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Evaluation of the Crude Protein and Amino Acid Composition of Nigerian *Monodora myristica* (Ehuru)

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Abstract: Protein plays an important role in biochemical, biophysical and physiological processes. The deficiency of proteins leads to weakness, anaemia, protein energy malnutrition (Kwashiorkor and marasmus), delayed wound healing and fracture healing and also decreased resistance to infection. Proteins in the body come from both plant and animal source. Life without protein is not possible and amino acids are the building blocks of protein. The crude protein and amino acid composition of *Monodora myristica* seed was determined using standard analytical techniques with a view to further appraise the nutritive value. The results showed that crude protein content in percentage as 11.34%. The Total Amino Acid (TAA) of *Monodora myristica* seed was 65.60g/100g of crude protein. The Total Essential Amino Acid (TEAA, with Histidine) was calculated to be 47.64% of the crude protein while the Total non Essential Amino Acid (TNEAA) was calculated to be 52.36% of the crude protein. The predicted protein efficiency ratio (P-PER) was calculated to be 2.32. The content of total Essential Amino Acid (EAA) with value 26.85g/100g crude protein is lower than FAO/WHO recommended value of 36.0g/100g crude protein. *Monodora myristica* could be used as good sources of protein supplement in the human diet. *Monodora myristica* has been used as spice and condiment in food and also possess medicinal property.

Key words: *Monodora myristica, Ehuru,* amino acid, protein

INTRODUCTION

Spices are esoteric food adjuncts that have been used as flavouring agents and as preservatives for thousands of years in tropical Africa. They have also been recognized to possess medicinal properties and their use in traditional system of medicines has been on record for a long time. Spices are rich in several forms in human diet, as whole spices, ground spices, or isolates from their extract (Srinivasan *et al*., 2004).

Monodora myristica (Ehuru) belonging to the Anonaceae family is one of the most important trees of the evergreen forest of West Africa. It is most prevalent in the southern region of Nigeria. Almost every part of the tree has economic importance (Okafor, 1987; Okigbo, 1977). However, the most economically important parts are the seeds which are embedded in the white-smelling pulp of the sub-spherical fruit. It is harvested between April and September each year. The seed is obtained by cracking the nuts which is easier done by heating. The seed when ground to powder is a popular condiment used to prepare pepper soup as a stimulant to relieve constipation and to control passive uterine haemorrhage in women immediately after child birth (Okafor, 1957; Udeala *et al*., 1980). It also has diuretic properties and is used for mild fever.

The aim of the study is to determine the crude protein content and amino acid composition of *Monodora myristica* to augment the available information on *Monodora myristica* research.

MATERIALS AND METHODS

Collection and preparation of plant material: Fresh fruits of *Monodora myristica* were collected from local farmers in Owerri, Eastern Nigeria. The plant was authenticated by a taxonomist at the department of Botany, University of Nigeria Nsukka, Nigeria by comparison with voucher specimen deposited at the department. The seeds were separated from the fruits, washed and dried for two weeks at room temperature of 40°C. The dried seeds of *Monodora myristica* were ground into powder using a manual blender and stored in a cool dry container until analysis.

Crude protein determination: Crude protein content was determined by microkjedahl method as described by AOAC (1990). The microkjedahl method involves digestion, distillation and filtration.

Digestion: Small quantity of the *Monodora myristica* seed flour (0.1g) was weighed into a kjedahl flask with 2.0g catalyst (sodium sulphate). This was followed by the addition of 20ml concentrated H_2SO_4. The flask and its content were gently heated. The heating was increased until the content of the flask was completely digested to give a clear solution.

Distillation: The content of the flask was then washed with 200ml distilled water separately into a distillation flask and cooled under ice block. This was followed by

the addition of 100ml of 4% Boric acid poured into each of the flask and 3 drops of screened methyl red then added.

