**In-vitro** protein digestibility of selected under-utilized local wild beans and bio-availability in rats model

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**ABSTRACT**

The supplement of rich legumes is considered as one of the best solution to protein – calorie malnutrition in developing world. Hence, this study sought comparative proximate analysis and *in-vitro* protein digestibility (IVPD) of five varieties of wild beans seeds namely: *Otili, feregede* and *pakala*, and common edible beans. Proximate analysis and *in-vitro* protein digestibility of the seeds were carried out. The samples crude protein varies from 23.99% - 34.16% such that *otili* contain the least crude protein (23.99±1.61%) while common edible beans contain the highest recorded crude protein (34.16±0.28%). Carbohydrate of *feregede* is the highest 51.07±0.75% and the least carbohydrate content was recorded in common edible beans 44.40±0.37%. The samples crude protein varies from 23.99 to 34.16% such that *otili* had the least crude protein (23.99 ± 1.61%), while common edible beans contained the highest recorded crude protein (34.16 ± 0.28%) and carbohydrate of *feregede* contained the highest crude protein with a value of 51.07 ± 0.75% (0.72b); the least carbohydrate content was recorded in common edible beans 44.40 ± 0.37% (0.378a). The *in-vitro* protein digestibility (IVPD) varied from 31.39 to 48.66%, respectively such that common edible beans has the highest IVPD values of 48.66 ± 0.71% and *feregede* had the least IVPD value of 31.93 ± 0.10%. There was no significant difference (p< 0.05) recorded among the samples except in common edible beans with relatively high IVPD properties as deduced from the high value of digestibility. Higher digestibility could also indicate that normal presence of crude fiber in the beans is minimal and could not contribute to the bulkiness of the intestinal content of the consumer. Under the experimental conditions used, the chemical composition values indicated that the levels of moisture, protein, ether extract, ash, fiber and carbohydrate differed between the studied species. Nearly all the wild beans under the present study contained macro-nutrient, especially protein and carbohydrate. They provide significant amounts of protein and their bioavailability across vital organs and tissues in the system. It is therefore recommended that beans, especially common edible beans should be included in our diet as it would substantially aid in redressing the problems of malnutrition in our country.

**Key words:** *In-vitro* protein digestibility, wild beans, edible beans, ether extract, ash, fiber and carbohydrate.

**INTRODUCTION**

Bean seeds are rich sources of plant proteins that present a substitute for scarce animal proteins. Bean seeds consist of array of edible types otherwise known as common bean and non-common wild types otherwise known as non-edible types. The greatest impediment to utilize wild or underutilized legumes as food and feed is the presence of certain anti-nutritional factors, which may not only be toxic, but can also be lethal in extreme situations. For a food researcher, removal of the anti-nutrients from the wild and underutilized legumes with minimal compromise on the
nutritional qualities has been a great challenge (Gupta et al., 2001). Out of the many known of this legume specie, only few at the moment are used extensively as food (Brough et al., 1993; Chinedu and Nwinyi, 2012; Nwosu, 2013). The edible types are known to be used especially in developing countries as a staple of diet, due to their relatively very low cost, high nutritional value and health benefit (Adebowale and Adebowale, 2007; Adebowale et al., 2005). They are also more explored in the developed countries as ingredients primarily to provide a variety of functional properties, including desirable structure, texture, flavour and colour characteristics in formulated food products (Abbey and Ibeh, 1987; Adeyeye and Agesin, 2007). They are widely used in high protein foods including dairy foods, nutritional supplements, meat systems, infant formulas, nutritional beverages, cream soups, sauces and snacks and also as a protein source in milk replacers (Ahenkora et al., 1999; Agbede and Aletor, 2003).

