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Analysis of Amino Acid Composition in the Seeds of *Calliandra surinamensis* (Pink Powder Puff)

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Abstract
Calliandra surinamensis is a tropical legume shrub that is gradually gaining recognition for its pharmacological and medicinal potential. The amino acid content in the seeds was analyzed and the protein quality was determined. The seeds were found to contain all nine essential amino acids, with varying concentrations. The most abundant essential amino acids were leucine (7.25), methionine (6.04), lysine (4.96), isoleucine (4.90), threonine (2.41), and valine (2.24), while the limiting amino acids where: phenylalanine (0.82) tryptophan (0.27) and histidine (0.02). The non-amino acids found were; Alanine (3.30) Senine (0.84) Proline (0.18), Glutamate (0.05), Aspartate (6.28), Arginine (0.19), Glycine (2.74) and Cysteine (2.73). The protein quality analysis of *Calliandra surinamensis* seed revealed a Protein Efficiency Ratio (PER) value of 47.7, a high protein digestibility-corrected and amino acid score (PDCAAS) of 0.74, and an Essential Amino Acid Index (EAAI) value of 37.89 due to the restricted makeup in the key amino acids, particularly in phenylalanine, tryptophan, and histidine. The meager chemical score (1.05) indicates that histidine is a severe limiting factor in the protein quality. The EAA / NEAA Ratio was 0.94 suggesting a relatively balanced amino acid profile with a slight emphasis on non-essential amino acids The findings imply that the seeds of Calliandra Suriname are a valuable source of plant-based protein and the protein quality of *Calliandra surinamensis* seed could be used as a complementary protein source in combination with other proteins that have a more balanced amino acid profile.

Keywords: *Calliandra surinamensis* seed, Nutritional Potential, Protein quality, Amino Acid Profile, Essential Amino Acids.

Introduction

Legume seeds are well known for their outstanding nutritional value, especially for their well-balanced amino acid profile and high protein content. Many studies have been conducted on the amino acid composition of legume seeds, and the results have shown considerable species differences. Legume seed quality assessment depends largely on the amino acid composition of the seed (Singh et al., 2020). The content of amino acids, or essential amino acids (EAAs), is a critical factor in determining the quality of protein in legume seeds and is most significant for human nourishment (Boye et al., 2010). Animals eat proteins, not amino acids, which are not normally present in food. Organic substances with amino and carboxylic acid functional groups are called amino acids. These are the components of proteins, which are necessary for almost all biological processes within cells (Genchi, 2017). During the digestive process, the protein is broken down into amino acids which can then be oxidized to produce urea and carbon dioxide as a source of energy, or they can be utilized to create new proteins and other biomolecules (Kirschning, 2021). Some amino acids are necessary for various bodily functions and tissue repairs and must be obtained through diet or supplements because the human body cannot produce them (Kumar & Kumar, 2017). They are known as Essential Amino Acids (EAAs). There are nine essential amino acids which are: histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), valine (Val). Others are Non-Essential Amino Acids (NEAAs) because the human body can produce them from other amino acids.

Research has indicated that essential amino acids (EAAs) such as lysine, leucine, threonine, and valine are abundant in legume seeds (Kumar et al., 2020). However, limited methionine, cysteine, and phenylalanine have been reported (Singh et al., 2019). *Calliandra surenamensis* (commonly called Pink Powder Puff), is a leguminous flowering plant that is a South American native widely distributed in Nigeria, East and South Africa. The tree is recognized for its ornamental and horticultural purposes and valued for its year-round floral

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display (Falodun et al., 2010). The plant has also been gaining attention for its pharmacological therapeutic properties (Falodun et al., 2010) and potential medical uses (Irabor et al., 2020). The plant as a legume, is expected to have a unique amino acid profile which would invariably contribute to its nutritional and functional properties (Kumar et al., 2020). Due to the growing demand for plant-based protein sources, *C. Surinamensis* is one of the neglected legume species that researchers are examining. Although the seeds have been utilized historically, little is known about their protein quality and amino acid composition. This study attempts to fill that knowledge gap. Analyzing the amino acid composition of Calliandra surinamensis is essential for evaluating its nutritional value as a food or feed source (World Health Organization, 2007; Ganesan et al., 2018), exploring its potential for pharmaceutical applications (Sarker et al., 2018; Kumar et al., 2020) and help to investigate its functional properties for industrial uses (Singh et al., 2019; Patel et al., 2019). This study is therefore designed to analyze the amino acid composition of the seed of *Calliandra surinamensis* to provide insights into its amino acid profile, and potential applications which in turn can unlock the full potential of this tropical plant species and explore its benefits for various industries

Materials and Methods

Sample Preparation, Identification, and Collection.

The *Calliandra surinamensis* pods containing the dry seeds were collected from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University Benin, Benin-City, and identified by a plant taxonomist in Plant Biology and Biotechnology. The pods were cracked open to release the dry seeds and were pulverized into powder using a mechanical blender. The powder was stored at room temperature until analysis.

