International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 23-05-2022, Accepted: 08-06-2022, Published: 25-06-2022
Volume 7, Issue 3, 2022, Page No. 35-41

Comparative studies on amino acid profile and protein quality of *Chrysobalamus icaco, Afrostyrax lepidophyllus, Afromemum subsericeum* and *Ricinodendron heudilotti* used as soup spice in 'Ofeakwu'

Oganezi, Nuria C*, Osondu, Rejoice O
Lecturer, Department of Food Science and Technology, Abia State University, Uturu, Nigeria

Abstract
The amino acid profile and protein quality of seed spices namely *Chrysobalamus icaco, Afrostyrax lepidophyllus, Afromemum subsericeum* and *Ricinodendron heudilotti* used in the cuisine of ofeakwu were evaluated. The most abundant essential amino acid was leucine and it ranged between 5.75 and 6.80g/100protein. The total amino acid content ranged between 66.29 g/100protein for *Chrysohalamus icaco* and 74.65 g/100protein for *Ricinodendron heudilotti*. *Afrostyrax lepidophyllus* had the highest total sulphur amino acids with a value of 3.30 g/100protein. *A. subsericeum* had the highest predicted protein efficiency ratio while *A. lepidophyllus* had the highest essential amino index with a value of 60.05. *A. lepidophyllus and A. subsericeum* had the highest biological value. All the seed spices were sufficient in phenylalanine+tyrosine. The order of limiting amino acids in the spices was in this order: Lysine>Trptophan>Valine>Threonine>Methionine+Cysteine. Although the proteins of these seed spices can be classified as low biological value, a combination of them as it is used in the cuisine of 'ofeakwu' can result to a mix of protein with improved biological value.

Keywords: Ofeakwu, *Chrysohalamus icaco, Afrostyrax lepidophyllus, Afromemum subsericeum, Ricinodendron heudilotti,* amino acids

Introduction
Spices are finding more important roles in healthcare amidst their main use as organoleptic enhancers in culinary (Ogbunugafor *et al*., 2017) [30]. Many spices used for culinary come as seeds. They can be important source of nutrients such as amino acids, minerals and vitamins beyond playing the main role as flavour agents (Jiang and Wang., 2006) [25]. A number of essential amino acids such as histidine, cysteine, isoleucine, leucine, valine, threonine, phenylalanine plus tyrosine have bioactive activities as well as antioxidative activities such as histidine, methionine plus cysteine (Yoshinori and Mizue.' 1998) [40]. Certain spices contain more proteins than others but not as much you can get from meat, invariably they add some proteins to diet while adding great flavour as well(Hoover, 2020) [22]. Most seeds just like nuts are rich in protein, healthy fats, fibre, minerals such as magnesium, potassium, calcium, plant iron and zinc as well as vitamin B_1, B_2, B_3 and E (DHSGV, 2021) [15].

'Ofeakwu' also known as 'banga soup' is prepared from a decoction of boiled palm fruits to which various ingredients and certain spices are added to as flavour. 'Ofeakwu' as it is called by Ibo speaking tribes or 'banga soup' as it is called by tribes in the Niger Delta/Southern parts of Nigeria is served as sauce or soup. In the Niger Delta areas, it is commonly eaten with various stiff dough recipes such as starch, pounded yam, semolina, garri, fufu while in the South east, 'ofeakwu' is commonly eaten as stew for boiled white rice. Seeds are good sources of protein and a combination of different seeds as they are added for flavour in such dishes as 'ofeakwu' can provide a good supply of proteins. Some of the seed spices added in the preparation of 'ofeakwu' include *Chrysohalamus icaco, Afrostyrax lepidophyllus, Afronemum subsericeum, Ricinodendron heudilotti, Monodora myristica, Xylopia aethiopica* to mention but a few.

