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Evaluation of Amino and Fatty Acids Profiles and Sterols of Raw and Processed Conophor Nut (*Tetracarpidium Conophorum*)

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Abstract

Conophor nut oil is reported to be rich in sn-3 fatty acid (linolenic acid) with a protein- rich defatted residue. This study investigated the effect of toasting and cooking on the amino acid profile, the fatty acid profile and the content of sterols of conophor nut (*Tetracarpidium conophorum*). Freshly harvested conophor nuts were processed by cooking (at 100 ^{o}C for 90 min) and toasting (at 145 ^{o}C for 50 min). Raw nuts served as the control. The raw and cooked seeds were dried (60 ^{o}C for 8 h) and all samples were pulverized; oils were also obtained from the samples. Oils extracted from the seed flours were analyzed for physicochemical properties, amino and fatty acids profiles, and sterol composition. Glutamic acid was the most abundant ranging from 2.80 for raw to 3.31 g/100 g crude protein for processed flour. However, the highest value (3.31 g/100 g crude protein) was recorded in the cooked sample. Glutamic and aspartic acids in the samples made up to 6.60-6.77 g/100 g crude protein on an average basis with a percentage of 22.76-26.49. Leucine was the most concentrated essential amino acid with values of 1.93 g/100g in the raw and 1.88 g/100 g and 1.65 g/100g in the cooked and toasted flour samples respectively. The predominant fatty acid group in the oils was the polyunsaturated fatty acids (PUFAs) with values ranging from 77.11 to 79.65 %. Alpha-linolenic acid was found to be the most abundant fatty acid in the oils (61.96-62.64 %) followed by linoleic acid (14.37-16.12 %) and oleic (13.69-16.00 %). Other fatty acids occurred in trace amounts. Campesterol and stigmasterol were the two most abundant of the sterols identified. Cholesterol was not detected in the oil obtained from the toasted conophor nut but its precursor, desmosterol was present.

Keywords: conophor nut; amino acids; fatty acids; sterols; seed oil

Introduction:

The conophor plant (*Tetracarpidium conophorum* (Müll. Arg.) Hutch. And Dalz. (*Euphorbiaceae*), commonly called the African walnut is a perennial climbing shrub, open-branched and mainly tropical (Enujiugha, 2008). The nut is known as 'ukpa' in the Igbo and 'awusa' or 'asala' in the Yoruba speaking tribes of Nigeria. In Cameroon, it is known as 'kaso' or 'ngak' (Ajaiyeoba and Fadare, 2006). The plant is cultivated principally for the nuts which are cooked or toasted and consumed as snacks or along with boiled corn (Enujiugha, 2003). The freshly harvested mature fruits are yellowish green in colour and turn dark brown when left to age. The nuts are encased in carps which contain four (4), three (3), or two (2) nuts per pod. The seed is made up of two cotyledons enclosed in a hard brown shell-like case within the pods (Nkwonta *et al*.,2010). The leaves and young shoots of the plant are known to be edible (FAO, 2006). However, the nuts attract the most attention because of the high nutrient contents (Enujiugha and Ayodele-Oni, 2003).

A bitter after taste is usually observed upon drinking water immediately after eating conophor nut and this could be attributed to the presence of alkaloids and other antinutritional factors (Enujiugha, 2003). Significant concentrations of oxalates, phytates and tannins have been reported in raw conophor nut (Enujiugha and Ayodele – Oni, 2003), but the effect of processing on these factors has not been investigated. Sato *et al*., (1994) isolated isolectins from the nut cotyledons and evaluated the carbohydrate-binding specificity. Olabinri *et al*., (2010) reported on the chelating ability of *Tetracarpidium conophorum* nut *in vitro* and stated that the extract may be explored in the industrial production of iron chelators due to its high chelating ability *in vitro* at low doses, which would be of clinical relevance in the treatment of iron-overload disorders such as thalassemia (a group of genetically inherited blood disorders characterized by defective globin chain of hemoglobin and iron overload).

