

## Effect of Methods of Hydrolysis on the Composition of *Prosopis Africana* seed Hydrolysates

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**Abstract:** Variations in plant or animal species, climatic conditions as well as processing methods affects the reproducibility, quality and quantity of natural or industrial raw materials. In the present study, *Prosopis africana* seed was hydrolysed using two distinct methods. In a separate study, the hydrolysates were found to be good sources of peptone for microbial cultures. The proximate analyses of contents of hydrolysates obtained from enzyme-hydrolysed or acid-hydrolysed seeds were determined. The seeds obtained from a local market were thoroughly cleaned and boiled until the seed coats were softened. The softened seed coats were manually removed to reveal the cotyledons, which were washed, dried and powdered. The powdered cotyledon was subjected to hydrolysis using the enzyme, papain, or hydrochloric acid. Proximate analysis of the hydrolysates was determined using standard methods. The results revealed that papain-hydrolysed seeds (PHP) and acid-hydrolysed seeds (AHP) had moisture contents of 32.7 % and 83.3 %, respectively whereas the ash values were 3.05% and 0.15 %. Protein contents of PHP and AHP were 39.69 % and 12.78 % respectively. Crude fibre compositions were 2.01 % in PHP while AHP showed no presence of fibre. It was observed that methods of hydrolysis significantly affected the quality of *Prosopis africana* seed hydrolysates.

**Keywords:** *Prosopis africana* seed hydrolysates, papain, Hydrochloric acid.

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### I. Introduction

*Prosopis africana* is a perennial leguminous tree of the family *Mimosidea* that is mostly found growing in the savanna region of West Africa [1]. In Nigeria, the seeds are very important to the Idoma, Ighala, Ebirra and Tiv people who use them to prepare fermented soup condiment known as “ukpehe” which is rich in protein and fatty acids [2]. Related studies have further reported that *Prosopis africana* seeds also contain carbohydrate, crude fibre, mineral elements as well as vitamins [3]. Seeds of high nutritional value of this nature can be useful in developing industrial raw materials that will have vast array of application in human and animal nutrition, pharmaceutical and biotechnological industries. One of the most popular methods of processing this leguminous seeds is fermentation using microbial enzymes [4]. However, utilization of chemical or protease in the hydrolysis of this proteinous plant product could be useful in standardization of the optimum method of processing this seed for industrial uses. Hydrolysis of plants or animal materials of the same source using different methods can greatly affect the quality and quantity of product outcome. It is therefore the aim of this study to compare the effects of acid or papain (enzyme) methods of hydrolysis of *Prosopis africana* seed using the proximate analysis as the assessing criteria.

### II. Materials And Methods

#### Sample preparation

*Prosopis africana* seeds were purchased from a local market in Nigeria. The seeds were sorted to remove solid contaminants, and decaying seeds. The selected seeds were washed in de-ionized water and then boiled for 8 hours until the seed coats became soft and swollen. The seed coats were manually peeled off leaving behind the clean cotyledons. The cleaned cotyledons were dried in an oven at 40°C for 12 hours, weighed and pulverized using a Kenwood blender. The resulting powder was kept in an air-tight container and stored at 4°C prior to further procedures.

#### Acid hydrolysis

Two hundred grams of the *Prosopis africana* seed flour was soaked in 250 ml of 6 M HCl. The mixture was incubated at 80°C for 24 hours. At the end of the incubation period, the broth was diluted with 500 ml of de-ionized water and then boiled at 100°C for 1 hour. The solution was then cooled to room temperature, and the pH adjusted to 7.0 with 6 M NaOH. The mixture was filtered through Whatman’s No.1 filter paper. The filtrate was lyophilized using a freeze-dryer (Amsco/Finn-Aqua Lyovac GT3, Germany) to yield acid-hydrolysate of *Prosopis africana* seed (AHP).