Analysis of Amino Acid Profile

The Amino Acid analysis was performed using a gas chromatography (GC) analytical method. A BUCK M910 gas chromatography system with a flame ionization detector and a 15 m x 250 um x 0.15 um RESTEK 15 meter MXT-1 column was used.

The sample, weighing 5 grams, was combined with 10 milliliters of a 1:1 v/v methanol and water mixture, and then heated for 25 minutes at 60 degrees Celsius in a water bath. In this way, the amino acid was converted to volatile derivatives. Filtration was performed after the solution had cooled for fifteen minutes at room temperature. The final volume was adjusted to 15ml. The GC analytical method was then applied using a 3 ml solution with a split-less injection of 2 ml sample and a linear velocity of 30 cms-1. The Amino acid derivatives were separated and detected by the detector. Triplicate determinations were carried out.

Calculation of the Essential Amino Acid Index (EAAI) (using WHO/FAO (2013) reference values.)

AEEI = (∑ Ratios / Total Reference Value) x 100)

Calculation of Chemical Score (CS)

CS = (mg of limiting amino acid per g of protein/mg of same amino acid per g of reference protein) x 100

Calculation of Protein Efficiency Ratio (PER) (using WHO/FAO (2013) reference values.)

PER = (Essential Amino Acids / Total Amino Acids) x 100

Essential Amino Acids (EAAs) = Histidine + Isoleucine + Leucine + Lysine + Methionine + Phenylalanine + Threonine + Tryptophan + Valine.

Calculation of Protein Digestibility-Corrected Amino Acid Score (PDCAAS) (using WHO/FAO (2013) reference values.)

PDCAAS = (Essential Amino Acids / Reference Amino Acid Profile) x Digestibility

Statistical analysis

The amino acid composition data were analyzed using descriptive statistics. Mean and standard deviation (SD) were calculated for each amino acid.

Results

Table 1; Amino Acids Profile of *C. surenamensis* Seeds

Essential Amino Acids Composition (EAAs) of *C. surenamensis* Seeds

Amino Acid	Content (g/mg)
Histidine	0.02 ± 0.01
Isoleucine	4.90 ± 0.08
Leucine	7.25 ± 0.26
Lysine	4.96 ± 0.08
Methionine	6.04 ± 0.15
Phenylalanine	0.82 ± 0.03
Threonine	2.41 ± 0. 12
Tryptophan	0.27 ± 0.03
Valine	2.24 ± 0.10

Non-Essential Amino acids composition (NEAAs) of *C. surinamensis*

Amino Acid	Content (g/mg)
Alanine	3.30 ± 0.05
Arginine	0.19 ± 0.06
Aspartate	6.28 ± 0.21
Cysteine	2.73 ± 0.15
Glutamate	0.05 ± 0.02
Glycine	2.74 ± 0.15
Proline	0.18 ± 0.03
Senine	0.84 ± 0.06
Tyrosine	15.34 ± 0.15

Table 2; **Essential Amino Acid Index (EAAI) (using WHO/FAO (2013)** reference values

Amino Acid	Content (g/mg)	Reference Value g/mg	Score
Histidine	0.02	1.90	0.01
Isoleucine	4.90	2.80	1.75
Leucine	7.25	5.50	1.31
Lysine	4.96	5.10	0.97
Methionine	6.04	2.20	2.74
Phenylalanine	0.82	2.30	0.36
Threonine	2-41	2.50	0.96
Tryptophan	0.27	0.50	0.54
Valine	2.24	2.50	0.90

AEEI = (∑ Ratios / Total Reference Value) x 100

= (9.55 / 25.3) x 100

≈ 37.8%

Chemical Score (CS)

CS = (mg of limiting amino acid per g of protein/mg of same amino acid per g of reference protein**) x 100**

= (0.02 mg/g /1.90 mg/g) x 100

CS = 1.05

Protein Efficiency Ratio (PER)

PER = (Essential Amino Acids / Total Amino Acids) x 100

Essential Amino Acids (EAAs): 28.91

Total Amino Acids: 60.56

PER = (28.91 / 60.56) x 100 ≈ 47.7%

EAA / NEAA Ratio = Total EAA / Total NEAA

Essential Amino Acids (EAAs): 28.91

Non-essential amino acids composition (NEAAs) = 31.65

28.91 / 31.65 = 0.94

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Protein Digestibility-Corrected Amino Acid Score (PDCAAS)
PDCAAS = (9.55 / 23.3) x 0.9 ≈ 0.74

Table 3: Comparison of Amino Acid Composition with other selected Legume Seeds (per 100g serving); Soybeans, Lentils, Chickpeas, and Peas. (USDA, 2019; FAO, 2013).

