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Estimation of Protein Content and Amino Acid Compositions in Selected Plant Samples Using UV-Vis Spectrophotometeric Method

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Abstract

Plant food samples were selected and analyzed for their individual protein concentration and identification of aromatic amino acids using the Evolution 300 UV-Vis Spectrophotometer. The results revealed that the selected food samples which were grouped into two: legume seeds (blackeye bean [brown], *Vignaun guiculata*; soybean seed, *Glycine max*; black eye bean [white], *Vignaun guiculata*; bambara groundnut 'ọkpa', *Vigna subterranean*; and African black bean 'odudu', *Phaseolus vulgaris*) and oil seeds (groundnut seed, *Arachis hypogea*; melon seed 'egusi', *Citrillus vulgaris*; castor oil seed, *Ricinus communis;* dikanut 'ọgbọnọ', *Irvingia spp*) had varying protein concentrations. Soybean had the highest concentration of 8.59mg/ml among the legumes while bambara groundnut had the lowest concentration of 4.29 mg/ml. Among the oil seeds, dika nut had highest concentration of 18.75mg/ml while castor oil seed had the lowest concentration of 9.40 mg/ml. The statistical test (t-test) showed that there was no significant difference between the means. However, aromatic amino acids (tryptophan, tyrosine and phenylalanine) and the sulphide bond amino acid (cysteine) were identified in the various food samples. Histidine, which is another aromatic amino acid but does not contribute to the protein concentration of the plant sample, was also identified. These samples therefore contained different amino acids which imply that combination of different plants foods samples may provide the required protein for body functioning.

Keywords

Amino Acids, Concentration, Legumes, Oil Seeds and Protein

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1. Introduction

Proteins are essential nutrients for the human body. They are among of the building blocks of body tissue, and can also serve as a fuel source [1]. They are better definedfrom a nutritional standpoint based ontheiramino acid composition [2] that is, proteins are polymer chains made of amino acids linked together by peptide bonds. There are nine essential amino acids which humans obtain from their diet in order to prevent protein malnutrition. These includephenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine [3]. However, humans are able to synthesize in the bodyfive dispensable amino acids which arealanine, aspartic acid, asparagine, glutamic acid and serine. The six conditionally essential amino acids that their synthesis can be limited under special conditionsespecially in severe catabolic distress are arginine, cysteine, glycine, glutamine, proline and tyrosine [2]. Humans need the essential amino acids in certain ratios for proper body nutrition. Both plant and animal foods contain protein which may provide all the essential amino acids or may not [4]. During human digestion, proteins are broken down in the stomach to smaller polypeptide chains via hydrochloric acid and protease action [5].

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Protein can also be seen to be complete and incomplete. Complete protein foods include meats, poultry, fish, eggs, cheese, yogurt, soy and peanuts are considered. This means that these proteins contain a good balance of all of the essential amino acids that are needed by the body while incomplete protein foods include grains, beans, nuts, seeds and even some types of meats like rabbit. The protein sources are mainly from plants and animals.

Plant protein-containing foods have been roughly divided into three groups depending on how much protein they contain: (i) High protein plant foods which include nuts, seeds and beans. Others are wheat germ, brewer's yeast, nutritional yeast. These foods contain about 20% protein or more; (ii) Medium-protein plant foods which include grains like rice, wheat, oats, millet and barley that contain between 6 to 14% protein. This means they should be combined with other protein foods to provide complete protein; and (iii) Low-protein plant foods which include fruits, vegetables and juices. These contain less than 5% protein. This implies that living on a low protein diet of mainly fruit, for example, does not work well for human beings [6].

Animal protein-containing foods are classified as high quality protein sources. The essential amino acids in animal products are in the right balance. Plant foods contain lower quality proteins. Most fruits and vegetables are poor sources of protein. Other plant foods, like baked beans, split peas and lentils, peanuts and other nuts, seeds, and grains like wheat, are better sources. They contribute a lot to protein intake. However, each type of plant protein is low in one or more of the essential amino acids which makes them a lower quality protein but the combination of different plants proteinous foods can actually go along way in providing the required protein needs. Vegetarians get enough essential amino acids by eating a variety of plant proteins [7] and their sources of proteins include whole grains, pulses, legumes, soybeans, nuts, seeds and fruits. Legumes, some of which are called pulses have higher concentrations of amino acids and are more complete sources of protein than whole grains and cereals [8]. Examples of plant foods that contain protein include soybeans, lentils, kidney beans, white beans, cowpeas, lima beans, pigeon peas, lupines, wing beans, almonds, Brazil nuts, cashews, pecans, walnuts, cotton seeds, pumpkin seeds, sesame seeds, and sunflower seeds [9].