Oladiji *et al*., (2010) reported that *Tetracarpidium conophorum* oil did not adversely affect growth performance and the feeding appetite of experimental rats. Ndie *et al*., (2010) observed that defatted flour derived from conophor nut with high protein content, high water absorption capacity, high solubility and good pasting characteristics could be used as composite flour in the preparation of bread and confectionaries.

Materials and Methods

Sample Collection

One hundred kilograms (100 kg) of nuts were extracted from the *T. conophorum* fruits and transported from the plantation to laboratory within few hours of harvest. The fresh *T. conophorum* nuts were sorted to remove defectives and later washed using potable water. They were then divided into three potions (Raw, Cooked and Toasted). Raw was shelled and the seeds obtained were chopped and dried at 60 ^{o}C to a constant weight in a hot air oven (model HS60 manufacturer) for 8-10 h. A portion was cooked (at 100 ^{o}C for 90 min) and another toasted (at 145 ^{o}C for 50 min) respectively. The kernels obtained from cooked nuts were dried to a constant weight as for raw. The samples were dry-milled separately using a local attrition mill and the flour obtained in each case was packed in cellophane bags and kept frozen at -4 ^{o}C prior to further analysis.

Extraction of Conophor Oil

Samples of oils and defatted samples of the seed flours were obtained by continuous solvent extraction method using n-hexane for 8 h. Defatted meal was later dried at 60 ^{o}C in a hot air oven (model HS60) to remove residual solvent and later pulverized into powder, sieved and packaged in air tight container for further analysis. The oil extracted was dried to remove residual n-hexane and later stored in air-tight and amber containers and kept in the freezer for further analysis.

Amino Acid Analysis of Raw and Processed Conophor Seeds

Amino Acid profiles of the flour obtained from both raw and processed conophor seeds were determined using Amino Acid Analyzer (S4300, Amino Acid Analyzer, Sykam, Germany).

Procedure: The samples were hydrolysed for 24 h with 6 M HCl according to the method described by Bidlingmeyer, (1984). The cysteine and methionine contents were determined after performic acid oxidation as described by Gehrke, (1985). Typtophan content was determined after alkaline hydrolysis using the method described by Landry and Delhaye, (1992).

Fatty Acid Analysis of Conophor Oil

The fatty acid profile of the oils from raw and processed conophor seeds were determined using Gas Chromatography (Varian 450 GC, The Netherlands).

Methylation: About 0.11 g of the oil was weighed into a 5 ml vial and 1 ml of hexane was added and mixed thoroughly. A portion of the diluted oil (0.1 ml) was pipetted into an 8 ml (13 x 100 mm) screw top tube and the solvent was then evaporated under nitrogen in a warm water bath at 30-35 ^{o}C. Then 1 ml of toluene was added to the tube and vortexed for 5-10 s after which 1.2 ml of methanolic HCl was added. The tube was capped tightly with Teflon lined lids and vortexed for 5-10 s. The tube was removed from oven and allowed to cool to room temperature. Then 1 ml of distilled water was added and also 1 ml of hexane. The tube was capped and vortexed for 20 s. The aliquot was centrifuged at 2000 rpm for 3-4 min. The upper layer was transferred to a clean 8 ml tube and 2 ml of water was added to the hexane layer. The tube was capped and vortexed for 20 s. The aliquot was centrifuged for 3-4 min. Part of the hexane layer was transferred to a GC vial (approximately ½ full); the vial was capped.

GLC analysis: A Varian GC 450 gas chromatograph equipped with a flame ionization detector and an injector with a split/split less device for capillary column was used. The chromatographic column was of a chemically bonded fused silica DB225MS capillary column (30 m x 0.25 mm I.D) (Agilent J & W) and hydrogen was used as the carrier gas. The GLC operating conditions were as follows: injector and detector temperatures were 280 ^{o}C and 290 ^{o}C, respectively. After injection, oven temperature was kept at 70 ^{o}C for 2 min and then programmed at a rate of 30 ^{o}C/min to a temperature of 180 ^{o}C and held for 1 min followed by 10 ^{o}C/min to 200 ^{o}C and held for 2 min, followed by 200 ^{o}C/min to 220 ^{o}C and held for 10 min and finally 220 ^{o}C/min to 240 ^{o}C and held for 5 min.