Chrysohalamus icaco belongs to the family Chrysobalanaceae (Govaerts, 2020) [21]. It grows as a shrub/bushy tree near the sea and inland throughout tropical Africa, tropical Americas and the Caribbean, Southern Florida and the Bahamas (Govaerts, 2020) [21]. Both the ripe fruit of *C. icaco* and the seed inside the ridged shell are edible. It is commonly known as 'Abageru' in Brazil (Barbosa *et al*., 2013) [6], ' Gbafilo' in the middle belt/South East/Southern region of Nigeria. *Afrostyrax lepidophyllus* commonly known as 'country onion' belongs to the family *Huaceae* (Moukette *et al*., 2015) [28]. *A. lepidophyllus* has a more restricted distribution occurring in the tropical forest of some central and West African countries (Yirankinyuki *et al*., 2017) [39]. It is predominant in Cameroun where the seeds, bark and leaves are important as medicine (Thomas, 2009) [38]. It is used as one of the spices in the preparation of a traditional Cameroonian food 'Nkui' and is envisaged to have a significant effect on the female reproductive physiology (Tchoupang *et al*., 2016) [37]. Its fruits are used as condiment in several African dishes (Moukette *et al*., 2015) [28]. *Aframomium subsericeum* belongs to the family Zingiberaceae (Burkill, 2000) [11]. Just like its close relative *Aframomum melegueta* that has been well studied, it is a creeping

rhizome with brief 2 flowered inflorence at the base of stems and the plant has a smell of citron (Burkill, 2000)[11]. Igbos in South East Nigeria call it 'Ataiko'. *Ricinodenadron heudelotti* belongs to the family Euphorbiaceae (Burkill, 1985)[10]. *R. heudelotti* has been reported to have high protein, suitable to satisfy demand for human populations and animal foods (Coulibaly *et al*., 2018) [14] as well as appreciable quantities of fats. It has local names as 'Akpi' (Cote d'voire), 'Njansang' (Cameroon), 'Okwe'/'Akpi' (South east Nigeria), 'Bomoko' (Central Africa Republic), and 'Betratra' (Madagascar) (Assanvo *et al*., 2015) [5]. Seeds are good sources of proteins (Hornick and Yarnell. 2013) [23]. A mixture of seed proteins utilized in various cuisines can be of good nutritive value to man. This research aimed at evaluating the amino acid content and protein quality of these seed species used in the cuisine of 'ofeakwu'.

Materials and Methods

Chrysobalamus icaco, Afrostyrax lepidophyllus, *Afromemum subsericeum* and *Ricinodendron heudelotti* seeds were purchased from a local market in Aboh Mbaise, Aboh- Mbaise Local Government Area of Imo State, South East Nigeria.

Fig 1: *Ricinodendron heudelotti,*

Fig 2: *Chrysobalamus icaco*

Fig 3: *Afromemum subsericeum*

Fig 4: *Afrostyrax lepidophyllus*

Processing of Samples

Chrysobalamus icaco and *Afrostyrax lepidophylus* seeds were decorticated to remove the seed coat while *Afromemum subsericeum* and *Ricinodendron heudelotti* seeds didn't have any need for decortication. They respective seed spices were washed in clean cold water, air dried, milled into fine powder using a hammer mill, stored in labelled air tight containers. They were kept in a refrigerator prior to use for amino acid analysis.

