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Amino Acid Profile of Conophor Nut as Affected by Fermentation and Heat Treatment

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Abstract: ***Conophor plant (Tetracarpidium conophorum) is a tropical climbing shrub that belongs to the family Euphorbiaceae. It is one of the lesser known and underutilized oilseeds. Mature conophor nuts were processed in three ways namely cooking for 2hours, roasting in hot sand at 130 °C for 1 hour and natural open air fermentation for three days. Both raw and processed samples were dried, and milled into flour for the determination of amino acid composition. The most abundant amino acids in both the raw and processed conophor samples were glutamate (14.56 – 16.10 g/100g protein) and aspartate (7.07 – 10.74 g/100g protein). Out of the essential amino acids, Isoleucine concentration was significantly (p<0.05) reduced by roasting and fermentation while valine level was reduced by roasting. The total amino acids (TAA) in all the samples were in the range of 745.1 – 798.2 mg/g protein. The total essential amino acids (TEAA) content ranged between 281.5 – 327.7 mg/g protein. From the calculated amino acid scores, lysine was found to be the limiting amino acid in both the raw and the processed samples. Arising from the reduction in TEAA in the raw conophor sample from 41.5 % to 37.5 % due to roasting, it may not be a desirable method of processing of conophor nut. Boiling and fermentation are however recommended.***

Keywords: Amino acid, conophor nut, fermentation, roasting, boiling

1. Introduction

In developing countries, the high cost of animal proteins has made the search for cheap and abundant sources of proteins with desirable functional and nutritional properties highly imperative. In recent years, research attention has focused majorly on vegetable proteins as sources of low- cost proteins to supplement human diets. Due to increasing market demands on protein ingredients, underutilized oilseeds are now receiving considerable attention. Conophor nut (*Tetracarpidium conophorum*) is one of the neglected oil seeds in Nigeria but with great potential for increased utilization. It is a tropical climbing shrub and belongs to the family Euphorbiaceae. The fruit is a four winged and ribbed capsule, containing seeds with thin brown shell and yellowish kernel (Adebisoye, 1991). Its cultivation is mainly for the nuts which are usually eaten traditionally as snack in the boiled form. The nuts have been reported to have high nutrient content particularly protein and fat (Oke and Fafunso, 1975; Ogunsua and Adebona, 1983). The amino acid composition of the raw nut have been investigated (Ogunsua,1988, Asaolu, 2009). However, there is paucity of information on how processing could affect the amino acid profile hence the need for this study.

2. Materials and Methods

Collection and Preparation of Samples

Matured freshly harvested conophur nuts were purchased from Iruekpen market in Esan West Local Government Area, Edo State, Nigeria. Identification was carried out by an Agronomist and Botanist from the Department of Crop Science and Botany respectively, Ambrose Alli University, Ekpoma, Nigeria

Processing of the Seeds

The shelled nuts were removed from their pods and divided into four portions. One portion, in the raw state, was deshelled and cut into tiny pieces. The second portion was deshelled, cut into pieces and mixed with distilled water (1:2 w/v). It was allowed to stand for three days at room temperature (25 °C) for natural fermentation to take place. The third portion was boiled in water at 100°C for 2 hours before deshelling and cutting into pieces. The fourth portion was roasted in hot sand (while stirring continuosly) at 130 °C for 1 hour before deshelling and cutting into pieces. All the samples were dried in a hot air oven at 60°C for 24 hours before milling into flour. They were then packaged and kept in a refrigerator at 4°C for use.

Determination of Crude Protein

Protein (N x 6.25) was determined by the Kjedahl method as described by AOAC (2000).

Amino ACID DETERMINATION.

The amino acid profile in the differently processed conophor nut samples were determined using the ion exchange chromatographic method described by Spackman *et al* (1958). The samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential multi-sample amino acid analyzer (TSM-1, model DNA 0209 Technicon Instruments Corporation, New York).

Defatting

About 2.0g of each sample was weighed into the extraction thimble and the fat was extracted with chloroform/methanol mixture using Soxhlet extraction apparatus (AOAC, 2000). The extraction lasted for 10 hours.

Hydrolysis of Sample

Between 30 - 35mg of each defatted sample was weighed into the glass ampoule. 7ml of 6M HCI was added and oxygen was expelled by passing nitrogen gas into the ampoule. The glass ampoule was then sealed with bunsen flame and put in an oven preset at 105^0C for 22 hours. The ampoule was allowed to cool before breaking open at the tip and the content was filtered to remove the humins. The filterate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 4.5) and stored in plastic specimen bottles which were kept in the deep freezer.