Titration: About 50ml of cooled 40% NaOH was added and the distillate was then titrated against 0.5N Na_2SO_4 solution.

Determination of amino acid profile: The amino acid profile in the sample was determined using methods described by Benitez (1989). The sample was dried to constant weight, defatted and hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential multi-sample Amino acid analyzer (TSM).

Defeating of sample: A 2g of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006). The extraction lasted for 15 hours.

Nitrogen determination: A small amount (200mg) of ground sample was weighed, wrapped in whatman filter paper (No 1) and put in the kjedahl digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjedahl digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml 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:

$$\text{Percentage Nitrogen} = \frac{(a-b)\times 0.01\times 14\times v\times 100}{W\times C}$$

Where:

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

Hydrolysis: A 30-35mg of the defatted *Monodora myristica* seed was weighed into glass ampoule. 7ml 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 example methionine and cysteine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105±5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humans. It should be noted that tryptophan is destroyed by 6N HCl during hydrolysis.

The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles which were kept in the freezer.

Loading of the hydrolysate into TSM analyzer: The amount loaded was between 5 to 10 micro litres. This was dispensed into the cartridge of the analyzer. The TSM analyser is designed to separate and analyze free fatty acidic, neutral and basic amino acids of the hydrosylate. The period of an analysis lasted for 76 minutes.

Method of calculating amino acid values from the chromatogram peaks: the net height of each peak produced by the chart recorder of TSM (each representing an Amino acid) was measured, the half-height of the peak on the chart was found and width of the peak on the half height was accurately measured and recorded. Approximately 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 formula:

$$NE = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}}$$

A constant calculated S was calculated for each amino acid in the standard mixture as:

$$S_{std} = Ne_{std} \text{ x Molecular weight x } \mu MAA_{std}$$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

$$\text{Concentration (g/100g protein)} = NH\times W@NH/2\times S_{std}\times C$$

Where:

$$C = \frac{\text{Dilution}\times 16}{\text{Sample Wt (g)}\times N\%\times 10\times \text{Vol.loaded} + NH\times W\,(nLeu)}$$

Where:
NH = Net height
W = Width at half height
nLeu = Norleucine

Determination of quality of dietary protein and predicted protein efficiency ratio (P-PER): The quality of dietary protein was measured by finding the ratio of available amino acids in the protein concentrate compared with needs expressed as a ratio (Oshodi *et al*., 1998). Amino acid score (AMSS) was then estimated by applying the FAO/WHO (1991) formula:

$$AMSS = \frac{\text{mg of amino acid 1g of test protein}}{\text{mg of amino acid 1g refernce protein}} \times 100$$

The predicted protein efficiency ratio (P-PER) of the spice sample was calculated from their amino acid composition based on the equation developed by Alsmeyer *et al*. (1974) as stated thus: P-PER = -0.468+0.454 (Leu)-0.105 (Tyr).

RESULTS AND DISCUSSION

Table 1 showed the amino acids values of *Monodora myristica* flour (g/100g protein). The major abundant amino acids were aspartic acid, glutamic acid and leucine with values 8.84, 9.52 and 6.69 g/100g crude protein respectively. The Total Amino Acid (TAA) was 65.60g/100g crude protein. This value compare favourably with values obtained for selected spices (pepper, garlic, ginger, onion, curry leaf, tomatoes) ranging from 39.21-78.08 (Aremu *et al*., 2011).

Leucine was the most concentrated essential amino acid in *Monodora myristica* (Table 1). The leucine concentration of *Monodora myristica* (6.69g/100g crude protein) is comparable with values obtained for spices like pepper, ginger, onion and tomatoes with values of 5.08, 5.66, 4.09 and 3.24 g/100g crude protein respectively (Aremu *et al*., 2011) but slightly lower for values obtained for other spices like garlic and curry leaf with values 8.13 and 7.69 g/100g respectively (Aremu *et al*., 2011). This concentration of leucine in *Monodora myristica* is in agreement with the observations made earlier by some researchers (Aremu *et al*., 2006; Olaofe *et al*., 2008; Aremu *et al*., 2010) that leucine is the most concentrated essential amino acid in Nigerian Plant Products. Glutamic acid was the most concentrated amino acid in *Monodora myristica* seed. Tryptophan concentration could not be determined.