Amino Acid Analysis

Amino acid profile was done by Ion Exchange chromatography using the methods described by (Benitez, 1989) [8] while tryptophan was determined by the method described by (Robel, 1967)[35] using Technical Sequential Multi sample (TSM) amino acid analyser (Technicon Instruments cooperation, New York, USA). Two grams of each spice sample was hydrolyzed using Sulphuric acid. Subsequently, 10 µl of the hydrolyzed sample was loaded into the cartridge of the TSM amino acid analyzer respectively. The net height of each peak produced on the chart record of the TSM amino acid analyzer (each representing an amino acid) was measured and calculated. Tryptophan was hydrolyzed after de-fatting 2 g of the respective spice sample using chloroform/ methanol mixture (1:1). 10 ml of 4.2M NaOH was added to the defatted sample which destroyed all the other amino acids except tryptophan. Oxidation of tryptophan was inhibited by the addition of drops of thioglucollic acid to the mixture in the glass ampoule. Alkaline pH was maintained using 5 ml acetate buffer (pH8.0). 5 µl of each hydrolyzed spice sample was dispensed into the cartridge of the TSM amino acid analyzer respectively. The net height of the peak produced on the chart record of TSM amino acid analyzer (representing tryptophan) was measured and calculated. Norleucine was used as internal standard. Amino acid values from the chromatogram peaks were calculated whereby half height of each peak on the chart was found and width of the peak on the half height was accurately measured and recorded. Area of each peak was then obtained by multiplying the height by

the width at half height the Norleucine equivalent (NE) for each amino acid in the mixture. Norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

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

A constant S was calculated for each amino acid in the standard mixture:
Where $S_{std} = NE_{std}$ x Molecular weight x μMAA_{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:

Concentration (g/100g protein) = NH x W@NH/2 x S_{std} x C

$$\text{Where } C = \frac{\text{Dilution X 16}}{\text{Sample Wt (g) X N\% X 10 X Vol loaded}} \div \text{NH x W (nleu)}$$

Where:
NH = Net height
W = Width @ half height
nleu = Norleucine

Protein quality indices were determined on the basis of the amino acid content of the respective seed spices.

Determination of protein Quality Indices
Estimation of Amino Acid score and predicted protein Efficiency Ratio (p-PER): Amino acid scores of the respective flour samples were determined based on whole Hen's egg (Paul *et al*., 1976)[18]. Essential amino acids were grouped namely methionine+cysteine and phenylalanine+tyrosine were taken as two distinct units. Amino acid scores (AMSS) were estimated by using the formula stated by (FAO/UN/WHO, 1991)[19] as:

$$AMSS = \frac{\text{Mg of amino acid/g of test protein x 100}}{\text{Mg of amino acid/g of reference protein}} \quad \text{.....1}$$

Determination of Predicted Protein Efficiency Ratio (P-PER): The predicted protein efficiency ratio (P-PER) was calculated from the amino acid composition using the equation by (Alsmeyer *et al*., 1974) [1].

P-PER = - 0.468 + 0.454 (Leu) – 0.105 (Tyr).....2

E**ssential Amino Acid Index (EAAI):** The essential amino acid index was calculated using the equation stated by (Oser, 1959) [31] as:
EAA Index

$$\sqrt[n]{\frac{PhePxTryPxThrePxValPxHisPxIlePxLeuPxLysPxMetP}{PheSxTrySxThreSxValSxHisSxIleSxLeuSxLysSxMetS}} \quad \text{.....3}$$

Where P = food protein, S = standard protein (whole egg), n= number of amino acids (counting pairs such as methionine and cysteine as one).

Determination of Biological value (BV): The biological value (BV) of the respective seed spices were calculated using the equation stated by (Oser, 1959) [31].

Biological Value = 1.09 (EAAI) – 11:7.....4

Results and Discussion
Table1 shows the concentration of various amino acids in the respective seed spices used in the cuisine of 'ofeakwu': Results indicated that the most concentrated amino acid was glutamic acid while the least concentrated amino acid was tryptophan except that of *Ricinodendron heudelotti* which had a value of 1.10g/100g protein. Glutamic acid content of the seed spices ranged between 10.30 and 12.26g/100g protein. Glutamic acid content of *A. lepidophyllus* and *A. subscericeum* were comparable to glutamic acid content of *Monodora myristica* (9.52g/100g protein, another seed spice used in the cuisine of 'ofeakwu' (Ekeanyanwu, 2013)[16]. Glutamic acid is an exciting neurotransmitter for the central nervous system, the brain and the spinal cord (Bouba *et al*., 2016)[9].

