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Comparative Evaluation of Nutritional Qualities of *Nymphaea lotus* and *Nymphaea pubescens* Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MA, AAI and MKA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AZ managed the analyses of the study. Author NA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was aimed at evaluating the nutritional qualities of *Nymphaea lotus* and *Nymphaea pubescens* seeds.
Study Design: It was designed to determine the proximate and amino acids profiles of *Nymphaea lotus* and *Nymphaea pubescens* seeds to indicate their nutritional potentials.
Place and Duration of Study: Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University, Kano State-Nigeria, between August 2016 and January 2017.
Methodology: Fresh seeds were collected, dried and grounded to smaller particle size. Standard official methods were employed in the proximate analysis while amino acids analysis was carried out using Technicon Sequential Multisample Amino Acid Analyser.
Results: The result of the proximate composition revealed the richness of both seeds in carbohydrates, crude lipids and proteins. The amino acid analysis revealed that amino acids were more concentrated in *Nympahaea lotus* seed with the total value of 73.82 g/100 g than 70.70 g/100

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g of *Nymphaea pubescens* seed. The former also exceeded the later in total essential amino acids by difference of 1.12 g/100 g. The highest scoring amino acid was arginine in *Nymphaea lotus* seed and leucine in *Nymphaea pubescens* seed while methionine was the most limiting amino acid in both the samples. All the essential amino acids in both samples satisfied WHO/ FAO reference protein except histidine, valine and methionine. The P-PER of *Nymphaea lotus* seed and *Nymphaea pubescens* seed were found to be 1.69 and 1.54 respectively, meaning *Nymphaea pubescens* seed may be slightly more bioavailable than the *Nymphaea lotus* seed. The Lys/Arg ratio of *Nymphaea lotus* seed (0.84) and *Nymphaea pubescens* seed (1.09) indicate their low arthrogenic potential.
Conclusion: These results re-enforce the growing awareness that wild and semi-wild plant seeds can contribute useful amounts of essential nutrients to human diets.

Keywords: Proximate composition; amino acid; essential amino acid; protein.

1. INTRODUCTION

Malnutrition is a condition that results from eating a diet in which nutrients are either not enough or are too much such that the diet causes health problem. It may involve calories, protein, carbohydrates, vitamins and minerals [1]. Not enough nutrients is called undernutrition or undernourishment while too much nutrients is called overnutrition. Malnutrition is often specifically to refer to undernutrition where there is not enough calories, protein and micronutrients [2]. There are number of causes of malnutrition. It results from: inadequate or unbalanced diet, problems with digestion or absorption and certain medical conditions.

Nigeria has been ranked second after India in the list of countries with highest cases of malnourished children in the world as it accounts for 10% of the 160 million stunted children globally. The malnutrition in Nigeria is not very encouraging as about 37% of the children are stunted, children under the age of five have chronic malnutrition and 29% are underweight, while 18% have acute malnutrition [3]. However, protein energy malnutrition affects children the most because they have less protein intake. The few rare case found in the world are almost entirely found in children as a result of diets or ignorance in the nutritional needs of children particularly in the cases of milk [4].

Nymphaea lotus (white water lily) and *Nymphaea pubescens* (red waterlily) also known as *Bado gero* and *Bado dawa* in the native Hausa language, are perennial plant that grows up to 45 cm in height. They are herbaceous aquatic plants, whose leaves float or are submerged in water [5]. These plants prefer clear, warm and slightly acidic water and is localized to central and Southern Europe, Asia, the Middle East, Northern Africa, and Tropical Mountain of West Africa especially Nigeria [6]. *Nymphaea lotus* and *Nymphaea pubescens* fruits are either eaten raw or cooked for food when dried by local people of Northern Nigeria. This study evaluated and compared the nutritional qualities of *Nymphaea lotus* and *Nymphaea pubescens* seeds.