Amino Acid	Content (g/mg)	Soybeans g/mg	Lentils g/mg	Chickpeas g/mg	Peas g/mg
Histidine	0.02	2.90	2.50	2.60	2.30
Isoleucine	4.90	4.70	3.90	3.90	4.20
Leucine	7.25	7.90	6.70	6.70	7.20
Lysine	4.96	6.40	6.00	5.90	5.60
Methionine	6.04	1.40	1.00	1.30	1.10
Phenylalanine	0.82	4.90	4.30	4.60	4.50
Threonine	2-41	4.10	3.80	4.20	3.90
Tryptophan	0.27	1.30	1.00	1.30	1.20
Valine	2.24	5.10	4.30	4.50	4.80

Discussion

The findings from Table 1; showed that the seed of *C. surinamensis* contains the nine essential amino acids which are; leucine (7.25), methionine (6.04), lysine (4.96), isoleucine (4.90), threonine (2.41) and valine (2.24), and the values of isoleucine, leucine, and methionine are relatively higher than the reference values in Table 2. They are essential because the body is unable to produce them and can only be obtained through diet (Singh et al., 2019). Tyrosine (15.3), though not an essential amino acid since the body can produce it was the most abundant. Other nonessential amino acids that were also present are; alanine (3.3), senine (0.8), proline (0.2), glutamate (0.05), aspartate (6.3), arginine (0.2), glycine (2.7) and cysteine (2.7). The protein indices have limited amino acid content (EAAI; 37.8%) suggesting a potential deficiency in histidine (Tome, 2017), a moderate protein efficiency (PER; 47.7%) indicating decent growth and maintenance support, and a good digestibility and amino acid utilization (PDCAAS; 0.74) ensuring that most nutrients are absorbed. The EAA / NEAA Ratio of 0.94 suggests a relatively balanced amino acid profile with a slight emphasis on non-essential amino acids. In comparison with other selected legumes in Table 3, Leucine (7.2), an essential amino acid whose value ranked second is similar to the value reported for soybean (7.6-8.5), pea (6.7-7.5), lentil (6-3-7.1), chickpea (6.5-7.3) (Joshi, 2020; Kumar, 2020; Sharma, 2020) and a higher methionine content than soybean (1.3-1.5), pea (1.1-1.3), chickpea (1.2-1.5) and lentil (1.0-1.2) (Rathore, 2019; Tiwari, 2019; Sharma, 2020; Upadhyay, 2020). Analysis of the amino acid content of *C. surinamensis* seed revealed a moderate protein quality (Wang et al., 2017). The PER value of 47.7 suggested that the protein is moderately efficient in supporting growth and maintenance (Schaafsma, 2000; Food and Agriculture Organization of the United Nations /WHO, 1991), while the EAAI value of 37.89 indicates relatively low efficiency in providing essential amino acids (Leterme & Muylaert, 2017) by the insufficient amounts of the key amino acids, particularly phenylalanine, tryptophan, and histidine (Schaafsma, 2000). The low chemical score (1.05) value indicates that histidine is a significant limiting factor in the protein quality. However, the PDCAAS of 0.74 indicates a relatively high protein digestibility and amino acid score. The seed is high in leucine, methionine, and lysine and has little isoleucine, threonine, and valine. The relatively low EAAI value indicates a need for complementation with other protein sources like blending *C. surinamensis* seed protein with other protein sources to complement its amino acid profile (Bressani & Elias, 2017).

Conclusion

The thorough analysis of the protein sample's amino acid profile, protein efficiency ratio (PER), and protein digestibility -corrected amino acid score (PDCAAS) provide valuable insights into its nutritional quality. The protein quality of *Calliandra surinamensis* seed was revealed to be moderately low, limited by its amino acid composition. The Protein Efficiency Ratio (PER) value of 47.7 indicates a moderate protein efficiency, suggesting that the protein can support growth and maintenance to some extent and the limited essential amino acid index (EAAI) value highlights a significant limitation in essential amino acid composition in phenylalanine, tryptophan, and most especially histidine with a chemical score (CS) of 1.05. This therefore suggests areas for improvement such that the seed may not be suitable as a sole source of protein, but could be used as a complementary protein source in combination with other proteins that have a more balanced amino acid profile. The EAA / NEAA Ratio of 0.94 suggests a relatively balanced amino acid profile with a slight emphasis on non-essential amino acids. Further research on the in vivo protein digestibility and bioavailability of *C. surinamensis* seed protein is recommended to confirm its nutritional value. The seed has a moderate protein efficiency (PER; 47.7), relatively high protein digestibility-corrected and amino acid score (PDCAAS;

0.74), and limited essential amino acid efficiency (37.89), particularly in phenylalanine, tryptophan, and histidine with a CS of 1.05. The EAA / NEAA Ratio of 0.94 indicates a slight emphasis on non-essential amino acids.

Recommendation

The protein quality of *C. surenamensis* seed was moderately low and may not be suitable as a sole source of protein therefore, blending *C. surenamensis* seed with other protein sources to complement its amino acid profile will be most considerate. Amino acid supplementation in phenylalanine, tryptophan, and histidine is recommended. Also, further research on the bioavailability and in vivo protein digestibility of *C. surenamensis* seed protein is greatly recommended to confirm its nutritional value.

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