

Comparative Study on the Nutrient and Antinutrient Composition of the Seeds and Leaves of *Uziza* (*Piper Guineense*)

¹Ojinnaka, M. C., ²Odimegwu, E. N., ¹Chidiebere, F.E.

¹Department of Food Science & Technology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria

²Department of Food Science & Technology, Federal University of Technology Owerri, Imo State, Nigeria

Abstract: The nutrient and antinutrient compositions of the leaves and seeds of uziza (*Piper guineense*) were investigated to compare the differences in the nutrient properties determined. The result of the proximate analysis shows uziza leaves had significantly higher crude protein and fibre content values of 11.32% and 18.19% while those of the seeds had protein and fibre values of 7.82% and 2.88% respectively. The carbohydrate content of the seeds (70.51%) were significantly higher than the leaves (42.23%). The result of the nine mineral analysis performed also shows that iron, zinc, lead, calcium and potassium contents of the uziza leaves were higher than those of the seeds with values 3.12mg/100g, 0.39 mg/100g, 0.06 mg/100g 466.39 mg/100g and 116.52 mg/100g respectively. Copper, magnesium, sodium and phosphorus content values were higher in the seeds than in the uziza leaves. The uziza leaves showed a higher vitamin C content of 2.51mg/100g while the seeds had vitamin C value of 1.94mg/100g. The oxalate content for the uziza leaves and seeds were the same with a value of 0.55 mg/100g each. The tannin and phytate values were more in the uziza leaves than the seeds while saponin content was lower in the uziza leaves (3.26 mg/100g). Results from the amino acid composition shows the leaves and seeds of uziza (*Piper guineense*) contained sufficient amount of amino acids. The essential amino acids (EAA₇ and EAA₁₀) in the seeds of uziza (*Piper guineense*) were more than those in the leaves. The highest amino acid content was observed in glutamic acid. The uziza seeds contained 113.50 µg/100g glutamic acid while the uziza leaves contained 100.75 µg/100g. This was closely followed by the aspartic acid and lysine in both the leaves and seeds of uziza. Tryptophan was however not detected in the seeds and leaves of uziza.

Keywords: seeds, leaves, uziza, *Piper guineense*, nutrient, antinutrient

I. Introduction

The knowledge and use of plants as spices is as old as the history of mankind [1] and plants used as spices are usually aromatic and pungent [2]. Spices are known as products of plants, which are mostly used for seasoning, flavouring and thus enhancing the taste of food, beverage and drugs [3].

Uziza (*Piper guineense*) is a spice plant from the family, *piperaceae* and from the genus *piper*. It is a West African spice plant and is commonly called "Black pepper". *Uziza* (*Piper guineense*) is a climbing perennial plant of the family *Piperaceae*. It is used in the preparation of pepper soup in the Southern part of Nigeria. In the Eastern parts, the fruits are used to prepare soups for mothers from the first day of delivery to prevent post partum contraction [4]. It is also widely used in insect pest control.

In traditional herbal medicine, the seeds of *P. guineense* are put into a variety of uses; for instance, in some parts of Nigeria, the seeds are consumed by women after child birth,[5] to enhance uterine contraction for the expulsion of placenta and other remains from the womb [6] as an adjuvant in the treatment of rheumatic pains and as an antiasthmatics [7] and also for the control of weight [8]. The seed and leaf extracts are capable of exhibiting a depolarizing neuromuscular activity in a concentration related manners. The antiparasitic, antimicrobial and antifungal activities of the leaf and seeds of *P. guineense* have also been reported [9].

This present study was undertaken in order to compare the nutrient and antinutrient compositions of the leaves and seeds of *uziza* (*Piper guineense*) plant.

II. Materials And Methods

2.1 Collection of plant materials: The seeds and leaves of *uziza* (*P. guineense*) were purchased from Umuahia Modern Market, Ubani Ibeku Abia State, Nigeria. The leaves and seeds of *uziza* (*P. guineense*) were separated from the stem, sorted to remove debris. The leaves and seeds were washed separately using clean tap water, and oven dried at 65°C for 4hours and milled into powder before analysis.

2.2 Nutrient analysis

The uziza leaves and seeds were analyzed for moisture, ash, crude protein, crude fibre and crude fat contents according to the method of AOAC [10] while carbohydrate content was determined by difference.

2.3 Mineral Analysis : The mineral components were analyzed using an Atomic Absorption Spectrophotometer (AAS, Model SP9, Pychicham UK).