Protein can be found in a wide range of foods. Some foods are high in certain amino acids, but their digestibility and the anti-nutritional factors present in them make them of limited value in human nutrition. Therefore, one must consider digestibility and secondary nutrition profile such as calories, cholesterol, vitamins and essential mineral density of the protein source. Plant protein foods on average contribute also to percent proteinrequirements [9]. Proteins are the most abundant kind of molecules in the body aside from water [1, 10]. Protein, when broken down into amino acids are used as precursors to nucleic acid, co-enzymes, hormones, immune response, cellular repair, and other molecules essential for life [10].

In many applications involving peptides or proteins, it is important either to identify fractions containing protein or to estimate the concentration of a purified sample. Amino acids containing aromatic side chains (i.e., tyrosine, tryptophan and phenylalanine) exhibit strong UV-light absorption. Consequently, proteins and peptides absorb UV-light in proportion to their aromatic amino acid content and total concentration. Once an absorptivity coefficient has been established for a given protein (with its fixed amino acid composition), the protein concentration in solution can be calculated from its absorbance. For most proteins, UV-light absorption allows detection of concentration down to 100μg/ml. Nevertheless, estimation of protein concentration by UV-light absorption is not accurate for complex protein solutions but for aqueous protein solutions commonly used in the research laboratory setting, interference from other compounds is minimized by measuring absorbance at 280 nm. Only the amino acids tryptophan (Trp, W) and tyrosine (Tyr, Y) and to a lesser extent cysteine (Cys, C) contribute significantly to peptide or protein absorbance at 280 nm [11]. Phenylalanine (Phe, F), absorbs only at lower wavelengths (240-265 nm).

This work therefore, determined the protein concentration andidentified the aromatic amino acids selected plant samples using UV-Visible Spectrophotometer.

2. Materials and Methods

The plant food samples analyzed includes, groundnut (*Arachis hypogea*), blackeye bean (white) (*Vignaunguiculata*), black eye bean (brown) (*Vignaunguiculata*), African black bean 'odudu' (*Phaseolus vulgaris*), melon seed 'egusi' (*Citrillusvulgaris*), bambara groundnut 'ọkpa' *(Vigna subterranean)*, castor oil seed 'ogiri' *(Ricinuscommunis)*, Soy bean *(Glycine max)* andDika nut 'ọgbọnọ' (*Irvingiaspp*) were all purchased from the New Main Market in Aba South L.G.A., Abia State, Nigeria. The samples were dried and labeled properly before taken for analysis.

The samples were ground to powdered form using a new mortar and pestle and much care was taken to avoid the contamination of the various samples. Five (5) grams of the each of the sample was weighed into a 250 ml beaker. The sample was extracted using methanol. 50ml of methanol was added to the sample and warmed in an electro-thermal heater while stirring which was then allowed to stand for 15 minutes, after which, the solution was filtered off into a 100ml volumetric flask, then methanol was used to make up to the mark of the solution.

During the sample preparation, the UV-Vis spectrophotometer was warmed up and set-up, by inputting the necessary values and details of the sample, with the wavelength range set at 200nm to 600nm, and auto-tracking component activated. The equipment was zeroed to adjust to the imputed parameters, before the sample was put into the sample chamber. Once the sample preparation and equipment set-up were completed, the methanol was put into the curvette and placed in the inner sample chamber to serve as a blank, while the sample solution was also carefully poured into a curvette and placed in the outer sample chamber. The run button was put which initiates the analysis, and after some seconds the graph had been plotted and the peak of the result auto-tracked showing the points of amino acid absorption at its specific wavelengths of absorption, then the displayed result on the screen was printed out.

Calculation of the Percentage (%) Concentration and Concentration in mg/ml

To obtain the percent concentration of protein contents, a percent solution extinction coefficient ($\varepsilon_{percent}$) was used. In most proteins, the extinction coefficients ($\varepsilon_{percent}$) range from 4.0 to 24.0. Therefore, although any given protein can vary significantly from $\varepsilon_{percent} = 10$, the average for a mixture of many different proteins will likely be approximately 10 [12].

