



Proximate, Functional Properties and Amino acid Composition of *Piper guineense* (Uziza) Leaves

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Abstract The proximate analysis, functional properties and amino acid composition of *Piper guineense* leaves were investigated. The results showed that *Piper guineense* leaves contained moisture (11.51%), total ash (13.94%), crude protein (3.87%), crude fat (5.48%), crude fibre (12.60%) and carbohydrate (53.15%). The sample contained oil absorption capacity (106.75%), water absorption capacity (187.5%), emulsion capacity (250.5%), foaming stability (3.75%) and foaming capacity (94%). The water and oil absorption capacities were relatively good. Glutamic acid was the most abundant amino acid while cystine (1.09g/100g crude protein) was the least amino acid. The total essential amino acid of the sample was calculated to be (51.46g/100g crude protein) which showed that the sample contained moderate quantities of essential amino acids.

Keywords Functional Properties, Amino acid, *Piper guineense* (uziza) leaves

Introduction

Vegetables are unavoidable components of human diets because of their ethno-medicinal and nutritional potentials. They generally contain low fat and protein contents [1] and varying levels of vitamins, minerals, phytochemicals, antioxidants, anti-carcinogenic agents and fibre for gastro intestinal functions [2]. The cultivation of green leafy vegetables proliferate different races because of their various and health benefits [3]. It constitutes an indispensable constituent of human diet, especially in local delicacies. Vegetables are those herbaceous plants whose parts are eaten as supporting food or main dishes and they may be aromatic, bitter or tasteless [4].

Vegetables are included in meals mainly for their nutritional value; however, some are reserved for the sick and convalescence because of their medicinal properties. They are the cheapest and most available sources of both macro and micro nutrients useful for body development. The nutritionists have recommended that public should eat more of vegetables and fruits [5]. This will prevent or treat several chronic non-communicable diseases that may cause by oxidative imbalance. *Piper guineense*, popularly known as African black pepper or hot leave and is widely consumed in some parts of Africa especially eastern Nigeria, where it is known as 'Uziza' and Ghana. It belongs to the family Piperaceae or Sapotaceae [6]. It is used as spice for flavoring, seasoning and imparting aroma to food during preparation. They can be consumed as vegetable in human meals. It has preservative and antioxidant properties [7]. In some parts of Nigeria, the seeds are consumed by women after child birth [8] to enhance uterine contraction for the expulsion of placenta and other remains from the womb [9], for the treatment of rheumatic pains, as an anti-asthmatics and also to control weight [10]. The seed and leaf extracts are capable of exhibiting a depolarizing neuromuscular activity in a concentration related manners [11]. The antimicrobial and antifungal



activities of the leaf and seeds of *P. guineense* have also been reported [11]. The leaf is also used traditionally to cure some respiratory diseases and correction of female infertility problems [12], and widely used for various therapeutic purposes locally, but there is scanty information on the nutritional compositions. Therefore, the objectives of this study are to determine the proximate, functional properties and amino acids of *Piper guineense* (Uziza) leaves.

Materials and Methods

Sample Collection

The sample (*Piper guineense*) was collected from a farm in Ado Ekiti, Ekiti state, Nigeria. The leaves were air-dried and then processed into flour. The flour was kept in a freezer before analyses.

Determination of Proximate Composition

The moisture was determined using air-oven at temperature of 105 °C for 1 h while the ash content was analyzed using a muffle furnace at 550 °C for 6 h [13]. The sample was analyzed for crude fat and crude protein according to the methods described by AOAC [14]. The crude fiber was determined by adding 2g of the sample into 500mL conical flask; 200mL of boiling 1.25% H₂SO₄ was added and boiled for 30min. The mixture was filtered through muslin cloth and rinsed with hot distilled water. The sample was scraped back into the flask and 200mL of boiling 1.25% NaOH was added and allowed to boil again for another 30 min; filtered and then rinsed with 10% HCl twice with industrial methylated spirit and allowed to drain and dry. The residue was scraped into a crucible, dried in an oven at 105 °C, allowed to cool in a desiccator and weighed; then placed in muffle furnace at 300 °C for 30 min and finally allowed to cool at room temperature and weighed again [13]. The carbohydrate content was calculated by difference.