2.4 Vitamin determination: The Spectrophotometric Method described by Pearson [11] was used for the determination Vitamin A, Riboflavin (Vit B2) and Niacin (Vit B3) while the spectrophotometric method described by Okwu [12] was used for the determination of thiamine (vit B1). Vitamin C was determined using the method of the Association of Vitamin Chemist as described by Kirk and Sawyer [13].

2.4 Evaluation of anti-nutritional factors

2.4.1 Determination of Oxalate

The permanganate titration method described by Onwuka [14]. A measured weight of the sample was suspended in 100mls of distilled water and 5mls of 6mHCl was added. The mixture was digested by heating at 100⁰C for an hour. It was cooled and filtered. Then the pH was adjusted by adding 2 drops of methyl red indicator followed by drop wise addition of concentrated aqueous ammonia solution (NH₄OH) until a faint yellow colouration was obtained, at pH between 4-4.5. The mixture was heated to 90⁰C in a water bath, cooled and filtered (to remove ferrous ion precipitates). The filtrate was again heated 90⁰C and 10mls of 5% CaCl₂ solution was added with constant steering. It was allowed to cool and then allowed to stay overnight in the refrigerator (5⁰C) the mixture was centrifuged at 3000xg for 6 minutes. The supernatant was decanted and the precipitate was dissolved in 10mls of 20% H₂SO₄. The solution was made up to 100mls with distilled water and was titrated against 0.05 KM_nO₄ solutions to a faint pink colour which persisted for 30 seconds. The oxalate content was given by the relationship that 1ml of 0.05m

KM_nO₄ solution = 0.00225g oxalate

Calculation of oxalate content

$$\% \text{ oxalate} = \frac{100 \times \text{titre} \times 0.00225}{W}$$

Where W = weight of sample used

2.4.2 Determination of Phytate

The oberlease Spectrophotometer method described by Onwuka [14] was used. A weighed processed sample (2g) was extracted by mixing it with 50mls of 0.2N HCl solution and shaken for 30mins. It was filtered through whatman No. 42 filter paper to obtain the extract. Meanwhile standard phytate solution (sodium phytate), was prepared and diluted to a chosen concentration. An aliquot, 0.5mls of the extract as well as 1ml of the standard phytate solution was put in separate test tubes and treated with 1ml ferric solution (ferric ammonium sulphate). The tubes were corked with stoppers and boiled in a water bath for 30mins. They were cooled in ice for 15mins and then allowed to attain room temperature, then 2.0mls of 2,2 -Bipyrimidine solution was added to each tube, mixed well and their respective absorbance was read in a spectrophotometer at 519 nanometer wavelength. Calculation of phytate content of a sample

$$\% \text{ phytate} = \frac{100 \times \frac{a_u \times C}{a_s} \times \frac{v_t}{v_a}}{W}$$

Where W = weight of sample

Au = absorbance of sample

As = absorbance of std phytate solution

C = concentration of std phytate (mg/ml)

Vt = total extract volume

Va = volume of extract used

2.4.3 Determination of Tannin

Tannin content of the sample was determined by Folin Denis Colometric method Krik and Sawyer [13]. A measured weight of the processed sample (5.0g) was mixed with distilled water in the ratio of 1:10 (w/v). The mixture was shaken for 30 minutes at room temperature filters the obtain the extract

A standard tannic acid solution was prepared, 2ml of the standard solution and equal volume of distilled water was dispersed into a separate 50ml volumetric flask to serve as standard and reagent blank respectively. Then 2mls of each of the sample extract were put in their respective labelled flask. The content of each flask was mixed with 35ml distilled water and 1ml of the Folin Denis reagent was added to each. This was followed by 2.5mls of saturated Na₂CO₃ solution. There after each flask was diluted to the 50ml mark with distilled water and incubated for 90 minutes at room temperature. Their absorbance was measured at 760 min in a Spectrophotometer with the reagent blank at zero

Calculation of tannin content

$$\% \text{ tannin} = \frac{100 \times \frac{au}{x} \times \frac{C}{x}}{w \times \frac{as}{x} \times \frac{Vt}{Va}}$$