*Sphenostylis stenocarpa* (Africa Yam bean, *Otili*), and other types of wild beans, *Cajanus cajan* Pigeon Pea; (*Feregede*), *Phaseolus lunatus* Lima bean/Butter bean; (*Pakala*) presented in this report are grossly underutilized tropical legumes. They are grown in tropical countries as a green manure/cover and there have been reports in Nigeria that the wild bean—African yam bean, pigeon bean and lima bean are popular legumes consumed in many communities (Aletor and Aladetimi, 1989; Edem et al., 1990; Anderson and Moore, 2004). Chemical composition of these grain legumes were shown to contain high quantities of proteins, amino acids, fiber and minerals (Arinathan et al., 2003, Campos-Vega et al., 2009). Their high intake is associated with reducing the risk of developing diabetes, hypertension, colon cancer and hypercholesterolemia (Geil and Anderson, 1994; Awoyinka et al., 2016). Attempts have been made to improve their utilization in human diet due to increasing need for cheaper and available plant proteins to meet the increasing demand of the Nigerian populace. For example, reducing cooking time and acceptability have been achieved for pigeon pea through dehulling process (Fasoyiro et al., 2010). Despite all these aforementioned findings, these wild beans are still considered an orphan crop, with a huge untapped potential for improvement both in quantity and quality of food products. In this respect, the edible bean types appear to be the most explored among the beans. Although as other plant foods that have high nutrient contents our digestion system cannot absorb it fully. Hence, it becomes necessary to determine their protein bioavailability.

**MATERIALS AND METHODS**

**Collection of cultivar**

The legumes (beans) used in this work are of two types; Wild-type beans *Sphenostyles stenocarp* (*Otili* African yam bean), *Cajanus cajan* (*Feregede* Pigeon pea), *Phaseolus lunatus* (*Pakala* Lima beans) and Edible bean *Phaseolus vulgaris* (Oloyinkidney bean). They are gotten from the farmers in Ado-Ekiti.

**Proximate analysis**

Analyses of spices for crude protein, nitrogen, ash and moisture contents were carried out essentially according to the standard AOAC methods [2000]. Flour samples were acid-hydrolyzed and the reducing sugar known as available carbohydrates determined by the dinitrosalicic acid (DNS) method.

For the determination of lipid content, the cold extraction method was used. Following this procedure, 50 g of spice powder samples were introduced in volumetric flasks containing 300 ml of a mixture of chloroform/methanol (200 ml/100 ml) and mixed for 20 min using an agitator. The mixture was filtered under N2 and the residue re-extracted in 200 ml of the same solvent and filtered. The extracts were then mixed and allowed to separate after the addition of 0.2 ml of NaCl solution 0.7 g/100 ml. The lipid in the lower phase solution was flushed out and recovered by rotary evaporation at 50°C using liquid nitrogen. The flasks containing the extracted oil were weighed and the difference in weight expressed as percentage oil content.

**Determination of in-vitro protein digestibility**

*In-vitro* protein digestibility of sample was determined using the modified procedure of Hsu et al. (1977). The enzymes used include porcine pancreatic trypsin and pepsin. The activity of the enzymes was initially determined using the modified procedure of Hsu et al. (1977). The enzymes used include porcine pancreatic trypsin and pepsin. The activity of the enzymes was initially determined before use by using them to digest casein. 100 mg of the sample were dispersed in 1.0 ml of phosphate buffer pH 7.4. 25 mg of both porcine pancreatic trypsin and pepsin were dissolved in 1.0 ml of distilled water. 200 µl of the sample was dispersed into test-tube and 200 µl of the enzymes added to it and incubated at 37°C for 30 min. 2.0 ml of copper alkaline solution was added to it and allowed to stand for 10 min. immediately after 10 min, 0.5 ml of folin ciocalteau was added and incubated at room temp for 30 min. The absorbance was measured at 700 nM against reagent blank. The standard calibration curve was prepared using 100 µg/ml of BS.

**Experimental animals**

Twenty four albino rats were obtained from the College of Medicine Animal House, Ekiti State University, Ado Ekiti, Ekiti state. Their weight ranged between 50 to 100 g. The animals were acclimatized to the environment for 7 days. The rats were then randomly divided into 6 groups (4 rats
Table 1: Experimental design.