The Predicted Protein Efficiency Ratio (P-PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 1991). The P-PER of *Monodora myristica* was 2.32. This value compared favourably with P-PER value of some spices; Pepper: *Capsicum spp*, Onion: *Allium cepa*, Garlic: *Allium sativum*, Ginger: *Zingiber officinale*, Tomato: *Solanum lycopersicum* (Table 4) (Aremu *et al*., 2011). Crude protein content of *Monodora myristica* was found to be

Table 1: Amino acid composition (g/100g crude protein) of *Monodora myristica*

Amino acid	*Monodora myristica*
Lysine (Lys)[a]	3.25
Histidine (His)[a]	2.79
Arginine (Arg)[a]	4.60
Aspartic acid (Asp)	8.84
Threonine (Thr)[a]	2.51
Serine (Ser)	2.19
Glutamic acid (Glu)	9.52
Proline (Pro)	3.02
Glycine (Gly)	3.95
Alanine (Ala)	3.83
Cysteine (Cys)	0.57
Valine (Val)[a]	3.59
Methionine (Met)[a]	0.62
Isoleucine (Ile)[a]	3.22
Leucine (Leu)[a]	6.69
Tyrosine (Tyr)	2.42
Phenylalanine (Phe)[a]	3.98
(P-PER)	2.32
Crude protein	11.34

[a]essential amino acid, P-PER- calculated predicted protein efficiency ratio

Table 2: Classification of amino acid composition (g/100g crude protein) of Monodora myristica

Amino acid	*Monodora myristica*
Total Amino Acid (TAA)	65.60
Total Non-essential Amino Acid (TNEAA)	34.35
% TNEAA	52.36%
Total Essential Amino Acid (TEAA) (with Histidine)	31.25
TEAA (without Histidine)	28.46
% TEAA (with Histidine)	47.64%
% TEAA (without Histidine)	43.38%
Essential Aliphatic Amino Acid (EAAA)	9.91
Essential Aromatic Amino Acid (EArAA)	3.98
Total Neutral Amino Acid (TNAA)	4.70
%TNAA	7.16%
Total Acidic Amino Acid (TAAA)	18.36
%TAAA	27.99%
Total Basic Amino Acid (TBAA)	10.64
%TBAA	16.22%
Total Sulphur Amino Acid (TSAA)	1.19
% of Cysteine in TSAA	47.90%

Table 3: Amino acid score of *Monodora myristica*

EAA	PAAESP[a]	EAAC	AMSS
Ile	4.0	3.22	0.81
Leu	7.0	6.69	0.96
Lys	5.5	3.25	0.59
Met+Cys (TSAA)	3.5	1.19	0.34
Phe+Tyr	6.0	6.40	1.07
Thr	4.0	2.51	0.63
Try	1.0	ND	ND
Val	5.0	3.59	0.72
Total	36.0	26.85	5.12

EAA = Essential amino acid, PAAESP = Provisional amino acid (Egg) scoring pattern, EAAC = Essential amino acid composition, AMSS = Amino acid scores, ND = Not detected

Table 4: Amino acid composition (g/100g crude protein) of selected spices in literature