The most abundant essential amino acid in these seed spices was leucine and it ranged between 5.75 and 7.21g/100g protein. Leucine is a branched chain amino acid and is reported to be important in glucose homeostasis by promoting glucose uptake and increasing insulin sensitivity in skeletal muscles (Liu *et al*., 2014) [27]. Glycine, isoleucine, leucine and tyrosine content of the seed spices used in the cuisine of "ofeakwu' are sufficient to meet with human nutritional needs based on (FAO/UN/WHO, 1991)[17] reference pattern for amino acids. Arginine from *C. icaco, A. subsericeum* and *R. heudelotti* had values of 5.34, 6.28 and 6.71g/100g protein respectively and can meet the nutritional needs of humans. Arginine rich proteins have been reported to have a variety of activities such as bactericidal, membrane–penetrating, antimicrobial anti-hypertensive, pro-angiogenin (Chandana and Venkatesh, 2016)[12] and it regulates blood pressure (Goke, 2004)[20]. Arginine is essential for children up 5 years old and the elderly-sixty and up (Sowers, 2009)[36]. The presence of these amino acids in substantial quantities that meet with (FAO/UN/WHO,1991)[17] reference pattern and can confer health benefits associated with them.

Table 1: Results on the Amino Acid Content of *Chrysohalamus icaco, Afrostyrax lepidophyllus, Afromemum subsericeum* and *Ricinodendron heudilotti* seeds

Amino Acid (g/100g protein)	*C. icaco*	*A. lepidophyllus*	*A. subsericeum*	*R. heudelotti*	FAO/WHO (1991) Reference Pattern (g/100g protein)
Lysine*	4.30	3.29	3.02	3.34	5.80
Histidine*	1.85	1.72	2.01	2.27	2.50
Arginine	5.34	4.82	6.28	6.71	5.20
Aspartic acid	3.23	8.00	7.62	8.93	7.70
Threonine*	2.97	3.02	3.49	3.19	3.40
Serine	3.62	3.40	4.00	3.51	7.00
Glutamic acid	10.30	9.61	8.78	12.26	14.70
Proline	3.35	3.15	3.55	3.04	10.70
Glycine	4.01	3.66	4.32	3.85	2.20
Alanine	4.17	4.44	3.94	4.02	6.10
Cysteine	1.39	1.75	1.57	1.27	3.00
Valine*	3.54	3.80	3.51	3.01	5.00
Methionine*	1.47	1.55	1.07	1.17	2.50
Isoleucine*	3.21	3.57	4.02	3.27	2.80
Leucine*	5.75	6.30	7.21	6.80	1.10
Tyrosine	3.44	2.93	2.58	3.10	1.10
Phenylalanine*	3.64	3.99	3.63	3.81	6.30
Tryptophan*	0.71	0.66	0.58	1.10	1.10

*Essential amino acids

Table 2 shows results on the different classes of amino acids of these seed spices used in the cuisine of 'ofeakwu'. Amino acids present as well as their concentrations are very much important in defining protein quality (Amaechi *et al*., 2016) [3]. Protein quality is very important when considering the nutritional benefits it can provide (Hoffman and Falvo, 2004) [24]. Total amino acids content ranged between 66.29g/100g protein for *Chrysobalamus icaco* and 74.65g/100g protein for *Ricinodendron heudelotti*. The total amino acids of *A.lepidophyllus, A. subsericeum* and *R. heudelotti* were higher than the total amino acids of *Monodora myristica* (65.60g/100g protein) (Ekeanyanwu, 2013)[16]. *R. heudelotti* had the highest total non-essential amino acids content with a value of 39.98g/100g protein. The total essential amino acids of the spices with and without histidine ranged between 27.44 -28.54g/100g protein and 25.59 - 26.53g/100g protein respectively. Histidine is used by the body to synthesize histamine which is a neurotransmitter needed for immune response as well as sleep-wake cycles (Kubala and Ritcher, 2022) [26] and it is critical for maintaining the myelin sheath which is a protective barrier that surrounds the nerve cells (NCBI, 2022) [29].