Determination of Functional Properties

The water and oil absorption capacities of the sample were determined using the method of Beuchat [15]. 10mL of water was added to 1.0g sample in a centrifuge tube. The suspension was mixed vigorously using vortex mixer. This was then centrifuged at 15,000 rpm for 15 min and the volume of the supernatant left after centrifuging was noted. Water bound was calculated from the difference in the initial volume of the solvent used and the final volume after centrifuging. The same procedure was used for oil absorption capacity by replacing oil with water in above process. Emulsion was prepared according to method of Lin *et al.* [16]. A 2.0 g sample flour was weighed with 100mL distilled water and blended for 30 sec using Kenwood food mixer at a high speed. After complete dispersion, vegetable oil of density 0.880 g/mL was added in 5mL portions from a burette with continuous blending until the emulsion break point (i.e. a separation into two layers) was observed. Emulsion capacity and stability determinations were carried out at 25°C and the value obtained was expressed as gram of oil emulsified by 1 gram sample. The emulsion stability was determined as the amount of the water separated after 24 h at room temperature. The slight modified procedure of Sathe *et al.* [17] was used to determine the least gelation concentration. Sample slurries of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20% were prepared in 5mL of distilled water. The test tubes containing these slurries were heated for one hour in boiling water followed by rapid cooling (2 h at 4°C). The least gelation concentration was determined as concentration which did not slip when the test tubes were inverted. The method of Coffman and Garcia [18] was employed to determine foaming capacity and stability. 1g of the sample was whipped with 50mL distilled water for 5 min in a Kenwood blender and later poured into a 100mL graduated flask to study the foaming stability.

Determination of Amino Acid

The amino acid profile was determined using the method described by Spackman [19]. The sample was dried to constant weight and then defatted using Soxhlet extractor. After the defatting process, the defatted sample (2g) was weighed into a glass ampoule; 7mL of 6M HCl was added and oxygen was expelled by passing nitrogen into the glass ampoule sealed with Bunsen burner flame and placed in an oven at 105±5°C for 22 h. The ampoule was allowed to cool before it was broken at the tip and the content was filtered to remove the organic matters. The



filtrate was then evaporated to dryness at 40°C under vacuum in a rotavapor. The residue was dissolved in 5mL of acetate buffer (pH 2.0) and stored in specimen bottles which were kept in the freezer. The hydrolysate (7.5µL) was dispensed into the cartridge of the Technicon Sequential Multi-Analyser (TSM) using a syringe. The TSM analyzer is designed to separate and analyze neutral, acidic and basic amino acids of hydrolysate. The amount of amino acid was obtained from the chromatogram peaks. The whole analysis lasted for 76 min and the gas flow rate was 0.50mL per minute at 60°C with reproducibility consistence at $\pm 3\%$.

Results and Discussion

Table 1: Proximate Analysis of *Piper guineense* Leaves

Proximate	Composition
%Moisture	11.51
%Protein	3.87
%Fat	5.48
%Ash	13.94
%Crude fibre	12.06
%Carbohydrate (by difference)	53.15

Table 1 shows the values (%) of the proximate composition of *Piper guineense*. % Carbohydrate has highest value (53.15%) followed by %Ash (13.94%), crude fiber (12.06%), moisture (11.51%), fat (5.48%) and protein (3.87%). These results were higher than the previous reported by Morufu *et al.*, [20]. Crude protein was 16.67 \pm 0.00%. This value was higher than that of bread produced from indigenous yeast isolates (3.325-5.425%) as reported by Balarabe *et al* [21] but lower than that reported for *G. latifolium* (5.57%) by Ughegbu *et al.* [22]. It is to be noted that the present value was higher than those reported by Ughegbu *et al.* [22] for *Piper guineense* and 0.23% reported by Ogungbenle and Omodara [23] for *Ectadasegigas* seeds but lower than those by Ogungbenle and Ebadan [24] for *Dalium guineense* pulp (24.3%) and *Parinari curatellifolia* reported by Ogungbenle and Atere [25]. The moisture content of any food is an index of its water solubilisation and the measure of stability and susceptibility to microbial activities. High amount of moisture in crops makes them vulnerable to microbial attack, hence, spoilage and deterioration. The moisture content of *Piper guineense* leaves analyzed was 11.51%. This value is lower than those of Sclerotium of *Pleurotus tuber-regium* with the value of 41.6% reported by Adeyeye [26] and stingless bee honey (23.1-43.5%) reported by Fabiola *et al.* [27]. The low moisture content of *Piper guineense* is an indication of the fact that these spices can be stored for a long period without deterioration in quality. The fat content of *P. guineense* (5.48%) is lower than those for fermented *Parkia biglobosa* (25.1 \pm 0.11%) [28] and raw sesame flour (40.97%) [29] but higher than that of *Celosia spicata* (1.15 \pm 0.01%) [1]. The ash content (13.94%) was higher than those of *Uacapa guineense* (1.7% and 0.80%) and *Zanthoxylus zanthoxyloides* (1.30% and 1.40%) as reported by Ogunka-Nnoka and Mepba [30]. The fibre content of *P. guineense* (12.06%). This value was higher than those of quinoa seeds (9.6 \pm 0.1%) reported by Oshodi *et al.* [31], dry Nigeria okra (8.85%) reported by Ogungbenle and Omosola [32] and bread produced from different indigenous yeast isolates (1.49-3.50%) reported by Balarabe *et al.* [21].

