of protein isolation. Furthermore, the solubility profile also gives an indication of the type of foods into which the protein could be incorporated.

The protein and moisture content of the defatted *Terminalia catapa* kernel meal are shown in Table 1. Results show that *Terminalia catapa* has a high amount of protein value of 40.5% and the moisture was 9.1%. The moisture and the protein content obtained from defatted seed were compared to that of 8—10% protein reported for wheat flour and 14% moisture (Figoni, 2010). The high protein content in seed of *Terminalia catapa* shows that it can be use as a supplement in food and feed formulation. Table 2 shows the various fractions in which the proteins are segmented to during extraction, in which each proteins fractions has a protein percentage.

The influence of extraction time on protein extractability from defatted seed of *Terminalia catapa* using varying extraction times (15, 30, 45 and 60 min) were shown in Table 2. Virtually, the percentage extraction is increasing with increasing extraction time. Govardhan *et al*. (2011) had earlier reported that, most of the protein extracted in the initial 10 min and maximum protein extraction would be obtained at 20 min. This result agreed with the findings of Thompson (1977) for mung bean protein, reporting that the time of extraction did not have much influence on nitrogen extractability. Jyothirmayi *et al*. (2006) had also reported that extraction of proteins increased till 35 min after which it remained constant.

The effect of solid-solvent ratio on the extraction of proteins from *Terminalia catapa* meal is shown in table 3 above. There was an increase in the yield of protein with increasing solid-solvent ratio. The result however, is in agreement with the earlier reports of Adebowale (2008) on mucuna isolates. The mechanism governing the extraction of proteins must follow the dissolution and/or diffusion kinetics (Adebowale, 2008). This kinetics is governed by a driving force related to the gradient of the component concentration between the solid and liquid phases (Lawhon *et al*., 1981).

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