Aliphatic amino acids have a large hydrophobic side chain with the branched chain amino acids (BCAAs) namely leucine, isoleucine and valine making up the bulk of it (Amaechi *et al*., 2017) [4]. BCAAs supplementation have been proved to alleviate symptoms of exercise induced muscle damage and facilitate acute performance recovery (Osmond *et al*., 2019) [32]. The concentration of essential aliphatic amino acids was in the order *A. subsericeum >A. lepidophyllus = R. heudelotti > C. icaco*. Total acidic amino acids in these spices were higher than the total basic amino acids. *R. heudelotti* had the highest concentration of both acidic and basic and amino acids. *A. lepidophyllus* had the highest concentration of total sulphur amino acids with a value of 3.30g/100g protein while *R. heudelotti* had the highest concentration of aromatic amino acids with a value of 8.01g/100g protein. The total sulphur amino acids of these seeds spices were higher than the total sulphur amino acids of *M. myristica* (1.19g/100g protein) (Ekeanyanwu, 2013) [16]. Sulphur amino acids provide sulphur for sulfating reactions in the body with cysteine having sparing effects for methionine (Fukagawa *et al*., 1998) [19]. Sulphur amino acids are involved in the synthesis of intracellular antioxidants such as glutathione and N-acetyl cysteine, they may provide a chelating site for heavy metals (Colovic *et al*., 2018) [13].Dietary aromatic amino

acids are required to supply phenylalanine and tyrosine needed for protein synthesis with tyrosine sparing phenylalanine during metabolism (Pencharz and Hsu, 2007) [34].

The predicted-protein efficiency (P-PER) ratio of these seeds spices ranged between 1.78 for *C. icaco* and 2.53 for *A. subsericeum*. Leucine content above 5.0g/100g protein often results to appreciable P-PER (Amaechi *et al*., 2015) [2]. A protein efficiency ratio below 1.5 depicts a protein of poor quality (Freidman, 1996) [18]. It entails that the proteins in these seed spices used in the cuisine of 'ofeakwu' are not very poor quality proteins. Essential amino acid index (EAAI) is an index of protein quality. The EAAI of these seed spices ranged between 51.85 for *R. heudelotti* and 60.05 for *A. lepidophyllus*. The EAAI was in this order: *A. lepidophyllus* > *A. subsericeum* > C. *icaco*> *R. heudelotti*. The biological value of a protein describes the amount of amino acids in a protein that is utilized by the body for protein synthesis. The BV of these seed spices ranged between 44.82 and 53.76. *R. heudelotti* had the lowest score for biological value. This implies that the proteins of *C. icaco*, *A. lepidophyllus* and *A. subsericeum* will be utilized better by the body than proteins from *R. heudelotti*. However, all the proteins of these seed spices are of low biological value.

Table 2: Evaluation of Amino Acid Classes, predicted Protein Efficiency Ratio, Essential Amino acid index and Biological value of *Chrysobalamus icaco, Afrostyrax lepidophyllus, Afromenum subsericeum, Ricidendron heudeiotti* used as spices in 'ofeakwu'.

