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Fatty Acid and Amino Acid Composition of Protein Concentrate from Cashew Nut (*Anarcadium occidentale*) Grown in Nasarawa State, Nigeria

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Abstract : Fatty acids; proximate and amino acid composition of *Anarcadium occidentale* protein concentrate were investigated. The three most abundant fatty acids were C18:1ω9 > C16:0 > C18:3ω3. Unsaturated fatty aids predominated in the sample with adequate amounts of essential fatty acids. Proximate analysis of protein concentrate revealed high percentage crude protein of 69.6g/100g protein. Ash and crude fibre were low while ether extract was not detected. The protein concentrate had a balanced content of some of the essential amino acids, with respect to the FAO/WHO provisional pattern however supplementation may be required in valine and threonine. The calculated isoelectric point (pI) was 4.25 while the first limiting amino acid was valine.

Key words: *Anarcadium occidentale* oils, protein concentrate, fatty acids, amino acids

Introduction

The cashew tree is a tropical tree and is found between the tropics of Cancer and Capricorn. It is a hardy and drought resistant tree thriving in a variety of soil and climatic conditions. It is unselective in soil requirement, except like most trees, it will not grow on marshy land. It will grow in areas of rainfall of as low as 87.5cm up to 375cm but requires a long dry pell during which it crops. It grows well in on sandy soils and on lateric soils and thrives where other more sophisticated commercial crop trees would not. It has a strong root structure with at least as long as the height of the tree and for this reason, it is ideal for erosion arresting purposes and has been utilized for this in various countries (Carneiro, 1992).

Nasarawa State is one of the 36 States in the Federal Republic of Nigeria. It is an agrarian. The state is located in the North-Central geo-political zone of Nigeria otherwise known as the Middle Belt region. Cashew tree can be described as cash crop in the state and hold special position in the agriculture of Nasarawa State. Its production has an important economic activity for the State.

The reported works on the products from cashew included fermentation of cashew juice into wine (Aderiye *et al*., 1991); preparation of jam and preserves from the pulp (Ogunmoyela, 1983); evaluation of alcoholic drink from cashew biomass extract (Aderiye and Mabadiwe, 1993). Processing treatment which has improved functional properties of cashew nut flour has been reported by Fagbemi *et al.* (2004). Also compositional studies and physicochemical characteristics of cashew nut flour have been presented by Aremu *et al.* (2006a).

Fat provides a major portion of man's energy supplies, giving weight-for-weight more than twice as energy as proteins or carbohydrates (Osborne and Voogt, 1978). Fats in foods are not composed of a single type or category of fatty acids, rather each dietary fat is a complex mixture of different fatty acids (Wardlaw and Kessel, 2002; Enwere, 1998). It has been reported that the total lipid content of plant foods varies with variety, origin, location and climate as well as seasonal and environmental conditions; and the type of soil in which they are grown (Worthington *et al*., 1972). There is a growing interest in the utilization of plant proteins for the formulation of new food products. The most commonly used method for preparing plant protein concentrates or isolates is alkaline extraction followed by precipitation of the extracted protein either by decreasing the pH to the isoelectric point or by heating. Chemical evaluation of protein concentrate prepared from cashew nut (*Anarcadium occidentale*) is sparse in the literature.

Therefore, the present work involved the chemical investigation of fatty acid composition of *Anarcadium occidentale* oils and nutritive value assessment of protein concentrate from its flour through alkali solubilization followed by acid precipitation. It is hoped that the data generated from our investigation will provide information on utilization of *Anarcadium occidentale* oils and protein concentrate in various food applications.

Materials and Methods

Collection and preparation of sample: Mature disease-free cashew nuts were obtained directly from the farmers in Garaku Village, Kokona Local Government Area of Nasarawa State, Nigeria. The nuts were thoroughly screened to remove the stones. Preparation of the nuts into powdered sample was done according to the method of Aremu *et al.* (2006a).