Amino acid	Pepper	Garlic	Ginger	Onion	Curry leaf	Tomatoes
Lysine (Lys)	2.52	4.48	1.61	2.26	5.32	2.04
Histidine (His)	1.88	2.07	0.53	1.25	2.44	1.69
Arginine (Arg)	3.57	4.59	2.72	2.89	4.94	3.40
Aspartic acid (Asp)	5.70	8.66	3.18	3.37	9.78	4.36
Threonine (Thr)	2.00	3.53	2.30	1.55	3.27	1.89
Serine (Ser)	1.94	2.70	1.11	1.40	2.70	1.78
Glutamic acid (Glu)	6.26	9.33	4.13	6.87	11.12	7.49
Proline (Pro)	2.23	2.55	6.85	2.02	3.08	1.49
Glycine (Gly)	3.16	1.94	1.70	2.04	4.01	2.16
Alanine (Ala)	2.90	4.48	1.08	2.12	4.17	2.17
Cystein (Cys)	0.66	0.79	0.53	0.46	0.99	0.40
Valine (Val)	3.40	3.66	2.35	2.99	5.14	2.59
Methionine (Met)	0.60	0.78	0.47	0.39	1.17	0.31
Isoleucine (Ile)	2.26	2.26	1.00	4.09	7.69	3.24
Leucine (Leu)	5.08	8.13	5.66	4.09	4.30	1.54
Tyrosine (Tyr)	2.20	2.42	1.29	2.04	3.22	1.77
Phenylalanine (Phe)	3.04	3.89	2.70	2.54	4.73	2.28
P-PER	1.84	2.97	1.97	1.15	2.69	0.82
Crude protein	20.11	19.94	11.43	10.36	25.67	19.83

Source: Aremu *et al*. (2011)
(Pepper: *Capsicum spp*, Onion: *Allium cepa*, Garlic: *Allium sativum*, Ginger: *Zingiber officinale*, Tomato: *Solanum lycopersicum*)

11.34% (Table 1). This is comparable with the crude protein content of spices such as Ginger (11.43) and Onion (10.36) but lower than values for Pepper (20.11), Garlic (19.94), Curry leaf (25.57) and Tomatoes (19.83) as reported by Aremu *et al*. (2011). This shows that *Monodora myristica* seeds are low protein containing spices.

The total essential amino acid (with Histidine) of *Monodora myristica* was found to be 31.25 (Table 2). This is comparable with values obtained for spices like Garlic (33.38) and Curry leaf (39.00) by Aremu *et al*. (2011) suggesting that *Monodora myristica* can effectively serve as a food supplement. Essential Aliphatic Amino Acid (EAAA), Ile, Leu and Val content which constitute the hydrophobic regions of proteins was found to be 9.91. This is lower than values for other spices reported by Aremu *et al*. (2011). This means that better emulsification property may not be expected in *Monodora myristica* seed flour. Table 2 also depicts that the percent of Total Acidic Amino Acid (TAAA) of 27.99% for *Monodora myristica* was greater than the percent of Total Basic Amino Acids (TBAA) (16.22%) indicating that the protein is probably acidic in nature. Total Sulphur Amino Acid (TSAA) of *Monodora myristica* was found to be 1.19g/100g crude protein. The TSAA is lower than 5.8g/100g crude protein recommended for infants (FAO/WHO/UNU, 1985). This confirms many reports on spices that they are used as food additive for the purpose of flavour, medicine, colour or as a preservative that kills harmful bacteria or prevent their growth (Eshbaugh, 1975; Augusti, 1996; Grontved and Pittler, 2000).

The content of some essential amino acids was lower than FAO/WHO (1991) recommendations (Table 3). However, *Monodora myristica* was adequate only in Leu and Phe+Tyr. Thus, based on the findings, *Monodora myristica* may be used as a food supplement for any food material that is not adequate in essential amino acid. It has been reported that the essential amino acids most often acting in a limiting capacity are Met (and Cys), Lys and Try (Aremu *et al*., *2006*). The first limiting amino acid in this study was Met+Cys. Tryptophan (Try) could not be determined.

This study showed that *Monodora myristica* contained nutritionally useful quantities of most of the essential amino acids and can serve as food supplement for food materials that are not adequate in essential amino acid based on FAO/WHO provisional pattern.

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