Sterol Composition of Conophor Oils

The sterol composition of the oils from the raw and processed conophor seeds was determined using Gas Chromatography (Varian 450 GC, The Netherlands).

Sterol extraction from conophor oils: Oil (100 µl) was taken in 1 ml of 0.5 N sodium hydroxide and heated at 80 ^{o}C for 1 h. After cooling, the neutral sterols and stanols were extracted with n-hexane (3x3 ml). Hexane was evaporated to dryness and the residual product dissolved in 2 ml chloroform. Subsequently 0.5 ml aliquot was transferred to a 2 ml GC vial and evaporated to dryness.

Procedure: trimethysilyl (tms) ether derivatization of sterols and stanols: Aliquots of the biological extracts that contained the sterols (and stanols) were treated with 100 µl of Sil-Prep for 30 min at 55 ^{o}C in a screw cap tube. Solvent was evaporated at 55 ^{o}C under nitrogen, the reaction product is dissolved in 100 µl hexane and an aliquot (1-5 µl) is used for gas liquid chromatography.

GLC analysis: A Varian GC 450 gas chromatograph equipped with a flame ionization detector and an injector with a split/ split less device for capillary columns was used. The chromatographic column consisted of a chemically bonded fused silica DB17 capillary column (30 m x 0.25 mm ID) (Agilent J and W) and hydrogen was used as the carrier gas. The GC operating conditions were as follows: injector and detector temperatures are 280 ^{o}C and 300 ^{o}C, respectively. After injection, oven temperature were kept at 130 ^{o}C for 2 min and then programmed at a rate of 30 ^{o}C/min to a final temperature of 290 ^{o}C.

Statistical Analysis

All analyses were carried out in triplicate and data were subjected to Analysis of Variance (ANOVA) using SPSS version 19.0 (for windows). Statistical differences between mean values were determined by Duncan's Multiple Range Tests and accepted at $P < 0.05$.

Results and Discussion

Amino Acid Content of Raw and Processed *T. conophorum* Seed Flours (g/100 g protein)

The results of the amino acid profile of raw and processed *T. conophorum* seed flours and the summary of amino acid nutritional indices are presented in Tables 1 and 2. Glutamic acid was the most abundant amino acid. In general, the amino acid content was significantly higher ($p < 0.05$) in the raw seeds.

Glutamic acid was the most abundant ranging from 2.80 for raw to 3.31 for processed. However, the highest value was recorded in the cooked sample. This followed similar trend in the amino acid profile of a plant source Ogunlade *et al*., 2011.

Amino acids	samples		
	Raw	Cooked	Toasted
Lysine*	1.36±0.02	1.30±0.02	1.09±0.05
Histindine*	0.83±0.00	0.76±0.02	0.74±0.01
Arginine*	3.01±0.01	2.80±0.20	2.56±0.20
Aspartic acid	3.42± 0.10	3.29±0.11	2.97±0.03
Threonine*	1.63±0.30	1.77±0.10	1.39±0.02
Serine	1.90±0.10	2.02±0.03	1.63±0.00
Glutamic acid	3.20±0.20	3.31±0.40	2.80±0.10
Proline	1.48±0.02	1.77±0.03	1.26±0.02
Glycine	2.78±0.01	2.59±0.02	2.36±0.01
Alanine	1.01±0.20	0.95±0.03	0.84±0.01
Cystine	0.68±0.01	0.68±0.01	0.59±0.02
Valine*	1.56±0.10	1.43±0.02	1.31±0.03
Methionine*	0.31±0.01	0.26±0.01	0.28±0.01
Isoleucine*	1.21±0.02	1.15±0.20	1.02±0.30
Leucine*	1.93±0.30	1.88 ±0.20	1.65±0.02
Tyrosine	1.27±0.01	1.24±0.02	1.07±0.01
Phenylalanine*	0.85±0.01	0.80±0.01	0.71±0.02
Tryptophan*	0.91±0.10	0.70±0.11	0.76±0.01
Crude protein (g/100g)	0.60±0.30	0.49±0.21	0.53±0.20

Values in the table are means of three determinations ±SD. Means in the same row with different superscripts are significantly different ($p<0.05$).