Given that 1% solution equals 1g/100ml measure in a 1 cm curvette

$$\text{The percentage concentration} = \frac{\text{Absorbance}}{\varepsilon_{percent}} \quad (1)$$

Then, to correct and report in mg/ml, an adjustment factor must be made when using the percent solution extinction coefficients.

i.e. For 1g/100 ml (1% solution)

$$\text{Then: } \frac{1\text{g}}{100\text{ml}} \times \frac{1000\text{mg}}{1\text{g}} = 10\text{mg/ml}$$

For 5g/100ml (5% solution) which was the solution used

$$\text{Then: } \frac{5\text{g}}{100\text{ml}} \times \frac{5 \times 1000\text{mg}}{1\text{g}} = 50\text{mg/ml}$$

$$\therefore \text{ Concentration in mg/ml} = \left(\frac{\text{Absorbance}}{\varepsilon_{percent}}\right) \times 50$$

$$\text{or Concentration in mg/ml} = \%\text{ Concentration} \times 50$$

3. Results and Discussions

The results shown in Tables 1 and 2 revealed the percentage (%) concentration and concentration in mg/ml of the selected legume and oil seed plant samples respectively. The results for the legumes in Table 1, showed that among the legumes, soybean (*Glycine max*) had the highest protein concentration of 8.59 mg/ml, while between the two varieties of black eye beans that were analyzed, the brown coloured bean, showed a higher concentration than the white colored bean, with varying concentrations of 6.59 mg/ml and 5.59 mg/ml respectively, followed by African beans with a concentration of 4.35 mg/ml and the least in the group with a lesser concentration was bambara groundnut with a protein concentration of 4.29 mg/ml.

Table 1. Percentage concentration and concentration in mg/ml of the selected legume seeds.

Legume seeds	Concentration (%)	Concentration (mg/ml)
Bambara groundnut *(Vigna subterranean)*	0.086	4.29
Soybean *(Glycine max)*	0.172	8.59
Black eye bean (white) *(Vignaun guiculata)*	0.112	5.59
Blackeye bean (brown) *(Vignaun guiculata)*	0.132	6.59
African black bean *(Phaseolus vulgaris)*	0.087	4.35

Means were not significantly different at P< (0.05)

Also, the results for the oil seeds in Table 2, showed that Dika nut (‘*ọgbọnọ’* or *Irvingia spp*), had the highest protein concentration of 18.75 mg/ml. Groundnut and melon seeds had a very close range of concentration, with values of 11.38 and 11.13 mg/ml, respectively, followed by castor oil seed with concentration of 9.40 mg/ml.

Table 2. Percentage concentration and concentration in mg/ml of the selected oil seeds.

Oil seeds	Concentration (%)	Concentration (mg/ml)
Dikanut *(Irvingia spp.)*	0.375	18.75
Groundnut seed *(Arachis hypogea)*	0.228	11.38
Melon seed *(Citrillus vulgaris)*	0.223	11.13
Castor Oil seed *(Ricinus communis)*	0.188	9.40

Mean were not significantly different at P <(0.05)

Although the calculated concentrations tend to show a wide range of differences between the means of the results but statistical tests (t – test), showed a negligible significant difference between the mean of the oil seeds when grouped into two and also that of legumes when grouped into twoat p < 0.05 probability. The legume seeds also showed that the mean were not significantly different at that same level of probability. The same applied to the oil seeds. Though, the spectral analysis helped in calculating the plant protein content in the selected food samples, it is important to note that their concentrationsare in relation to the tryptophan, tyrosine, and to a lesser extent on the phenylalanine or cysteine amino acids, contained in the various plant samples.

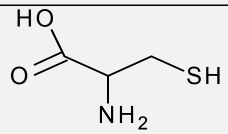
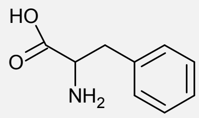
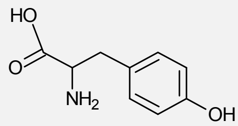
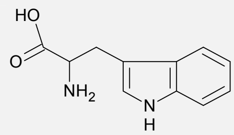
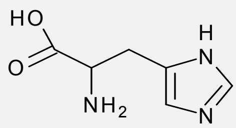
Table 3, showed the list of identified amino acid contained in each of the various plant food samples and the wavelength at which each of them was observed in the various samples while the structure of the identified amino acids are shown in Table 4. The table revealed that different aromatic amino acids were identified in the various plant samples signifying that the samples contained different amino acids. Also, it was observed that each of the plants samples contained at least more than two aromatic amino acids. In dika nut, the following aromatic amino acids: cysteine (Cys), phenylalanine, tryptophan and traces of histidine were identified. In bambara groundnut only phenylalanine (Phe) and Tyrosine (Tyr) were identified.

Table 3. Amino acids identified at different wavelengths from the various plant samples.