### **Papain hydrolysis**

Two hundred grams of powdered *Prosopis africana* seed was weighed and suspended in 800ml of distilled water. This was stirred to form slurry. The pH of the slurry was adjusted to 6.5 with 1 M HCl. Commercially obtained papain (BDH Chemical Ltd, Pools, England) was added to the slurry to make a concentration of 0.25 %. Digestion was allowed to take place for 9 hours at a temperature of 60 °C, and the pH was controlled using 1 M NaOH. The temperature was then raised to 80°C for 15 minutes to inactivate the enzyme. Liquor from the hydrolysed material was separated from the protein/enzyme slurry by repeated centrifugation. The clear solution was then freeze-dried to obtain papain-hydrolysate of *Prosopis africana* seed (PHP).

### **Proximate analysis**

#### **Moisture content**

The standard method of the Association of Official Analytical Chemists [5] no. 925.10, using hot air oven was adopted. Three porcelain dishes were thoroughly washed and dried in a hot air oven at 100 °C for 30 minutes. They were cooled in a desiccator to ensure absence of surface moisture. The dishes were then weighed, and 2 grams of the sample were placed in each of them and heated in an oven set at 105 °C for six hours. The containers were removed from the oven and cooled in the desiccator, to prevent moisture re-absorption. After cooling, the porcelain dishes were weighed again until constant weights were obtained and the percentage moisture content was calculated as follows:

% Moisture content =  $\frac{(X-Y)}{Z} \times \frac{100}{1}$  - where X= Sum of weight in gram of each of the dishes and sample before drying; Y= Weight in gram of the dish and sample after drying and Z= Initial weight of the sample in grams.

#### **Determination of ash value**

The ashing crucibles were washed and dried in a hot air oven at 100 °C for 25 minutes. They were then cooled in desiccators and weighed. Two grams of dry samples were each placed in the porcelain crucibles and transferred into a muffle furnace set at 600 °C. The material was burnt until ashing was completed. The crucibles and contents were then cooled in the desiccators, and reweighed [5].

Percentage ash was calculated as follows:

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times \frac{100}{1} -$$

#### **Determination of lipid content**

Two grams of the sample were placed in a weighed thimble and introduced into a dry Soxhlet column attached to a weighed extraction flask containing 100 ml of petroleum ether. Extraction was continued until the refluxing liquid became colourless. The thimble and its content were removed from the chamber, cooled in desiccators and re-weighed [5]. The percentage of fats for each sample was obtained, as:

$$\% \text{ Fat} = \frac{X - Y}{Z} \times \frac{100}{1}$$

where X = Weight of thimble and sample before extraction (g); Y = Weight of thimble and sample after extraction (g) and Z = Weight of the sample (g).

#### **Determination of total protein**

The micro-Kjedahl method described by [5] was used in the determination of the total crude protein. This involves three stages:

(a) Digestion: 10 g of each sample was digested with a mixture of concentrated sulphuric acid; a pinch each of copper sulphate and sodium sulphate crystals, to act as catalysts, were added. The mixtures were heated till the black liquid cleared. Heating was continued till the sample became completely digested. The digested samples were transferred into a 100 ml volumetric flask and the volume made up to the mark with distilled water.

(b) Distillation: Kjeldahl distillation apparatus was used. Ten ml of 4 % boric acid was combined with two drops each of methyl blue and methyl red indicators in 100 ml conical flask. The conical flask was positioned at the receiving side of the distillation unit using a clamp. Five milliliters (5 ml) of the digested samples were first introduced into the distillation unit followed by addition of 10 ml of 50 %, NaOH gradually. The distillation process lasted between five to ten minutes, during which ammonia (NH<sub>3</sub>) was trapped in excess boric acid. The presence of ammonia changed the purple colour of boric acid to green, which is a common characteristic of alkaline gases.