Loading of the Hydrolysate into the TSM Analyzer

Between 5- 10 microlitre of each hydrolysate was dispensed into the cartridge of the analyzer. The TSM analyzer separates and analyzes free acidic, neutral and basic acids of the hydrolysate. The period of each analysis lasted for 76 minutes. The net height of each peak produced by the chart record of the TSM (each representing an amino acid) was measured and calculated.

Statistical analysis

All analyses were done in triplicate and data expressed as Mean ± SD. Data were subjected to the principle of single factor analysis of variance (ANOVA) and the differences between means at 5% was determined by the Turkey Krammer's test using a software, Graphpad instat 3.1 (Graphpad Software Inc., USA).

3. Results

The individual amino acids present in the conophor nut samples are shown in Table 1. The most abundant amino acids in both the raw and processed conophor samples were glutamate (14.56 – 16.10 g/100g protein) and aspartate (7.07 – 10.74 g/100g protein). Out of the essential amino acids, Isoleucine concentration was significantly ($p<0.05$) reduced by roasting and fermentation while valine level was reduced by roasting. The total amino acids (TAA) in all the samples were in the range of 745.1 – 798.2 mg/g crude protein as shown in Table 2. The total essential amino acids (TEAA with histidine) content ranged between 281.5 – 327.7 mg/g which accounted for 37.5 – 43.0%. The percentage of TEAA (with histidine) in the raw conophor sample was 41.5 %. This was reduced to 37.5 % due to roasting. From the calculated amino acid scores, lysine was found to be the limiting amino acid in both the raw and the processed samples (Table 3).

Table 1: Amino acid composition of raw and processed conophor nuts (g/100g protein)

Amino acid	Raw	Cooked	Roasted	Fermented
Lysine	3.69 ± 0.50	3.89 ± 0.63	3.75 ± 0.40	3.63 ± 0.59
Histidine	2.66 ± 0.47	3.07 ± 0.05	2.65 ± 0.05	2.65 ± 0.88
Arginine	5.97 ± 0.22	5.21 ± 0.13	5.60 ± 0.79	6.13 ± 0.48
Aspartate (Asn)	9.37 ± 0.18^{ab}	7.07 ± 0.28^{b}	8.97 ± 0.06^{ab}	10.74 ± 0.26^{a}
Threonine	3.62 ± 0.14	2.70 ± 0.46	2.92 ± 0.09	3.94 ± 0.14
Serine	3.12 ± 0.29	3.61 ± 0.58	3.08 ± 0.08	2.59 ± 0.16
Glutamate (Gln)	15.09 ± 1.29	14.56 ± 1.50	16.10 ± 1.12	15.43 ± 1.83
Proline	3.80 ± 0.55	3.70 ± 0.46	3.02 ± 0.52	2.98 ± 0.46
Glycine	4.62 ± 0.64	4.71 ± 0.85	5.19 ± 0.17	4.71 ± 0.61
Alanine	4.18 ± 0.17^{ab}	3.62 ± 0.54^{b}	4.94 ± 0.39^{a}	4.60 ± 0.46^{a}
Cysteine	1.32 ± 0.41	1.39 ± 0.48	1.20 ± 0.13	1.20 ± 0.32
Valine	4.08 ± 0.17^{a}	4.02 ± 0.01^{a}	3.20 ± 0.18^{b}	3.89 ± 0.34^{a}
Methionine	1.53 ± 0.25	1.10 ± 0.16	1.50 ± 0.14	1.62 ± 0.34
Isoleucine	3.35 ± 0.23^{a}	3.31 ± 0.45^{a}	2.55 ± 0.16^{b}	2.71 ± 0.26^{b}
Leucine	6.74 ± 0.27	6.95 ± 0.54	5.18 ± 0.34	6.22 ± 0.56
Tyrosine	2.57 ± 0.21	2.41 ± 0.15	2.23 ± 0.25	2.54 ± 0.31
Phenylalanine	3.21 ± 0.19	3.19 ± 0.22	2.97 ± 0.68	3.04 ± 0.22

Values are expressed as Mean ± SD of triplicate determinations.
Means on the same row with different superscripts are significantly ($p<0.05$) different.