Amino Acid Description	*C.icaco*	*A. lepidophyllus*	*A. subsericeum*	*R. heudeiotti*
Total Amino Acids (g/100gprotein)	66.29	69.66	71.18	74.65
Total Non Essential Amino Acids (TNEAA)	33.51	36.94	36.36	39.98
% TNEAA	50.55	57.34	51.08	53.56
Total Essential Amino Acids (TEAA) with Histidine	27.44	27.90	28.54	27.96
Without Histidine % TEAA	25.59	26.18	26.53	25.69
With Histidine	41.39	40.05	40.10	37.46
Without Histidine	38.60	37.58	37.27	34.41
Essential Aliphatic Amino Acid (EAAA)	15.47	16.69	18.23	16.27
% EAAA	23.34	23.96	25.61	21.80
Total neutral Amino Acids (TNAA)	41.27	42.22	43.47	41.14
% TNAA	62.26	60.61	61.07	55.11
Total acidic Amino Acids (TAAA)	13.53	17.61	16.40	21.19
% TAAA	20.41	25.28	23.04	28.39
Total basic Amino acids (TBAA)	11.49	9.83	11.31	12.32
% TBAA	17.33	14.11	15.89	16.50
Total Sulphur Amino Acids (TSAA)	2.86	3.30	2.67	2.44
% Cysteine in TSAA	48.60	53.03	58.80	52.05
Total Aromatic Amino Acids (TArAA)	7.79	7.58	6.79	8.01
% TArAA	11.75	10.88	9.54	10.73
Predicted Protein Efficiency Ratio (P-PER)	1.78	2.04	2.53	2.24
Essential Amino Acid Index (EAAI)	59.34	60.05	59.67	51.85
Biological Value (BV)	52.98	53.76	53.34	44.82

The essential amino acids scores (EAAS) based on provisional amino acids scoring pattern stated by (Belschant *et al*., 1975) [7] is shown in Table 3. All the seed spices used in the cuisine of 'ofeakwu' were all sufficient for phenylalanine+tyrosine. A. *subsericeum* was sufficient for isoleucine and leucine while *R. heudelotti* was sufficient for tryptophan. The limiting amino acids in these seed spices was in this order: Lysine> Tryptophan>Valine>Theronine> Methionine + cysteine. There were variations in limiting amino acids in the various spices, but a combination of these seed spices in 'ofeakwu' can result to improved essential amino index and biological value.

Table 3: Essential Amino Acid Scores of *Chrysobalamus icaco, Afrostyrax lepidophyllus, Afromemum subsericeum and Ricinodendron heudelotti* used as spices in 'ofeakwu'

EAA	PAAESP[a] g/100g Protein)	*C. icaco*		*A. Lepidophyllus*		*A. Subsericeum*		*R. heudelotti*	
		EAAC	AMSS	EAAC	AMSS	EAAC	AMSS	EAAC	AMSS
Ile	4.0	3.21	0.80	3.57	0.89	4.02	1.01	3.27	0.82
Leu	7.0	5.75	0.82	6.30	0.90	7.12	1.03	6.80	0.97
Lys	5.5	4.30	0.78	3.29	0.60	3.02	0.55	3.34	0.61
Met + Cys (TSSA)	3.5	2.86	0.82	3.30	0.94	2.64	0.75	2.44	0.70
Phe + Tyr	6.0	7.08	1.18	6.92	1.15	6.21	1.04	6.91	1.15
Thr	4.0	2.97	0.74	3.02	0.76	3.49	0.89	3.19	0.80
Try	1.0	0.71	0.71	0.66	0.66	0.58	0.58	1.10	1.10

Val	5.0	3.54	0.71	3.80	0.76	3.51	0.70	3.01	0.60
Total	36	30.42	6.56	30.86	6.66	30.68	6.53	30.06	6.75

EAA = Essential Amino Acid, PAAESP[a(Belschant *et al.*,1975)] = Provisional Amino Acid (Egg) Scoring Protein, EAAC = Essential Amino Acid Concentration, AAS = Amino Acid Score.

Conclusion

The studies on amino acids content and protein quality of these seed spices used in the cuisine of 'ofeakwu' revealed that they had appreciable quantities of some amino acids which can meet the nutritional needs of man. Some of the seed spices have good quantities of the various classes of amino acids. Results on their protein quality indicated that these seed spices are of low biological value. However, a combination of them as it is used in the cuisine of 'ofeakwu' may result to a mix of protein with improved biological value.

Competing interests: The authors declare that they have no competing interests.

Funding: No funding was received.

Authors' contributions: ONC devised the main conceptual ideas, proof project outline and initiated, wrote and revised the paper with input from all authors. ORO worked out the technical details and performed information collection. The author(s) read and approved the final manuscript.

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