2. METHODOLOGY

2.1 Collection and Identification of Plant Materials

Nymphaea lotus and *Nymphaea pubescens* seeds were obtained from Guzu- Guzu Dam in Kabo Local Government Area, Kano state of Nigeria. The samples were authenticated by the Department of Plant Biology, Bayero University Kano with accession number BUKHAN 0356.

2.2 Preparation of Samples

The samples were thoroughly washed to remove sand and the drained parts were air dried later using drier (Drier, AD61, UK). The samples were grounded using wooden mortar and pestle until powder was obtained to ensure homogeneity. The fine powdered samples were then stored at 25°C into labeled plastic containers prior to use.

2.3 Proximate Analysis

2.3.1 Determination of moisture content [7]

Three clean dried petri dishes were weighed (W_1) using weighing balance (A & D weighing, HR250AZ, USA) and 5 g of the pulverized sample was placed in each of them and weighed (W_2). They were placed in an oven (Tricity Bendex, S1530RD, UK) at 100°C for 17 hours. The dishes were removed and cooled in a dessicator for 30 minutes and finally weighed (W_3).

Calculation

The loss in weight due to drying is equal to the moisture content of the sample,

$$\%\text{moisture} = \frac{\text{loss of weight}}{\text{weight of sample taken}} \times 100 = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where

W_1 = weight of empty petri dish
W_2 = weight of empty petri dish + sample
W_3 = weight of empty petri dish + sample after drying

2.3.2 Determination of ash content [8]

Porcelain crucible or silica disc was ignited in a hot Bunsen burner flame or in a muffle furnace for about one minute; it was then transferred into a dessicator for cooling and weighing (W_1). The sample (2 g) was placed into the crucible and weighed again (W_2). A crucible containing the sample was heated gently on a muffle furnace at 550-570°C to burn off all the organic matter. The carbon char burn off as $C0_2$ leaving a white ash. The crucible taken out was immediately covered and placed in a dessicator for cooling and then weighed (W_3).

Calculation

$$\text{Ash \%} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where

W_1 = weight of crucible
W_2 = weight of crucible + sample
W_3 = weight of crucible after all organic matter was burnt off

2.3.3 Determination of crude fat using soxhlet extraction method [7]

Exactly 3 g of the sample was carefully weighed (W_1) into a folded fat-free filter paper using weighing balance (A & D weighing, HR250AZ, USA). This was properly tied with a thread at both ends and weighed (W_2). This was carefully placed in the extraction thimble and small cotton wool placed on top. The whole apparatus was then connected after the addition of 300 ml of hexane into the weighed extraction flask. The extraction was then carried out for 3 hours using the heating mantle at 60-80°C making sure there was continuous flow of water in the condenser (which served as the cooling system). The sample was then removed, air-dried and then placed in an oven (Tricity Bendex, S1530RD, UK) at 80°C until a constant weight was obtained (W_3).

Calculation

$$\text{Crude fat (\%)} = \frac{(W2 - W3)}{W1} \times 100$$

Where:

W_1 = Weight of sample
W_2 = Weight of sample and filter paper
W_3 = Weight of sample after lipid extraction and filter paper

2.3.4 Determination of crude protein [7]

Exactly 0.15 g of dried (moisture free) sample was weighed and transferred into the Kjeldahl digestion flask (30-35 ml). 0.8 g of catalyst (0.7 g sodium sulphate, 0.06 g copper sulphate and 0.04 g mercury (II) oxide red) was added into the digestion flask. To this mixture was added 2 ml of concentrated sulphuric acid of which color turned black. The mixture in the digestion flask was heated on the heating mantle for 1 hour until liquid became clear green. The digest was cooled and made alkaline with 15 ml 40% NaOH. The digest was then transferred to the steamed out apparatus using minimum volume of water. The ammonia steamed is distilled into 10 ml 2% boric acid solution with 5 drops of methyl red indicator for 15 minutes. The distilled ammonia was titrated with 0.02M hydrochloric acid. The same procedure was carried out for the blank.