Table 2: Levels of total, essential and nonessential amino acids in the raw and differently processed conophor nut

Amino acid	Raw	Cooked	Roasted	Fermented
TAA (mg/g protein)	789.2	745.1	750.5	798.2
TNEAA (mg/g protein)	461.5	424.8	469.0	471.8
TEAA (+ histidine) (mg/g protein)	327.7	320.3	281.5	326.4
TEAA (- histidine) (mg/g protein)	303.1	289.6	255.0	299.9
% TNEAA (%)	58.5	57.0	62.5	59.1
% TEAA (+ histidine) (%)	41.5	43.0	37.5	40.9
% TEAA (- histidine) (%)	38.4	38.9	34.0	37.8

TAA= Total amino acids, TNEAA= Total non essential amino acids, TEAA= Total essential amino acids

Table 3: Amino acid scoring pattern and amino acid scores of the different samples of conophor nut

Essential Amino acid	*Requirement pattern (mg/g protein)*	*Score*			
	2-5 yr old	*Raw*	*Cooked*	*Roasted*	*Fermented*
Isoleucine	28	1.19	1.18	0.91	0.97
Leucine	66	1.02	1.05	1.09	1.09
Lysine	58	0.64	0.67	0.65	0.63
Met + Cys	25	1.14	0.97	1.08	1.13
Phe +Tyr	63	1.04	1.06	1.03	1.11
Threonine	34	1.06	0.79	0.86	1.16
Tryptophan	11	-	-	-	-
Valine	35	1.17	1.15	0.91	1.11

Amino acid scores based on FAO/WHO/UNU (1985) requirement pattern for 2-5 years old children.

4. Discussion

The most abundant amino acids in both the raw and processed conophor samples were glutamate and aspartate. This is similar to what was reported for some oilseeds (melon seed, pumpkin seed and gourd seed) by Olaofe *et al (*1994). Asaolu (2009) however found glutamate and arginine as the most abundant amino acids in conophor nut. By and large, the individual amino acids were largely unaffected by the processing methods used except for valine and isoleucine. The former was significantly ($p<0.05$) reduced by roasting while the latter was significantly ($p<0.05$) reduced by both roasting and fermentation.

Conophor nut has good content of both essential and non essential amino acids. The total amino acids (TAA) in all the samples were in the range of 745.1 – 798.2 mg/g protein. This agrees with the 573mg/g protein reported by Asaolu (2009). The total essential amino acids (TEAA with histidine) content ranged between 281.5 – 327.7 mg/g protein which accounted for 37.5 – 43.0%. The percentage of TEAA in the raw conophor sample was reduced from 41.5 % to 37.5 % as a result of roasting. This may be due to certain changes during the roasting process such as Maillard reaction. As is evident here, although the individual amino acid content did not change much, cumulatively, there were significant changes in the total essential amino acids.

Using the scoring pattern proposed by FAO/WHO, 1985 and the data obtained from this study, the amino acid scores were calculated (Table 3). Some amino acid scores were close to unity (some even higher) which is an indication of the high quality of protein in African walnut. The limiting amino acid in the raw and differently processed conophor samples was found to be lysine. This is at variance with Ogunsua (1988) and Asaolu (2009) who reported methionine and leucine respectively as the limiting amino acid.

Conclusively, when the limiting amino acid is corrected for, conophor nut could still be used to fortify maize food products which are widely used as weaning foods for children in most African countries. In doing that however, roasting may not be a desirable method of processing of conophor nut. Boiling and fermentation are however recommended.

References

[1] Adebisoye, H.O. (1991). The effect of processing and storage on the chemical and sensory quality of conophor nut. *Nigerian Food Journal* **9**: 33 – 38.
[2] AOAC (2000). Official Methods of Analysis,17th edition. Association of Official Analytical Chemists, Washington DC.
[3] Asaolu, S.S. (2009). Amino acid composition of *Tetracarpidium conophorum. Oriental Journal of Chemistry*. 25(1): 63 – 66.
[4] FAO/WHO/UNU (1985). Expert consultation. Energy and protein requirements. Techical Report Series 724. World Health Organisation, Geneva.
[5] Ogunsua, A. O. (1988). Amino acid determination in conophor nut by gas-liquid
[6] chromatography. *Food Chemistry*. **28:** 287-298.
[7] Ogunsua, A.O. and Adebona, M.B. (1983). Chemical composition of *Tetracarpidium conophorum*. *Food Chemistry*. **10**: 173-177.
[8] Oke, O.L. and Fafunso, M.A. (1975). Lesser known oil seeds; the nutritive value of conophor seeds *in vitro. Nutrition. Reports. International*. **12**: 41 – 49.
[9] Olaofe, O., Adeyemi, F.O. and Adediran, G.O.(1994). Amino acid and mineral compositions and functional properties of some oilseeds. *Journal of the Agriculture of Food andChemistry*, **42**: 878-881.
[10] Spackman, D.H., Stein, E.H. and Moore, S. (1958). Automatic recording apparatus for use in the chromatography of amino acids. *Analytical Chemistry* **30**: 1191.