(c) Titration: Fifty ml of the distillate was titrated against 0.01 M HCl after which crude protein content of the samples was calculated by the formula:

$$\% \text{ Nitrogen} = \frac{\text{Titre} \times 0.01 \times \text{DF} \times \text{MWN}}{\text{Weight of sample (g)}} \times \frac{100}{1}$$

where DF= dilution factor (100 ml/5 ml) = 20; and MWN = Molecular weight of nitrogen = 14.01; % Total Protein = % nitrogen × 6.25.

#### Determination of crude fibre content

Two grams of sample was hydrolysed in a beaker with 200 ml of near-boiling 1.25 % of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) for 30 minutes. The content was filtered under suction, washed with 40-50 ml portion of near boiling water and was boiled for 30 minutes with 200 ml of 1.25 % NaOH. The digest was washed with 25-30 ml portion of near-boiling 1.25% H<sub>2</sub>SO<sub>4</sub> and then washed with 25-30 ml portion near-boiling water and filtered. The residue was collected and put into weighed crucible and dried at 130 °C for 2 hours. The crucible plus the residue were cooled in a desiccator and re-weighed. The dried digest was ashed in a muffle furnace at 550 °C for 5 hours [5]. The ash was cooled, weighed and the percentage crude fibre was calculated as follows.

$$\% \text{ Crude fibre} = \frac{\text{Weight after drying} - \text{weight after ignition}}{\text{Weight of sample (g)}} \times \frac{100}{1}$$

#### Determination of total carbohydrate

Two hundred milligram (200 mg) of the sample was mixed with 50 ml of boiling distilled water in a beaker. Three millilitres (3 ml) of 5 M hydrochloric acid was added and the mixture boiled for 30 minutes. The content of the flask was allowed to cool and was neutralized with 5 M NaOH solution. The hydrolysate was then transferred into a 100 ml volumetric flask and the volume made up to 100 ml with distilled water. Using 10 fold serial dilutions, 0.2 ml of the solution was pipetted and made up to 2 ml with distilled water for spectrophotometric determination of 512 nm [7].

% Total carbohydrate = mg of glucose equivalent to sample absorbance from graph × conversion factors (25) × dilution factor

### III. Results

**Table 1: Proximate composition of PHP and AHP**

Media	Composition in g/100 g					
	Moisture	Crude protein	Fat	Total carbohydrate	Crude fibre	Ash
PHP	32.70±0.01	39.69±0.001	8.68±0.002	16.97±0.01	2.01±0.01	3.05±0.01
AHP	83.30±0.03	12.78±0.01	1.02±0.003	2.98±0.04	0.00±0.00	0.15±0.01

PHP: Papain hydrolysed *Prosopis africana* seed

AHP: Acid hydrolysed *Prosopis africana* seed

### IV. Discussion

In the proximate composition of *Prosopis africana* seed as reported by [7], a percentage moisture of 1.9±0.03 % was recorded and adjudged low though comparable to rear cowpea (1.8±0.25 %), cranberry bean (1.7±0.51 %) and kersting's groundnut (1.7±0.12 %). They further opined that the value of 1.9±0.03 % was within the range expected of most legumes and that the low moisture content will afford a long shelf life for *Prosopis africana* [7]. On the contrary, the moisture content obtained in the proximate composition of fermenting *Prosopis africana* cotyledons in the laboratory into *okpehe* by Ogunshe [8] were between 68.0 % on the first day of fermentation and 72.8 % after the fifth day of fermentation. The moisture content of 40.7-60.0 % was recorded in *ukpehe* sample from market sources [9].

In the present study, the moisture content of 32.7 % and 83.3 % for PHP and AHP respectively were obtained. Such moisture as obtained in this *Prosopis africana* seed hydrolysate can afford organisms free-water (water that is not bound in its complex structures and can provide enabling environment for the transfer of nutrients and toxic waste). This "free" water however can result in material degradation and as such need to be adequately evaporated or dried for proper preservation of the hydrolysate prior to use.