Calculation

$$\%\text{Nitrogen} = \frac{0.00056 \times V \times 100}{W}$$

Where:
V= volume of acid used (titre value)
W= weight of sample= 0.15 g

%Crude protein= %Nitrogen × 6.25 (multiplication factor)

2.3.5 Determination of crude fiber content [7]

The sample (1.00 g) was weighed into a flat-bottom flask, 100 cm^3 of 20% H_2SO_4 was added

to the sample and was boiled on hot plate for 30 minute. The content was filtered with filter paper and the residue was boiled again in 100 cm3 2M NaOH solution for 30 minutes and filtered. The residue was then dried, weighed and ashed. The crude fibre content was then calculated using the formula:

$$\text{Crude fiber (\%)} = \frac{\text{Weight loss on ignition}}{\text{Weight of sample}} \times 100$$

Where:

W_1 = Weight of sample extracted + filter paper
W_2 = Weight of "W" after ashing.

2.3.6 Estimation of total carbohydrates

Total carbohydrate was estimated by calculation/ difference [8]. The available carbohydrate of samples was estimated by 'difference'. In this, the sum of the percentages of all the other proximate components was subtracted from 100. i.e.

% Carbohydrates = 100- (% Crude Fat + % Crude Protein + % Ash + % Crude fibre).

2.4 Analysis of Amino Acid Profile of *Nymphaea lotus* and *Nymphaea pubescens* Seeds

The amino acid profile of the *Nymphaea lotus* and *Nymphaea pubescens* seeds were determined as previously described by [9]. The samples were dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and then loaded into the Technicon Sequential Multisample Amino Acid Analyser (TSM). (Technicon Instruments Corporation, New York).

2.4.1 Defatting of sample

A known weight of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol mixture in ratio of 2: 1 using soxhlet extraction apparatus as previously described by [7]. The extraction lasted for 15 hours.

2.4.2 Percentage nitrogen determination

The sample (2 g) was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5) containing sodium sulphate (Na_2SO_4), cupper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ration of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added. The flask was then placed in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide was placed into the Markham distillation apparatus and distilled with 10 ml of 2% boric acid containing 4 drops of methyl red indicator until 70 ml of distillate was collected. The distillate was then titrated with standardize 0.01N hydrochloric acid to grey coloured end point, the percentage nitrogen in the original sample was calculated using the formula:

$$\% \text{ N} = \frac{(a-b)\times 0.01\times 14\times v}{w\times c} \times 100$$

Where:

a = Titre value of the digested sample
b = Titre value of blank sample
v = Volume after dilution (100 ml)
w = Weight of dried sample (g)
c = Aliquot of the sample used (10 ml)
14 = Nitrogen constant in g

2.4.3 Hydrolysis of the samples

The defatted sample (1.204 g) was weighed into glass ampoule. 7 ml of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (This is to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoule was then sealed with Bunsen burner flame and placed in an oven preset at 105°C ± 5°C for 22 hours to effect hydrolysis. The ampoule was allowed to cool before it was broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in deep freezer.

2.4.4 Loading of the hydrolysate into the TSM analyzer

The amount loaded was 60 microlitres. This was injected into the cartridge of the analyzer. The TSM analyzer is designed to analyse free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 75 min. The

amino acids present in the samples were identified by matching their peak retention time in the chromatogram with those peaks of standard mixture of amino acids with norleucine as internal standard.

2.4.5 Calculating the amino acid values from the chromatogram peaks

An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids. The net heights of each peak produced by the chart recorder of the TSM (each representing an amino acid) were measured. The half-height of the peak on the chart was found and the width of the peak at the half- height was accurately measured and result recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formular:

$$NE=\frac{(area\ of\ norleucine\ peak)}{(area\ of\ each\ amino\ acid)}$$

Constant S was calculated for each amino acid in the standard mixture:

Sstd. = NEstd. × Mol. weight × µMol.AAstd.