Table 2: Functional properties of *Piper guineense* leaves

Functional properties	Value (%)
Water absorption capacity	187.5
Oil absorption capacity	106.75
Emulsion capacity	250.5
Foaming capacity	94
Foaming stability	3.75

The functional properties of *Piper guineense* sample leaves are shown in Table 2. The value of water absorption capacity (187%) was higher than those of date palm fruit (45.0%) [33], soy flour (160%) (Paul *et al.*, 1985) and Lima bean (142.2%) [34]. The oil absorption capacity (106.75%) was lower than that of *Azelia africana* (588.49%)



[34], African nutmeg (256%) [35] and conophor nut (108.13%) but higher than those values obtained for sesame protein concentrates (25.70%) [36]. It is an indication that the *Piper guineense* leaves can act as flavour retainer and improve the mouth feels of foods [37]. The emulsion capacity (250.5%) of *Piper guineense* leaf was higher than those values obtained for *Parkia biglobosa* protein isolate ($82.5 \pm 0.15\%$) [38], *Celosia spicata* (56%) [1], African Nutmeg (45.6%) [35], *Afzelia africana* (35.25%) [33] and quinoa flour (45.0%) [39]. The foaming capacity and foaming stability of *Piper guineense* leaves were 94% and 3.75% respectively. The values were higher than those reported for raw (3.25%, 1.50%) and defatted (11.0%, 3.75%) African mango seeds reported by Ogungbenle [40] and *Pleurotus tuber-regium* (8.25 ± 0.35 , $1.50 \pm 0.71\%$) [26]. The water absorption capacity (187.5%) is higher than that of *Pleurotus tuber-regium* ($136 \pm 1.41\%$) [26]. It indicates that *Piper guineense* leaf can be used in the formulation of soups and processed cheese and that the protein concentrate has high potential for development of different food products in a critical viscous foods as a good additive and flavor retainer.

Table 3: Amino acid composition of *Piper guineense* (g/100g)

Amino acid	Composition (g/100g)
Glycine*	6.00
Alanine	6.03
Serine	4.28
Proline	6.16
Valine*	6.77
Threonine*	4.60
Isoleucine*	4.43
Leucine*	5.94
Aspartic acid	8.96
Lysine*	6.42
Methionine*	1.50
Glutamic acid	12.46
Phenylalanine*	5.18
Histidine*	6.68
Arginine*	6.96
Tyrosine	1.85
Tryptophan*	1.16
Cystine	1.09
Total	96.47

(*) –Essential Amino acid

The amino acid composition of the sample is shown in Table 3. The result showed that glutamic acid (12.46g/100g crude protein) was the major abundant amino acid. The value was lower than those reported for *Celosia spicata* (12.8g/100g) [1], *Amarathus hybridus L.* (15.79g/100g crude protein) [41], velvet tamarind (14.8g/100g crude protein) [24], *Pleurotus tuber-regium* (13.5g/100g) [26], and *Moringa oleifera* leaves (8.22g/100g crude protein), but higher than those stem (1.77g/100g crude protein), root (1.93g/100g crude protein) of *Moringa Oleifera* [42] and common maize (5.70 g/100g) [43]. It is worth noting that glutamic and aspartic acids were the most abundant amino acid in agricultural products. The value of histidine (6.68g/100g) was higher than those of maize (0.2 g/100g crude protein) [44], gourd seed (0.21g/100g crude protein), common and quality protein maize (2.00 g/100g) reported by Abiose and Ikujeniola [43]. Histidine is an essential amino acid for growth and development of infants. The methionine content (1.50g/100g crude protein) was higher than those of *Bidenspilosa* leaf meal (0.54g/100g crude protein) [45] and leaves of *Moringa oleifera* (0.99g/100g crude protein) [42] but lower than that of *Afzelia Africana* (13.8g/100g crude protein) [33] and dried okra seeds (10.4 g/100g) reported by Ogungbenle and Arekemase [46]. Methionine is required for choline synthesis and also may hinder fat deposition in liver [47].