The crude fibre of 2.1 % for PHP is low compared to 3.3 % obtained by [10] where he recorded. Crude fibre of 3.3 % was obtained in proximate composition of *Prosopis africana* flour [7]. Crude fibre was, however, not detected in the acid hydrolysate of the *Prosopis africana* seed. This is an indication that different methods of hydrolysis can affect a product or products from same source. It is very possible that, in the course of hydrolysis, the harsh attack of acids on the polymeric structure of the hydrolysate may have led to the loss of crude fibre in the final product. Enzyme hydrolysis of both PHPs could be the reason crude fibre was detected in sample. There is evidence that dietary fibre has a number of beneficial effects. Some soluble fibre which dissolve in water are readily fermented in the colon into gases and physiologically active by-products which can

be prebiotics. Table 1 reveals fat content of 8.6 % and 1.02 % for PHPs and AHPs respectively. In food and other related industries where rancidity is a threat to product stability, exploration of enzymatic hydrolysis portends high risk. Therefore, it becomes imperative that *Prosopis africana* seeds should be completely defatted prior to processing of the final product(s). Ash as one of the components in proximate analysis of *Prosopis africana* seed hydrolysate consists mainly of salty, inorganic constituents. Ash values of 3.05 % in PHP and 0.15 % in AHP, clearly depicts reasonable availability of mineral elements in the hydrolysates. These values are slightly lower than 5.01-7.07 % recorded by [3] and 4.4 % as was obtained by [7]. Lower value of 0.15 % recorded in AHP negates the expectation that neutralization reaction could produce more salts. Protein hydrolysates are major sources of nitrogen and vitamins in microbiological media. In the analysis of fermenting *Prosopis africana* seed, [4] observed high proteinase activity which was attributed, in part, to the high protein content of the seeds. Protein in *Prosopis africana* seeds constitutes about 60 % or more of the seed kernel's weight [11]. In the present study, papain hydrolysate recorded crude protein value of 39.69 % which was higher than the values estimated from percentage nitrogen calculated from micro-Kjedahl as documented in the above cited works. This depicts its high potential as source of nitrogen, amino acid and other protein derivatives in animal feeds as well as biochemical industries. This value obtained from the enzyme digest of *Prosopis africana* seed also compares favourably well with the results of [12] obtained in soybean 47.73 % and African locust bean 30.69 % while exploring the possibility of developing peptone from enzyme hydrolysis of soya beans and African locust bean. On the contrary, the value of 12.78 % for AHP was low relative to PHP. This can be attributed to the effect of acid in breaking some peptide bonds and, in the process some amino acids are lost in forms of soluble salts and are not detected. From this study, it can be seen that enzyme digest of same or similar plants or animal product yields higher protein values than similar results obtained from estimation or acid hydrolysis. The ability of a raw material to provide readily available and easily metabolizable carbohydrate to furnish the cellular energy needs in biotechnological exploration is indispensable. In this study, total carbohydrate value of 34.33 % was recorded for PHP. Ajiboye *et al.*, [10] recorded 1.9 %, while Gberikon *et al.*, [3] had 26.01-28.17 %. Reduction of carbohydrate to more soluble components like glucose in part, explains the lower value recorded in AHP. Similarly, other related researches [13] obtained lower values for their acid hydrolysates. Therefore, methods employed in the processing of similar plant or animal raw materials determine to a reasonable extent the product outcome.

## V. Conclusion

Adequate knowledge guided by informed choice of industrial processes greatly determines the outcome of the quality and quantity of finished products. Acid method of hydrolysis is a harsh process and as such could lead to loss of certain essential ingredients in raw materials. The enzyme, papain, exhibited protein sparing effect as shown in the proximate composition of the hydrolysates. This is essential in bio pharmaceutical industries where amino acids and other protein derivative are manipulated and modified into useful products. This work has successfully provided base-line information for further research on this plant seeds hydrolysates.

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