Finally the amount of each amino acid present in the samples was calculated in grams per 100 g protein using the following formula:

Concentration (g/100 of protein) = NH × NH/2 × Sstd. × C

Where:

$$C = \frac{[Dilution \times 16]}{Sample\ Wt(g) \times Vol.\frac{Loaded}{NH} \times W(nleu)}$$

Where Wt= weight, NH = net height,
W = width, nleu = norleucine

2.4.6 Determination of tryptophan

Tryptophan is a difficult amino acid to determine in proteins and peptides because it chemically decomposes during acid hydrolysis. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis. Alkaline hydrolysis was improved by using sodium hydroxide (NaOH) instead of barium hydroxide to prevent problems with both precipitation and adsorption of tryptophan.

The tryptophan in the known sample was hydrolyzed with 4.2 M Sodium hydroxide. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer as described above.

2.4.7 Estimation of seed samples protein quality

To estimate the quality of dietary protein in the seed samples, the Total Essential Amino Acid (TEAA), Total Non-Essential Amino Acid (TNEAA), Total Sulphur Amino Acid (TSAA), percentage of cysteine in TSAA (%Cys/TSAA), Total Aromatic Amino Acid (TArAA), Leu/Ile ratio, Lys/Arg ratio etc, were calculated as described by FAO [10]. The Predicted Protein Efficiency Ratio (P-PER) was also determined using the equation of Alsmeyer's. I.e. P-PER = - 0.468 + 0.454 (Leu) – 0.105 (Tyr) [11]. The percentage amino acid scores of the seed samples were also calculated using the formula:

% Amino acid score = *[Amount of amino acid per test protein (g/100 g)/ Amount of amino acid per protein in FAO/WHO (1995) reference pattern (g/100 g)] × 100*

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

Table 1 presents the proximate composition of the *Nymphaea lotus* and *Nymphaea pubescens* seeds. The moisture, crude fibre contents of *Nymphaea lotus* and *Nymphaea pubescens* seeds are not significantly different ($p>0.05$). However, ash, lipid, crude proteins and carbohydrates contents of the *Nymphaea lotus* seed and *Nymphaea pubescens* seeds recorded significant difference ($p<0.05$).

The percentage of ash, moisture, crude protein, crude fat (lipids), crude fibre and carbohydrate contents of *Nymphaea lotus* and *Nymphaea pubescens* seeds are presented in Table 1. The *Nymphaea pubescens* seed had higher percentage value of ash content compared to *Nymphaealotus* seed. This is higher than the reported value of *Nymphaea lotus* seed by [12,5]. Thus, the result indicated that *Nymphaea lotus* and *Nymphaea pubescens* seed could serve as good source of mineral element since ash content measured the minerals content of food substances and were found to be within the

range reported for some leguminous seed [13]. However, the percentage for the moisture content in *Nymphaea lotus* and *Nymphaea pubescens* seeds were 5.31% and 6.20% respectively which is less than 8.5% in *Annona muricata* and 9.7% in pride of Barbados seeds reported by [13,14] respectively. These results are comparable to the literature value of 6.00% reported for *Nymphaea lotus* seed [12]. However, the results were lower than 8.20% reported by [5]. The low moisture content of both *Nymphaea* seeds may signify longer shelf life since seeds with moisture content greater than 15% are subjected to deterioration from mould growth, heat, insect damage and sprouting [13]. Moreover, the percentage of crude fat (lipids) in *Nymphaea lotus* seed was found to be higher than in *Nymphaea pubescens* seeds. In addition [12] had earlier reported that *Nymphaea lotus* seeds have a lipid or crude fat content of 9.33% which was lower than value reported in the present study. Therefore, both *Nymphaea* seeds could serve as the secondary source of energy.