W = weight of sample

Au = absorbance of test sample

As = absorbance of test sample

C = concentration of standard tannin solution

Vt = total volume of extract

Va = volume of extract analyzed

2.4.4 Determination of saponin

This was done by the double solvent extraction gravimetric method Harborne [15]. 5.0g of the processed sample was mixed with 50mls of 20% aqueous ethanol solution and incubated for 12h at temperature of 55°C with constant agitation. After that, the mixture was filtered through Whitman No 42 grades of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 minutes and the extracts were weighed together. The combine extract was reduced to about 40 mls by evaporation and then was transferred to a separating funnel and equal volume (40mls) of diety) ether was added to it. After mixing, there were partitioned and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 with drop wise addition of dilute NaOH solution Saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of named butanol. The combine entrant (ppt) was washed with 5% NaCl solution and evaporated to dryness in a previously weighed evaporation dish. The Saponin was dried in the oven at 60°C (to remove any residual solvent) cooled in a dessicator and re-weighed

Calculation of Saponin content

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W}$$

W = weight of sample used

W₁ = weight of empty evaporation dish

W₂ = weight of dish + Saponin extract.

2.5 Amino Acid Profile Analysis

2.5.1: Extraction of Amino acid: Five grammes (5.0g) of the samples were weighed using digital chemical balance (model OHAUS precision plus. The sample were blended in a mortal and pestle and transferred into 250ml beaker, 20ml of 0.2m phosphate buffer solution pit 7.0 added to into the mixture was stirred for about 3 minutes and the resulting mixture was centrifuged at 200rpm for 10 mins. The superntant was shaken three times (3×) with 10ml portion of petroleum ether to remove organic pigments. The top phase was discarded and the aqueous phase which contained protein and amino acid was retained. Protein was precipitated from the aqueous phase by adding 5.0ml of 10% ^W/₀₀₁ trichloroacetic acid (TCA) to 5.0ml extract. The precipitate formed was removed by centrifugation (200g) and the filtrate was used for amino acid profile determinants.

2.5.2: Chromatographic Analysis of Amino Acid (Tcl technique) :The amino acids contents in the extract were separated by thin-layer chromatography method (TCL technique). Aliquots of 50µl of the extract were spolted on Avicel microcrystalline cellulose (whatman analytical plates) thin-layer plates along with 20µl of reference standard mixture. The reference mixture contain lysine, Histidine, phenylalanine, methionine, clycine, cysteine, proline, leucine, Isoleucine, Threonine, tyrosine, valine, Arginine, tryptophan and cilutamic acid (BOH and sigma chemical) each present at a concentration of 0.1% 10/100. One dimensional ascending chromatography was done, the solvent system employed for the separation was n-butanol-glacid acetic acid water at a ratio of 4:1:2 ^v/_v. After 4hrs separation, the chromatograms were air dried and the amino acids were located by spraying with locating reagent of 0.22% of Nintrydrin in ethanol. The sprayed chromatograms were allowed to air dried and later oven dried at 100°C for 5 minutes for the spots identified using the reference standard spotted along side.

2.5.3: Quantitative determination of Amino Acid profiler :The quantitative estimation or determination of the amino acids (profiles) was done through colorimetric method of Rosen [16].

Elution: The estimation of the amino acids by use of the guide strip technique where a developed thin layer chromatography plate was used in locating the portions of amino acids in unsprayed plates. The squares containing amino acids were cut out and eluted with 5ml distilled water at 70°C for 2hrs, the cellulose powder

was removed by centrifugation at 5,000rpm for 5minutes. The supernatant were decanted and kept for the colorimetric analysis of amino acid profiles.

2.5.4: Colorimetric analysis of amino acid profiles :The extracts obtained above from the samples were used for amino acid profiles analysis using the modified ninhydrin colorimetric analysis method of Rosen [16].

To 1ml of the diluted extract of each amino acid was added 0.5ml cyanide acetate buffer (PH 5.4) and 0.5ml 3% w/v ninhydrin in methylcellulose. The mixture was heated in a boiling water bath at 100^oc for 15mins, immediately after the mixture was removed from the water bath, 5.0ml Iso-propyl alcohol water mixture mixed by shalten vigorously cooled to room temperature (25^oc). The amino acid profiles was estimated by determining the optical density at 570nm wavelength using pye-unica uv/visible spectrophotometer (model 5625 uV/vis). The blank was similarly treated same as sample above and use in the control to set the absorbance to zero (distilled water). The amount of each amino acids (profiles) concentrate was calculated from the standard curve of known concentration of Tyrosine (10mg/ml).