<table>
<thead>
<tr>
<th>S/No</th>
<th>Experimental group</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive control</td>
<td>Chow</td>
</tr>
<tr>
<td>2</td>
<td>Negative control</td>
<td>Chow + High fat diet</td>
</tr>
<tr>
<td>3</td>
<td>Feregede</td>
<td>Chow + HFD + Feregede</td>
</tr>
<tr>
<td>4</td>
<td>Otili</td>
<td>Chow + HFD + Otili</td>
</tr>
<tr>
<td>5</td>
<td>Pakala</td>
<td>Chow + HFD + Pakala</td>
</tr>
</tbody>
</table>

Table 2: Proximate analysis of the edible beans.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Moisture (%)</th>
<th>Fibre (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Carbohydrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otili</td>
<td>6.92±0.023ab</td>
<td>5.33±0.433a</td>
<td>2.76±0.000ab</td>
<td>4.12±0.026b</td>
<td>23.99±1.61a</td>
<td>50.14±0.30b</td>
</tr>
<tr>
<td>Feregede</td>
<td>5.81±0.057a</td>
<td>7.75±0.428ab</td>
<td>2.95±0.064ab</td>
<td>3.72±0.220ab</td>
<td>28.95±0.15a</td>
<td>51.07±0.75b</td>
</tr>
<tr>
<td>Pakala</td>
<td>6.61±0.341ab</td>
<td>7.43±0.130ab</td>
<td>2.90±0.000ab</td>
<td>5.38±0.052b</td>
<td>32.10±0.18a</td>
<td>45.99±0.200a</td>
</tr>
<tr>
<td>Common edible</td>
<td>8.67±0.092b</td>
<td>7.18±0.011b</td>
<td>2.25±0.162a</td>
<td>3.21±0.244a</td>
<td>34.16±0.28b</td>
<td>44.40±0.37a</td>
</tr>
</tbody>
</table>

abc: varietal means with different superscripts in the same column are significantly (P<0.05) different from each other.

RESULTS AND DISCUSSION

Table 2 shows the percentage composition of beans varieties - Otili, Feregede, Pakala and Oloyin. The moisture contents ranged from 5.81 to 8.67%, common edible beans contained the highest amount of moisture, 8.67 ± 0.92%, while Feregede 5.81 ± 0.057% had the least value of moisture. These values are close to those found by Oliveira et al. (2008) and Ramirez-Cárdenas et al. (2008), who found 9 to 11% on a dry basis in raw beans. The moisture level of common edible beans is significantly (P<0.05) higher and different from all the other beans. Feregede having the least moisture content suffice to deduce that it will have a longer shelf life than the other beans varieties. This result corroborate with the report of Chinedu and Nwinyi (2012), since higher moisture content will enhance microbial actions which may lead to spoilage of the food during storage.

The ash content of the sample ranged from 3.13 to 5.38% on dry weight basis whereas pakala has the highest value of 5.38 0.052%. The ash value varied significantly (p<0.05) only between pakala and otili compared to the other samples, such that they both possess higher and significantly (p<0.05) different ash content compared to other samples. Since a high ash concentration in sample signifies a high mineral content, the high ash level in Pakala and Otili to the other samples is indicative of a high mineral composition in these samples.

Crude fiber content of the samples ranged from 5.33 to 7.84%, while otili possess the least crude fiber content 5.33 ± 0.433%. Crude fiber theoretically means materials that are indigestible in human and most animals (Fasoyiro et al., 2008).
The samples of crude protein varies from 23.99 to 34.16% such that *Otili* contain the least crude protein (23.99%), while common edible beans contain the highest recorded crude protein (34.16%), although, there was no significant difference (p < 0.05) recorded between the beans samples.

The primary national importance of protein is as a source of amino acid which are essential to good physical and mental health, thus, from these results it can be inferred that beans are rich sources of protein. The relatively high percentage of protein in beans implies that these beans can contribute significantly to the daily human protein requirement, which is in line with the findings of Alabi et al. (2005). Crude fat in the beans samples ranged from 2.25 to 2.95% with common edible beans (2.25%) having the least crude fat; *Feregede* had the highest crude fat of 2.95 ± 0.064% and a significant difference (p < 0.05) was recorded only in *Feregede* having the highest fat content. Dietary lipid represents the most compact chemical energy available to man. They also contribute to the palatability of food substance since they have the ability to carry odors and flavours; they also contribute a significant amount to the calorific value of food.