Methionine was the least amino acid in the sample, and this value was higher than that of *Bidens pilosa* leaf meal (0.289g/100g crude protein) [45]. Comparison between the amino acid profile of *Piper guineense* leaves and the amino acid reference values reported by FAO/WHO/UNU [48]. It indicates that most of the amino acids in the sample meet the range of amino acid requirements recommended for infants, pre-school children and school going children as well as adults [49]. Both the histidine and arginine are essential for children [48]. *P. guineense* leaf, compared to other vegetables is very high and serves as a cheap and good source of both essential and non-essential amino acids. Therefore, the present results indicate that the sample is a good source of quality protein and essential amino acids.

Table 3 also summarizes the profiles of free amino acid (FAA) in the leave of uziza (*P. guineense*) in mg/g. Glutamic acid appears to be the most concentrated amino acid in the leave sample which had 12.46g/100g. The value of glutamic acid is higher than those of *Brachystegia eurycoma* (4.60g/100g) and *Piper guineense* (6.35g/100g) reported by Ajayi *et al.* [50]. Table 3 also shows aspartic acid is the next highest amino acid for the sample with concentration value of 8.96g/100g. Cysteine and methionine had the lowest concentrations of amino acids. It is known that cystine can pair with methionine in improving protein quality and has positive effects on mineral absorption, particularly zinc.

Table 4: Classification of Amino Acid Composition

Amino Acid	Value (mg/g)
Total amino acid (TAA)	964.70
% Total amino acid (%TAA)	100.00
Total non-essential amino acid (TNEAA)	468.30
% Total non-essential amino acid (%TNEAA)	48.54
Total essential amino acid (TEAA)	496.40
% Total essential amino acid (%TEAA)	51.46
Total essential amino acid with Histidine	496.40
%Total essential amino acid without Histidine	51.46
Total essential amino acid with Histidine	429.60
%Total essential amino acid without Histidine	44.53
Total neutral amino acid (TNAA)	549.90
% Total neutral amino acid (%TNAA)	57.00
Total acidic amino acid (TAAA)	214.20
% Total acidic amino acid (%TAAA)	22.20
Total basic amino acid (TBAA)	200.60
% Total basic amino acid (%TBAA)	20.79
Total sulphur amino acid (TSAA)	25.90
% Total sulphur amino acid (%TSAA)	2.74
Total Aromatic Aminoacid (TArAA)	81.9
% Total Aromatic Amino acid (% TArAA)	8.49
% Cystine in TSAA	42.08

Table 4 shows that the Total amino acid (TAA) is 964.70mg/g, Total non-essential amino acid (TNEAA) is 468.30mg/g, Total essential amino acid (TEAA) is 496.40mg/g, Total essential amino acid with Histidine is 496.40mg/g, Total essential amino acid without Histidine is 429.60mg/g, Total neutral amino acid (TNAA) is 549.90mg/g, Total acidic amino acid (TAAA) is 214.20mg/g, Total basic amino acid (TBAA) is 200.60mg/g, Total sulphur amino acid (TSAA) is 25.90mg/g, Total Aromatic Amino acid (TArAA) is 81.9 mg/g and % of Cystine in TSAA is 42.08.

The total amino acid, TAA (904.7mg/100g) is far higher than that of *Dalium guineese* (102 mg/g) reported by Ogungbenle and Ebadan [24] and also total essential amino acid, TEAA (496.40 mg/g) is higher than those of *Pleurotus tuber-regium* (416 mg/g) [26].



Conclusion

Piper guineense is a potential source of various nutrients and useful in allied industries for the formulations of human food. Large cultivation of this plant would greatly enhance economic development both locally and globally because of its nutritional and medicinal applicability.

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