III. Statistical Analysis

Statistical analysis of all the data were subjected to analysis of variance (ANOVA) using SPSS version 17.0 for windows, SPSS inc. Means were separated using least significant difference (LSD).

IV. Results And Discussion

4.1 Proximate composition of the leaves and seeds of uziza (*Piper guineense*)

The result of the proximate analysis of the leaves and seeds of uziza are shown in Table 1. There was a significant difference in the protein content in the leaves and seeds of uziza (*P. guineense*) with the leaves having the higher value of 11.32% . Uhegbu et al. [17] reported protein content of 1.17% for *G. latifolium* and 5.57% for *P. guineense* leaves. Contrary findings were made by Asaolu et al. [18] who reported a protein content of 62.71% for scent leaf. Ene-Obong [19] reported that diet is nutritionally satisfactory if it contains high calorie value and a sufficient amount of protein. The result from the proximate composition indicates that the moisture content of the leaves and seeds of uziza are slightly high (11.55% and 9.12%). The moisture content of any food is an index of its water activity [20] and is used as a measure of stability and susceptibility to microbial contamination [21]. Similar results have been recorded by Ajayi et al. [22] and Asaolu et al. [18]. High amount of moisture in crops makes them vulnerable to microbial attack, hence, spoilage [23]. The crude fiber and ash contents of *uziza* leaves were evidently higher than that of *uziza* seeds. Agostoni *et al.* [24] reported that non-starchy crops are the richest sources of dietary fiber. Crude fiber is the part of food that is not digested by human but the normal functioning of the intestinal tract depends upon the presence of adequate fiber. It increases stool bulk and decreases the time that waste materials spend in the gastrointestinal tract. Fiber helps in the maintenance of human health and has been known to reduce cholesterol level of the body [25].

4.2 Mineral composition of the leaves and seeds of uziza (*Piper guineense*)

Table 2 shows the mineral composition of both the leaves and seeds of uziza. The iron content in the leaves of uziza (3.12mg/100g) was significantly higher than that of the seeds. The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide [26]. The consequences of iron deficiency include reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection [27].

The calcium content of the uziza leaves is higher than those of the seeds with values 466.39mg/100g and 317.68mg/100g while the phosphorus values of the leaves and seeds of uziza were recorded as 131.90 mg/100g and 146.85mg/100g. A balance proportion of calcium and phosphorus is needed in the body. Phosphorus is essential component of bone mineral. Deficiency of phosphorus- calcium balance result in osteoporosis, arthritis, pyorrhea, rickets and tooth decay [18]. The mineral content for sodium were low in the leaves and seeds of uziza. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction [28]. The copper content of the leaves and seeds of uziza were in the values 0.06mg/100g and 0.03mg/100g. Ajayi et al. [22] could not detect copper in the mineral analysis of both *B. eurycoma* and *P. guineense* leaves.

4.3 Vitamin composition of the leaves and seeds of uziza (*Piper guineense*)

Table 3 shows the vitamin content of *uziza* (*Piper guineense*) leaves and seeds. The two samples had vitamin A, vitamin B₁, vitamin B₂, vitamin B₃, vitamin C and vitamin E were detected in the two samples. The vitamin content of the spice was quite low. The seed had the highest amount of vitamins. The most abundant vitamin in the leaves and seeds of uziza was observed to be vitamin A (9.31–17.48 IU /100g) followed by vitamin C (1.94–2.51mg/100g).The least of the vitamins was thiamine (vitamin B₁) with values 0.028 mg/100g and 0.082mg/100g for the leaves and seeds of uziza. Vitamin A is important for visual health, immune function

and fetal growth and development. Vitamin A deficiency is a public health problem in many parts of the world, particularly Africa and South-East Asia. The recommended daily intake of vitamin A for children (7 – 10years) is 400 μ g [29]. Higher levels of intake of vitamin C and folic acid are beneficial and protective to health. Some countries have already adopted higher levels of intake of these nutrients as desirable. The recommended daily intake of vitamin C is 30mg [30]. Antioxidants such as ascorbic acid and carotenoids coupled with dietary fibre have been associated with prevention of nutritionally related diseases such as cancers, diabetes mellitus, coronary heart disease and obesity [31].