* Essential amino acids

Table 1: *Amino acid Profile of Raw and Processed T. conophorum Seed Flours*

Amino acid	samples		
	Raw	Cooked	Toasted
Total amino acid (TAA)	29.34	28.7	25.03
Total non-essential amino acid (TEAA)	15.38	15.31	13.21
Total essential amino acid (TEAA)			
-with His	13.96	13.39	11.82
-No His	13.13	12.63	11.08
% TNEAA	52.4	53.3	52.8
% TEAA			
-With His	47.6	46.7	47.2
-No His	44.8	44.0	44.3
Total neutral amino acid (TNAA)	17.5	17.2	14.9
% TNAA	59.7	60.1	59.4
Total acidic amino acid (TAAA)	6.6	6.6	5.8
% TAAA	22.6	23.0	23.1
Total basic amino acid (TBAA)	5.2	4.9	4.4
% TBAA	17.7	17.0	17.5
Total sulphur amino acid (TSAA)	0.99	0.94	0.87
% TSAA	3.37	3.28	3.48

% Cys in TSAA	68.67	72 .34	67.82
Total aromatic amino acid (TArAA)	2.12	2.04	17.82
% TArAA	7.2	7.1	7.1
Leu/Ile ratio	1.06	1.63	1.62
Leu- Ile (difference)	0.72	0.73	0.63

*See Table 1.

Table 2: *Concentrations of essential, non-essential, acidic, neutral, sulphur, aromatic (mg/g crude protein) of Raw and Processed T. conophorum seed flours.*

Glutamic + aspartic acids in the samples made up to 6.60-6.77 g/100 g crude protein on an average basis with a percentage of 22.76-26.49. Similar observation has been reported by Olaofe and Akintayo 2000 and Adeyeye and Afolabi 2004. Leucine was the most concentrated essential amino acid with values of 1.93 g/100g in the raw and 1.88 and 1.65 g/100g in the cooked and toasted sample flours respectively which is greater than that of soya beans, 0.9 g/100g (Temple and Aliyu 1994) but lower than the FAO standard for preschool children (2 to 5 years) FAO 1985. Isoleucine, (essential amino acid) values in the flours ranged from 1.02-1.21 g/100g crude protein; these values are high when compared to the FAO standards of 2.8 g/100g crude protein FAO 1985. The lysine (Lys) content of between 1.09-1.36 g/100g crude protein was low compared to the reference egg protein of 6.3 g/100g crude protein Adeyeye and Afolabi 2004. The phenylalanine and tyrosine (Phe + Try) levels ranged from 1.78-2.14 g/100g crude protein in the processed samples, showing that the cooking and toasting gave values that were comparable with FAO/WHO/UNU standards (2.3 g/100g crude protein) suggesting that processed conophor nut seeds can be exploited to enhance the protein quality of weaning/complimentary feeding, especially in dry form.

The total amino acid (TAA) ranged between 25.56 to 29.94 g/100g crude protein. The values are lower than the value of 56.16 g/100g crude protein of the reference egg protein Paul et al., 1970 and compare favorably with 21.48 to 30.70 g/100g crude protein for guinea corn Adeyeye and Afolabi 2004. In general, the amino acid content was significantly higher ($P<0.05$) in the raw seeds.

Fatty acid composition of oils from raw and processed conophor oil

The results of the fatty acid profile of oils from raw and processed T. conophorum seeds are presented in Table 3. There was a significant difference ($P<0.05$) in the fatty acid composition among the samples. The unsaturated fatty acids were the most predominant fatty acids representing up to 94.68% (Table 3). Cooking improved the content of the unsaturated fatty acids. There was a significant difference ($P<0.05$) in the fatty acid composition among the samples; this may be due to the differences in the processing conditions. The predominant fatty acid group in the conophor seed oils (Table 3) was the unsaturated fatty acids.