Table 1. Proximate compositions of *Nymphaea lotus* seed and *Nymphaea pubescens* seeds

Parameters (%)	*Nymphaea lotus* seed	*Nymphaea pubescens* seed
Moisture	5.31±0.32	6.20±0.87
Ash	1.33±0.11[a]	3.00±0.30[a]
Proteins	4.92±0.34[a]	4.14±0.06[a]
Lipids	13.23±1.01[a]	9.28±0.15[a]
Fibre	5.17±0.51	5.00±0.26
Carbohydates	75.35±1.38[a]	78.58±0.89[a]

All values are expressed as mean ± standard deviation for three determinations. Rows with similar superscript are statistically significant ($p<0.05$)

Furthermore, *Nymphaea lotus* seed was found to have higher crude protein content than the reported value of (3.09%) by [5]. It was earlier reported that *Nymphaea lotus*seed could be good source of protein [15]. The percentage value of crude protein obtained for *Nymphaea pubescens* seed was slightly lower than in *Nymphaea lotus* seed. Thus, this study suggests that *Nymphaea lotus* seeds possess high protein content compared to *Nymphaea pubescens* seeds. Proteins ensure proper growth and also serve as source of some essential amino acids for body use. Analysis revealed slightly higher percentage of crude fibre in *Nymphaea lotus* seed than obtained in the *Nymphaea pubescens* seeds. However these values are within the range reported by [16,5,12] for *Nymphaea lotus* seeds. Thus, ingestion of foodstuff which has reasonable amount of dietary fiber reduce postprandial glucose response after carbohydrate rich meals as well as lowering total LDL cholesterol levels [15].

Similarly, the carbohydrate content of both *Nymphaea lotus* and *Nymphaea pubescens* seeds were lower compared to the reported value in *Nymphaea lotus* seed (80.96%) by [12]. The difference in carbohydrates contents could be attributed to difference in climate and soil condition. The carbohydrate composition present in both *Nymphaea* seeds is enough to prevent ketosis as breakdown of 1 gram of (glucose) carbohydrate yield 4 kcal of energy [17]. Similar carbohydrate content was reported by Mohammed et al. [16] which reported that, seeds of *Nymphaea lotus* serves as good source of carbohydrate and is enough to prevent ketosis. The role of Carbohydrate is not limited to energy intake as they also play a vital role in both the taste of the food and the pleasure of eating [18].

Therefore, the proximate composition of the *Nymphaea lotus* and *Nymphaea pubescens* seeds indicates they are highly nutritious as they contains high protein content hence could supplement other protein sources such as beans, peas and groundnuts especially in dry seasons and in arid regions [19] when or where most common protein sources might be scarce and expensive, hence could help to reduce malnutrition and food insecurity

3.2 Amino Acids Compositions

The amino acid composition and percentage Amino acid score of *Nymphaea lotus* and *Nymphaea pubescens* seeds are presented in Table 2. The result indicates the presence of all the essential amino acids while two non-essential amino acids were totally absence in the two samples screened.

From the various parameters presented in Table 2, total amino acids (TAA), total non-essential amino acids (TNEAA), total essential amino acids (TEAA), their respective percentages, the predicted protein efficiency ratio (P-PER), Leu/Ile ratio and Essential amino acid index (EAAI) values of *Nymphaea lotus and Nymphaea pubescens* seed were calculated and presented in Table 3.

The amino acids contents of *Nymphaea lotus* seed and *Nymphaea pubescens* seed were presented in Table 2 showing only eighteen amino acids out of the common twenty amino acids found in proteins, including ten essential amino acids and eight non-essential amino acids. This may be due to the conversion of the amide glutamine and asparagine to their corresponding amino acids, glutamate and aspartate respectively [20].

All essential amino acids in both *Nymphaea lotus and Nymphaea pubescens* seeds were found to satisfied the FAO/WHO ideal protein value in children except for valine, histidine and methionine which were closer to the value established by FOA/WHO ideal protein in children. Hence, with proper processing, the seeds could meet up with the WHO ideal protein value for both children and adults.