4.4 Antinutrient composition of the leaves and seeds of uziza (*Piper guineense*)

The result of the antinutrient composition of the leaves and seeds of uziza shown in Table 4 were observed to be significantly low. There were no significant difference in the oxalate content of the two samples. The oxalate content of both the leaves and seeds of uziza had the same value of 0.55mg/100g. Oxalates are known to sequester and precipitate some useful metallic elements, thus making them unavailable for absorption in human system [32].

The tannin and phytate contents of the leaves of uziza were significantly higher than those of the seeds of uziza with values 0.34mg/100g and 0.29mg/100g respectively. Ajayi et al. [22] reported tannin and phytate content of 0.039mg/100g and 0.026mg/100g; 0.296 and 0.183mg/100g for *B. eurycoma* and *P. guineense* leaves. Oboh et al. [33] reported that phytate has the ability to chelate divalent minerals and prevent their absorption. Phytic acid has complicated effect in human system including indigestion of food and flatulence [34].

The saponin content of uziza seeds (3.48mg/100g) were higher than the uziza leaves (3.26mg/100g). According to Harborne [35], saponins have anti-hypercholesterol, anti-inflammatory, cardiac depressant property and also appear to kill or inhibit cancer cells without killing the normal cells in the process [36].

4.5 Amino acid profile of the leaves and seeds of uziza (*Piper guineense*)

Table 5 summarizes the profiles of free amino acid (FAA) in the leaves and seeds of uziza (*Piper guineense*). Glutamic acid appears to be the most concentrated amino acid in the two samples with the seeds of uziza having glutamic acid content value of 113.50 μ g/100g while the leaves had 100.75 μ g/100g. Ajayi et al. [22] reported similar findings in the analysis and characterization of amino acid contents of the seeds of *Brachystegia eurycoma* and *Piper guineense* seeds. They reported glutamic acid concentration of 4.60g/100g and 6.35g/100g for the seeds of *Brachystegia eurycoma* and *Piper guineense*. The result of the amino acid composition in Table 5 also shows aspartic acid as the next high amino acid concentration recorded for the leaves and seeds of uziza (*Piper guineense*) with concentration values of 81.75 μ g/100g and 92.75 μ g/100g. Cysteine and methionine were the lowest concentration of amino acid recorded for the leaves and seeds of uziza (*Piper guineense*). The cysteine content values were 3.50 μ g/100g and 10.00 μ g/100g for the leaves and seeds of uziza (*Piper guineense*) while the methionine content values were 6.00 μ g/100g and 11.00 μ g/100g. It is known that cystine can pair with methionine in improving protein quality and has positive effects on mineral absorption, particularly zinc [37]. Ajayi et al. [22] reported low amino acid content values in histidine, cysteine, methionine, serine and proline in the seeds of *Piper guineense*. Leucine was the most concentrated essential amino acid in the leaves and seeds of uziza (*Piper guineense*) with values 84.75 μ g/100g and 78.87 μ g/100g. 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 insufficient choline may be formed; this may cause accumulation of fat in the liver [38]. Tryptophan was not detected in the leaves and seeds of uziza (*Piper guineense*). Similar findings were made by [22]. The profiles of essential amino acids (EAA₇) and (EAA₁₀) are also presented in Table 5. Interestingly, the leaves and seeds uziza (*Piper guineense*) contained sufficient amounts of all free amino acid (FAA). Based on Lee et al. [39], FAA can be classified in groups of either seven or ten amino acids. The EAA₇ for the leaves of uziza was 240.14 μ g/100g while that of the seeds had value of 320.26 μ g/100g. The EAA₁₀ for the leaves and seeds of uziza showed similar trend with EAA₇; the seed having a high amino acid content of 392.96 μ g/100g and the leaves 293.76 μ g/100g. The seeds of uziza contain higher amount of essential amino acid compared to the leaves.

V. Conclusion

The present study has shown that the leaves and seeds of uziza (*Piper guineense*) contain appreciable amount of valuable nutrients. The leaves and seeds of uziza (*Piper guineense*) can be used favourably having been found to contain important compounds needed for both the food and pharmaceutical industries as well as of great use in traditional medicine too.