	Cooked	Samples Raw	Toasted
Unknown	$0.37±0.00^a$	$0.34±0.00^b$	$0.34±0.00^b$
C12:0	$0.10±0.00^a$	$0.08±0.00^b$	$0.11±0.00^c$
C16:0	$1.66±0.00^a$	$1.65±0.00^b$	$1.65±0.00^b$
C16:1t	$0.06±0.00^a$	0.05±0.00	$0.05±0.00^b$
C16:1	$0.04±0.00^a$	$0.04±0.00^a$	$0.03±0.00^b$
C17:0	$0.08±0.00^a$	$0.07±0.00^b$	$0.07±0.00^b$
C17:1	$0.07±0.00^a$	$0.05±0.00^b$	$0.06±0.00^c$
C18:0	$2.71±0.00^a$	$2.82±0.00^b$	$2.59±0.00^c$
C18:1	$13.69±0.00^a$	$13.72±0.00^b$	$16.00±0.00^c$
C18:1n7c	$0.48±0.00^a$	$0.48±0.00^a$	$0.50±0.00^b$
C18:2	$16.12±0.00^a$	$16.01±0.00^b$	$14.37±0.00^c$
Unknown 2	$1.34±0.00^a$	$0.35±0.00^b$	$0.51±0.00^c$
C18:3n6	$0.62±0.00^a$	$0.63±0.00^b$	$0.62±0.00^a$
C18:3n3	$62.64±0.00^a$	$62.47±0.00^b$	$61.96±0.00^c$
C20:0	$0.11±0.00^a$	$0.12±0.00^b$	$0.10±0.00^c$
C20:1	$0.61±0.00^a$	$0.91±0.00^b$	$0.88±0.00^c$
C20:2	$0.13±0.00^a$	$0.10±0.00^b$	$0.08±0.00^c$
C20:3n3	$0.15±0.00^a$	$0.11±0.00^b$	$0.09±0.00^c$
C24:0	$0.04±0.00^a$	ND	ND

Values in the table are means of three determinations ±SD. Means in the same row with different superscripts are significantly different ($p<0.05$).

ND --- not determined

Table 3: *Fatty Acid Profile of Oils from Raw and Processed T. conophorum seeds*

The percentage unsaturated fatty acids ranged from 80.28 to 94.63 % consisting mainly-Linolenic acid (61.96-62.64 %); Linolenic (14.37-16.12 %) and oleic (13.69-16.00). The saturated fatty acid in the seed oils ranged from 4.32 to 4.73 %, consisting mainly of stearic acid (2.59-2.82 %) and palmitic acid (1.65-1.66 %). Other saturated fatty acids occured in insignificant amounts. From the results, conophor oil is a good source of polyunsaturated fatty acids (PUFAs) as presented in Table 4. High levels of blood cholesterol are associated with high intake of saturated fatty acids El-mallah *et al*., 2011. It has been concluded that relative to carbohydrate, the saturated fatty acids elevate serum cholesterol; while the polyunsaturated fatty acids lower serum cholesterol Hegested *et al*., 1993. High intake of conophor seed oil with high levels of unsaturated fatty acid may not lead to the risk of increased blood cholesterol in the body. Oils from the processed seeds were significantly higher in the unsaturated fatty acids than oil from the raw seeds. Alfawax 2004 also reported linolenic acid as the predominant fatty acid in pumpkin seed oil. Linolenic acid has been reported as the most important essential fatty acid required for growth, physiological functions and body maintenance Salunkhe *et al.*, 1985. Conophor seed oils will participate in these functions. The very low content of trans fatty acid (trans palmitoleic acid), 0.05-0.06 % supports further the nutritional integrity of conphor seed oil, since studies have shown that the consumption of trans fatty acids elevates LDL-cholesterol (bad cholesterol) and decreases the HDL-cholesterol. The linolenic acid contents were higher than (0.53-0.60 %) those reported by Unal and Yalcin 2008 for varieties of sesame seed oils and 0.14-0.21 % reported for saftflower oils Vosoughkia *et al*., 2011.