FOOD SAMPLES	OBSERVED WAVELENGTHS (nm)	IDENTIFIED AMINO ACIDS
DIKA NUT	216	Cysteine
	250	Phenylalanine
	308	Tryptophan
	346	Traces of Histidine
BAMBARA GROUNDNUT	228 and 238	
	250	Phenylalanine
	276	Tyrosine
SOYBEAN	216	Cysteine
	230	-
	296	Tyrosine and Tryptophan
GROUNDNUT	228	
	250	Phenyalanine
	294	Tyrosine and Tryptophan
MELON SEED	216	Cysteine
	236	-
	250	Phenylalanine
	298	Tyrosine and Tryptophan
CASTOR OIL SEED	204	Cysteine
	236	
	312	Tryptophan
AFRICAN BLACK BEAN	216	Cysteine
	228	-
	250	Phenylalanine
	276 and 296	Tyrosine and Tryptophan
BLACKEYE BEAN (BROWN)	216	Cysteine
	228	-
	276 and 292	Tyrosine and Tryptophan
BLACKEYE BEAN (WHITE)	228	-
	236	-
	276 and 294	Tyrosine and Tryptophan

Cysteine, tyrosine (Tyr) and tryptophan were identified in soybeans and black eye bean (brown) while black eye bean (white) cysteine was not identified revealing the difference in amino acid composition of the different varieties of the beans. African black eye beans in addition to the aromatic amino acids identified in soybean, black eye bean (brown) contained phenylalaninewhich is similar to the aromatic amino acids identified in melon seeds. Cysteine and tryptophan were only identified in castor oil seeds. This implies that different amino acids can actually obtainedby eating a variety of plant proteins [7]. Amino acids have been reported to be present in different parts of plant materials [13, 14, 15].

Table 4. Structure of all the amino acids identified in the various samples at different wavelengths.

Wavelength Range	Structure of identified amino acid
204 – 220	 Cysteine (Cys, C)
240 – 265	 Phenylalanine (Phe, F)
274 – 300	 Tyrosine (Tyr, Y)
275 – 312	 Tryptophan (Trp, W)
Above 312	 Histidine (His, H)

The comparison among each plant protein and its amino acid contentsis shown in Table 5, revealing the possibility of obtaining at least two different amino acids in any of the plant samples. From the selected plant samples that were analyzed, only melon seed '*egusi*' and African black bean '*odudu*' revealed the presence of the four identifiable amino acids, (Trp, Tyr, Cys and Phe) in the plant sample while dika nut revealled the presence of cysteine, tyrosine, tryptophan and histidine (which does not majorly contribute to the protein concentration) in the plant sample. Bambara groundnut and Groundnut seeds showed the presence of two and three aromatic amino acids excluding the sulphide bonded amino acid – cysteine Soybean seed and black eye bean (brown) revealed the presence of two aromatic amino acids (Trp and Tyr) in addition to cysteineand excluding phenylalanine. Also, castor oil (‘*ogiri’*) seed only revealed the presence of two amino acids (cysteine and tryptophan) present in the plant while Black eye bean (white) revealed the presence of two amino acids that istyrosine and tryptophan were present in the plant sample. Although Histidine does not contribute significantly to the protein concentration of each plant using the UV-Vis Spectroscopy method [11], research showed that Histidine can be observed at UV range above 340 nm as shown by the absorbance observed in dika nut at 346 nm.

Table 5. Comparison of amino acids identified at different wavelengths in each of the food samples.

Food Samples	200 – 220 nm Cysteine	240 – 265 nm Phenylalanine	274 – 300 nm Tyrosine	280 – 312 nm Tryptophan	346 nm Histidine
Dika nut	+	+	-	+	+
Bambara Groundnut	-	+		+	-
Soybean	+	-	+	+	-
Groundnut	-	+	+	+	-
Melon seed	+	+	+	+	-
Castor Oil seed	+	-	-	+	-
African Black bean	+	+	+	+	-
Blackeye bean (Brown)	+	-	+	+	-
Blackeye bean (White)	-	-	+	+	-

Note:‘+’ represents the presence of the amino acids identified at the wavelength indicated
‘-’represents the amino acids not present at the wavelength indicated

4. Conclusion

The selected plant protein foods analyzed showed varying protein concentrations and different aromatic amino acids includingsulphide amino acids compositions. The concentration of protein in the various food samples had been found to vary from oil seeds to legume seeds with statistically no significant difference between their mean at probability < 0.05. Also from this study, it has been observed that though plant sources of food contains protein, the necessary dietary recommendation for protein intake would involve a blend of various food sources to obtain the needed essential and non-essential amino acids in the right proportionrequired by body for proper functioning.

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