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Table 1: Proximate composition of the leaves and seeds of *uziza* (*Piper guineense*) (%)

Parameters	Leaves	Seeds
Moisture content	11.55 ^a ±0.39	9.12 ^b ±0.03
Ash	14.25 ^a ±0.09	6.51 ^b ±0.01
Protein	11.32 ^a ±0.02	7.82 ^b ±0.02
Crude fibre	18.19 ^a ±0.05	2.88 ^b ±0.02
Crude fat	2.45 ^b ±0.02	3.16 ^a ±0.06
Carbohydrate	42.23 ^b ±0.22	70.51 ^b ±0.02

Means on the same row with different superscripts are significantly different (P≤0.05)

Table 2: Mineral composition of the leaves and seeds of *uziza* (*Piper guineense*)

Minerals (mg/100g)	Leaves	Seeds
Zinc	0.39 ^a ±0.01	0.35 ^b ±0.06
Iron	3.12 ^a ±0.01	2.20 ^b ±0.07
Copper	0.08 ^b ±0.00	0.06 ^a ±0.04
Lead	0.06 ^b ±0.02	0.03 ^a ±0.02
Magnesium	138.66 ^b ±1.44	142.35 ^a ±0.15
Calcium	466.39 ^a ±0.04	317.68 ^b ±0.02
Sodium	12.94 ^b ±0.05	20.84 ^a ±0.02
Phosphorus	131.90 ^b ±0.05	146.85 ^a ±1.9
Potassium	116.52 ^b ±0.01	122.07 ^a ±0.06

Means on the same row with different superscripts are significantly different (P≤0.05)

Table 3: Vitamin composition of the leaves and seeds of *uziza* (*Piper guineense*)

Vitamins (mg/100g)	Leaves	Seeds
Vitamin A	9.31 ^b ±0.04	17.48 ^a ±0.10
Vitamin B ₁	0.028 ^b ±0.02	0.82 ^a ±0.04
Vitamin B ₂	0.029 ^b ±0.01	2.06 ^a ±0.04
Vitamin B ₃	0.0714 ^b ±0.06	0.91 ^a ±0.5
Vitamin C	2.51 ^a ±0.04	1.94 ^b ±0.04
Vitamin E	0.060 ^b ±0.04	0.48 ^a ±0.02

Means on the same row with different superscripts are significantly different (P≤0.05)

Table 4: Anti-nutrient composition of the leaves and seeds of *uziza* (*Piper guineense*)

Parameters	Uziza leaves	Uziza seeds
Tannins	0.34 ^a ±0.07	0.12 ^b ±0.01
Phytate	0.29 ^a ±0.01	0.11 ^b ±0.01
Saponin	3.26 ^b ±0.02	3.48 ^a ±0.05
Oxalate	0.55 ^b ±0.01	0.55 ^a ±0.01

Means on the same row with different superscripts are significantly different (P≤0.05)

Table 5: Free amino acid content of the leaves and seeds of *Uziza* (*Piper guineense*) (µg/ml)

Amino acid Profiles	Leaves	Seeds
Lysine	35.50 ^b ±0.00	53.62 ^a ±0.53
Histidine	15.75 ^b ±0.35	23.50 ^a ±0.35
Cysteine	3.50 ^b ±0.00	10.00 ^a ±0.35
Arginine	37.87 ^b ±0.17	49.20 ^a ±0.70
Threonine	20.25 ^b ±0.70	34.12 ^a ±0.53
Proline	27.00 ^b ±0.35	31.25 ^a ±0.35
Valine	31.00 ^b ±0.35	51.75 ^a ±0.35
Glycine	30.00 ^b ±0.35	41.25 ^a ±0.35
Serine	16.25 ^b ±0.35	26.50 ^a ±0.00
Tyrosine	22.75 ^b ±0.35	33.75 ^a ±0.35
Alanine	39.25 ^b ±0.35	43.25 ^a ±0.35
Leucine	84.75 ^a ±0.35	78.87 ^b ±0.35
Iso-leucine	30.34 ^b ±0.17	44.10 ^a ±0.53
Phenylalanine	32.30 ^b ±0.35	46.80 ^a ±0.35
Methionine	6.00 ^b ±0.35	11.00 ^a ±0.00
Glutamic acid	100.75 ^b ±0.35	113.50 ^a ±0.35
Aspartic acid	81.75 ^b ±0.35	92.75 ^a ±0.35
EAA ₇	240.14 ^b ±0.35	320.26 ^a ±0.35
EAA ₁₀	293.76 ^b ±0.35	392.96 ^a ±0.35

Means on the same row with different superscripts are significantly different (P≤0.05)

** *EAA, essential amino acids were calculated according to the method of Lee et al. [39]

** EAA7: Val+Leu+ Ie+Thr+Lys+Phe+Met;

**EAA10:EAA7+His+Arg+ Trp.