Sterol composition of *T. conophorum* seed oils

Sterols are components of unsaponifiable compounds in fats and oils and are important to indentify blends of fats and oils Mariani *et al*., 1994. Sterols are also of interest because of their ant oxidative activities Dutta *et al*., 1994. The conophor seed oils differ significantly (P<0.05) in their sterol composition (Table 5). Hyrothermal processing processing appreciably reduced the 5-α cholestane content of the oils (Table 5). The contents of phytosterols in the oils were improved upon processing as shown in Table 5. Cholesterol and latosterol were reduced below detection levels when the seeds were toasted. Campesterol contents of the seed oils ranged from 18.85 to 29.27 % in the raw and toasted seed oil samples.

Summary	Samples		
	Raw	Cooked	Toasted
Total unsaturated fatty acids	80.28	94.59	94.63
Total saturated fatty acids	4.73	4.70	4.52
Essential fatty acids (C18:2+C18:3)	79.11	78.90	76.95
Total monounsaturated fatty acids	14.82	14.94	17.51
Total diunsaturated fatty acids	16.11	16.24	14.45
Total triunsaturated fatty acid	63.21	63.41	62.66
Saturated/unsaturated	0.06	0.05	0.05
Oleic/linoleic	0.86	0.85	1.11
Polyunsaturated/saturated	16.76	16.96	17.05
Total trans fatty acids	0.05	0.06	0.05

Table 4: *Summary of the Fatty acid Composition (%) of Oils from Raw and Processed T. conophorum seeds.*

Sterols	Cooked	Samples Raw	Toasted
Unknown	16.26 ± 0.00^{a}	17.58 ± 0.00^{b}	17.97 ± 0.01^{c}
5-α Cholestane	4.69 ± 0.00^{a}	10.54 ± 0.01^{b}	8.81 ± 0.00^{c}
Cholesterol	2.47 ± 0.01^{a}	6.14 ± 0.00^{b}	ND
Latosterol	1.50 ± 0.00^{a}	5.58 ± 0.00^{b}	ND
Desmosterol	4.46 ± 0.00^{a}	7.52 ± 0.00^{b}	3.37 ± 0.00^{c}
Campesterol	29.27 ± 0.00^{a}	18.85 ± 0.00^{b}	28.68 ± 0.00^{c}
Stigmasterol	28.28 ± 0.00^{a}	23.78 ± 0.00^{b}	28.12 ± 0.00^{c}
β-Sitosterol	13.08 ± 0.00^{a}	12.50 ± 0.00^{b}	13.05 ± 0.00^{c}

Values in the table are means of three determinations ±SD. Means in the same row with different superscripts are significantly different (p<0.05).
ND --- not determined

Table 5: *Sterol Composition (%) of Oils from Raw and Processed T. conophorum seeds.*

The campesterol in the oil from the processed seeds are higher than in the oil from the raw seeds. The levels are higher than 9.45-14.17 reported for *Carthamus tinctorious* seed oil Vosoughkia *et al*., 2011. Campesterol has been reported to be involved in controlling cholesterol and lowering the risk of heart disease. Stigmasterol content of the oil ranged from 28.12 to 23.78 %. Processing improved the stigmasterol content of the oils. Plant sterols are essential components of the membranes of all eukaryotic organisms. The commonly consumed plant sterols are sitosterol, stigmasterol and campesterol which are predominiantly supplied by vegetable oils. The nutritional benefits derived from sterols include their ability to lower plasma cholesterol and LDL cholesterol Piironen *et al.*, 1999. A hypothesis to explain the effectiveness of sterols as antioxidants has been presented.

Conclusion

It can be concluded that lipid free radicals react rapidly with sterols are unhindered allylic carbon atoms forming relatively stable allylic tertiary free radical which are slow to react further and this interrupts the antioxidation chain. Cholesterol is injurious to health as it blocks the arteries leading to vascular arteriosclerosis. The levels of the cholesterol make the oils heart friendly.

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