Carbohydrate composition of the samples varied from 44.40 to 51.07% with carbohydrate of *Feregede* being the highest 51.07 ± 0.72%; the least carbohydrate content was recorded in common edible beans 44.40 ± 0.378%. The carbohydrate content in the beans is in line with those reported by Brigide and Canniatti-Brazaca (2006) and Sathe (2002). The samples in-vitro protein digestibility (IVPD) varied from 31.39 to 48.66% such that common edible beans has the highest IVPD values of 48.66% (± 0.707b) and *Feregede* possessed the least IVPD value 31.93 ± 0.102; no significant difference (p < 0.05) was recorded among the samples except in common edible beans with relatively high IVPD properties deduced from the high value of digestibility (Table 3); higher digestibility could also indicate that the normal presence of crude fiber in the beans is minimal and as such will not contribute to the bulkiness of the intestinal content of the consumer. Common edible beans are the most advisable bean specie for consumption since its digestibility rate is high.

The total decrease in body protein and increase that follows across the groups is the summations of dissimilar changes in the protein content of the various organs and tissues of the body. It is believed that some informations might be gained about the relation between the peculiarities of the organs and the part, whether active or passive as played by each organ in the process of protein anabolism and catabolism where observations were made on the protein content of the organs and tissues after feeding the rats with different wild beans in a high fat diet. The feeding pattern of each group shows that *Otili* group had more appetite for the feed and this is clearly seen in their body mass gain over the period of the experiment. There was a general similarity in the behaviour of the rats that had wild beans in their feed. Unlike the controls and *Otili* other groups did not huddle together but lay separately, often on their backs with their limbs fully extended.

Generally, the pattern in liver protein in the experimental groups- *Feregede, Otili* and *Pakala* as compared to the two controls may be ascribed to intake of the diet mixed with high fats. Total protein was found to be higher in the liver of the group fed with *Feregede* with the value of 84±8.3 (Table 4). In the heart there is about 20% decrease in protein but in the kidney reduction is not as great. It is sufficed to assume that the decrease in heart protein in the experimental groups is due to a diminution in the work of the heart. As with the heart, so also in the case of the kidney it is therefore possible to suppose that the changes we find in the kidney protein are results of changes in the amount of work imposed upon it. With respect to the large proportion of the protein in the liver compare to other

Table 3: *In-vitro* analysis of protein digestibility.

<table>
<thead>
<tr>
<th>Samples</th>
<th>IVPD (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Otili</em></td>
<td>35.64±0.02a</td>
</tr>
<tr>
<td><em>Feregede</em></td>
<td>31.93±0.10a</td>
</tr>
<tr>
<td><em>Pakala</em></td>
<td>41.12±0.48ab</td>
</tr>
<tr>
<td><em>Oloyin</em></td>
<td>48.66±0.71b</td>
</tr>
</tbody>
</table>

Table 4: Bioavailability of protein in rats tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein in blood</th>
<th>Total protein in liver</th>
<th>Total protein in heart</th>
<th>Total protein in kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77 ± 0.15</td>
<td>15.4 ± 0.04</td>
<td>62.5 ± 0.52</td>
<td>19.4 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>75 ± 0.10</td>
<td>77.31 ± 8.6</td>
<td>9.4 ± 1.2</td>
<td>16.78 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>59 ± 0.04</td>
<td>84.43 ± 8.3</td>
<td>16.38 ± 0.01</td>
<td>33.39 ± 0.32</td>
</tr>
<tr>
<td>4</td>
<td>70 ± 0.01</td>
<td>69.4 ± 6.7</td>
<td>26.9 ± 4.3</td>
<td>64.47 ± 4.8</td>
</tr>
<tr>
<td>5</td>
<td>69 ± 0.01</td>
<td>68.1 ± 6.2</td>
<td>14.52 ± 0.01</td>
<td>33.75 ± 0.13</td>
</tr>
</tbody>
</table>
organisms of the rats after five weeks of the experiment the grounds for any explanation are even less secured, but we are of the opinion that determinations of liver protein in varying metabolic states will be useful in trying to define the part played by the liver in the processes of protein metabolism. The effect on the blood tissue is in line with the view that all the differences are responses to changes in functions (Addis, 1938).

Conclusion and Recommendations

One among the issues is the dearth of information about the nutritional benefits of our underutilized wild bean. They are rarely consumed perhaps due to little knowledge of its nutritional wealth. The current examination on the nutritional profile of our three recommended varieties provides a substantial range of nutritional information, bringing to attention the richness of healthy nutrients present in the eatable portion. It is therefore recommended that wild beans, especially *Otili* should be included in our diet as a result of its rich constituent revealed in this study.

REFERENCES


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