Table 2. Amino acid composition and percentage amino acid score of *Nymphaea lotus* and *Nymphaea pubescens* seeds

Amino acid	Concentration (g/100 g)		FAO/WHO Ref.	%Amino acid score	
	N. lotus	*N. pubescens*	Standard	*N. lotus*	*N. pubescens*
Leucine	5.49	5.14	4.2	130.71	122.38
Lysine	4.48	4.88	4.2	106.67	116.19
Isoleucine	4.52	4.19	4.2	107.62	99.76
Phenylalanine	2.48	2.39	2.3	107.83	103.91
Tryptophan	1.21	0.92	1.1	110.00	83.63
Valine	4.01	3.71	4.2	95.48	88.33
Methionine	1.23	1.20	2.2	55.91	54.55
Arginine	5.33	4.47	4.0	133.25	111.75
Histidine	2.43	2.20	3.4	71.47	64.71
Threonine	3.16	3.25	2.8	112.86	116.71
Cysteine	1.15	1.27	2.0	57.50	63.50
Proline	3.55	3.96	-	-	-
Tyrosine	3.10	3.10	2.8	110.71	110.71
Alanine	4.02	3.41	-	-	-
Glutamic acid	12.11	11.58	6.3	192.22	183.81
Glycine	3.61	3.94	-	-	-
Serine	3.94	3.59	-	-	-
Aspartic acid	8.00	7.50	-	-	-

- Means not reported/Not determined

Table 3. Nutritional quality indices of protein

Amino acid grouping	*Nymphaea lotus* seed	*Nymphaea pubescens* seed
Total amino acid (TAA)	73.82	70.70
Total non-essential amino acid (TNEAA)	39.48	38.38
Total essential amino acid (TEAA)		
-with His	34.34	32.32
-without His	31.91	30.12
% TNEAA	53.48	54.29
% TEAA		
-with His	46.52	45.71
-without His	43.23	42.60
Total Sulphur amino acid (TSAA)	2.38	2.47
% TSAA	3.22	3.49
% Cys in TSAA	48.32	51.42
Total Aromatic amino acid (TArAA)	6.79	6.41
1.69	1.54	9.07
Leu/Ile ratio	1.21	1.23
Lys/Arg	0.84	1.09
Essential amino acid index(EAAI)	87.6	84.2

Among the essential amino acids, leucine had the highest concentration in both the samples, followed by isoleucine. The least essential amino acid is methionine with *Nymphaea lotus* seed having the higher content. *Nymphaea lotus* seed had higher concentration of all amino acids compared to *Nymphaea pubescens* seed except for lysine, cysteine, proline and glycine. This could be attributed to genetic difference between the samples.

The arginine content of *Nymphaea lotus and Nymphaea pubescens* seeds was higher than the FAO/WHO [21] recommendations for infants (Arginine 4.0 g/100 g). Arginine is good for children and it is considerably high in both the *Nymphaea lotus* and *Nymphaea pubescens* seeds with respectively. Isoleucine is an essential amino acid both for young and old. Methionine is needed for the synthesis of choline which in turn forms lecithin and other phospholipids in the body. When the diet is low in protein, for instance in alcoholism and kwashiorkor, insufficient choline may be formed; this may cause accumulation of fat in the liver [22].

The amino acid scores(a rating of the quality of a test protein by comparing its amino acid pattern with that of FAO/WHO reference ideal protein for pre-school children) of *Nymphaea lotus* and *Nymphaea pubescens* seeds are shown on Table 2. The highest scoring essential amino acid was Arginine (133.25%) in *Nymphaea lotus* seed and leucine in *Nymphaea pubescens* seed while methionine was the lowest scoring amino acid (55.91 and 54.55%) in both the samples making it the first limiting amino acid. This is in conformity with the report that the essential amino acids most often acting in a limiting capacity are Methionine and Cysteine, Lysine and Tryptophan [23]. Leucine, Isoleucine and Tryptophan are also present in significant quantities. Methionine is needed for the synthesis of other important substances including choline. The intake of methionine may prevent the fatty liver and causes development and growth [22].

Furthermore, Table 3 shows the nutritional quality determinations of *Nymphaea lotus and Nymphaea pubescens* seeds. The nutritive value of a protein depends primarily on the capacity to satisfy the needs for nitrogen and essential amino acids [24]. The total amino acids, TAA (73.82 g/100 g) of *Nymphaea lotus* seed is higher than *Nymphaea pubescens* seed (70.70 g/100 g). This difference could be attributed to genetic differences among the *Nymphaea* species and the values obtained in the present study are comparable with that of some plant foods which range between 39.3 – 76.5 g/100 g protein as reported by earlier workers [25,26].

The predicted protein efficiency ratio (P-PER) value is one of the quality parameters used in the evaluation of proteins [21]. The P-PER of *Nymphaea lotus* seed and *Nymphaea pubescens* seed were found to be 1.69 and 1.54 respectively, meaning *Nymphaea pubescens* seed may be slightly bioavailable than the *Nymphaea lotus* seed. This is slightly lower than the reported values for true digestible protein of whole dried honey bees (*Apis mellifera L.*), 2.47 and 2.50 for casein [27]. Due to the fact that the experimentally determined PER usually ranged from 0.0 for a very poor protein to a maximum possible value of just over 4 [28], the result of this work shows that both *Nymphaea lotus* and *Nymphaea pubescens* seeds could be efficiently utilized in human body.

Furthermore, the Leu/Ile ratio value of *Nymphaea lotus* and *Nymphaea pubescens* seeds were found to be 1.21 and 1.23 respectively, of which Ile was less than half that of Leu. It has been suggested that an amino acid imbalance from excess leucine might be a factor in the development of pellagra due to sorghum consumption [29]. High Leucine in the diet impairs tryptophan and niacin metabolism and is responsible for niacin deficiency in sorghum eaters [30].

The Lysine/Arginine (Lys/Arg) ratio has been reported to play a role in the artherogenocity of a protein. [31] Reported that a Lys/Arg ratio as high as 2.0, increases the artherogenic potential of a diet. The Lys/Arg ratio of the *Nymphaea lotus* (0.84) and *Nymphaea pubescens* seed (1.09) are much lower than that of casein with Lys/Arg ratio 2.0. A similar observation has been made for *Kerstingiella geocarpa* [32]. Thus, since *Nymphaea lotus* and *Nymphaea pubescens* seeds showed a low Lys/Arg ratio, the samples will possess a low artherogenic potential.

The essential amino acid index (EAAI) and nutritional index of *Nymphaea lotus* seed were higher than the *Nymphaea pubescens* seed. However, the nutritional indexes of the both *Nymphaea* seeds were much higher compared to the *A. bisporus* (13.69%) and *P. florida* (12.59%) as reported by [33]. The essential amino acid

index can be useful as a rapid tool to evaluate food formulations for protein quality [34]. However, it does not account for differences in protein quality due to various processing methods or certain chemical reactions [34]. Protein material is said to be of good nutritional quality when its essential amino acid index (EAAI) is above 90% and to be useful as food when the values are around 80% and are inadequate for food material when values are below 70% [35]. From the present study it is observed that the EAAI values were generally appreciable and could be attributed to the fact that *Nymphaea* seeds, a wild haebaceous plant, possessed most essential amino acids in appropriate quantities.

4. CONCLUSION

From the results obtained from this work, it can be concluded that *Nymphaea lotus* and *Nymphaea pubescens* seeds are of high nutritional qualities. Both the seeds are rich in essential amino acids that if consumed in sufficient amount, it could contribute to meeting human nutritional needs and helps to combat diseases associated with malnutrition. The level of arginine and histidine in the seeds warrants them to be recommended for children food since children need arginine and histidine in their foods. This nutritional information could be of great use to nutritionists, industrialists, researchers, policy makers development agencies and encourage the consumption of *Nymphaea lotus* and *Nymphaea pubescens* seeds so that they become part of normal diet rather than being considered as 'famine' or 'poor peoples' food especially in arid regions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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