Extraction of oils: Oils was extracted from the sample according to the method described by Akintayo and Bayer (2002). Oven dried sample was extracted in Soxhlet apparatus with chloroform-methanol mixture (2:1) for 20 hours under nitrogen atmosphere. Solvent was removed under reduced pressure in a rotary evaporator. Toluene was added to ensure removal of any water through azeotropic distillation with toluene.

Fatty acid analysis: Fatty Acid Methyl Esters (FAMEs) were prepared by direct transesterification of the oils according to the method described by Akintayo *et al.* (2004). 0.2 µL of the clear supernatant of FAMEs was injected into Hewlett-Packard 5890 Gas Liquid Chromatograph (GLC) (Hewlett-Packard Co, Palo Albo CA) equipped with Flame Ionization Detector (FID). The column was packed with J and W scientific fused silks column DB5 coated with cross linked 5% phenol + 95% polysiloxane, 25×0.25mm, 0.2 coating thickness. The column initial temperature was 120°C for 2min, temperature increased at the rate of 4°C/min up to 260°C and maintained at this temperature for 5min. Injector and detector temperatures were 230°C and 300°C respectively. The carrier gas, nitrogen was maintained at 50cm^3/min. FAMEs peaks were identified by comparison of their retention times with those of a standard mixture obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Protein isolation: The method described by Ige *et al.* (1984) was modified for the preparation of protein concentrate. Sample flour was defatted by the Soxhlet method using petroleum ether 60-80°C). 600g of the defatted flour was suspended in distilled water at room temperature (29±1°C) and at a flour-solvent ratio of 1:10. The slurry was stirred for 1h. The pH of the slurry was then adjusted to 4.5 (dropwise addition of 0.1 M HCl) being the pH value of which the protein was found to be most suitable. The extraction was allowed to continue with occasional shaking for 24h while maintaining the pH. The slurry was then centrifuged at 8000rpm for 30min. The extraction was repeated on the residue and supernatant was precipitated by drop-wise addition of 0.1M HCl at pH 4.5. The precipitate formed in each case was centrifuged at 8000rpm for 30min. The curd obtained was reslurried in distilled water and spray dried. The spray dried sample was used as protein concentrate.

Chemical analysis: The crude protein, ether extract, ash and crude fibre of protein concentrate were determined according to the method of the Association of Official Analytical Chemists, AOAC (1990). The amino acids were quantitatively measured by the procedure of Spackman *et al.* (1958), using automatic amino acid analyzer (Technicon TSM Sequential Multisample Analyzer). Sample was hydrolyzed for determination of all amino acids except tryptophan in consistent boiling hydrochloric acid for 22h under nitrogen flush.

Estimation of isoeletric point (pI) and quality of dietary protein: The predicted isoelectric point was evaluated according to Olaofe and Akintayo (2000);

$$pIm = \sum_{i=1}^{n} pI_i X_i$$

Where pIm is the isoelectric point of the mixture of amino acids, pI_i is the isoelectric point of the i^{th} amino acid in the mixture and X_i is the mass or mole fraction of the i^{th} amino acid in the mixture and there are n amino acids in the mixture. 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 (FAO, 1970; Bender, 1992). Amino acid score (AMSS) was estimated by applying the following formula (FAO/WHO, 1973).

$$AMSS = \frac{\text{mg of amino acid per g test protein}}{\text{mg of amino acid per g ref.protein}} x \frac{100}{1}$$

Results and Discussion

Table 1 presents the fatty acid composition of the oils from *Anarcadium occidentale*. In our result, the saturated fatty acids were myristic (C14:0), palmitic (C16:0) and stearic (C18:0); the monosaturated acid was oleic (C18:1ω9) while the polyunsaturated component were linoleic (C18:3ω3). The unidentified peaks were amount to 14.5%. The highest concentration was oleic (30.7%). This value compares favourably with African oil bean (30.3) reported by Achinewhu (1998) however higher than most legume seed oils reported by Adeyeye *et al.* (1999), Oshodi *et al.* (1993), Paul and Southgate (1985) and Ihekoronye and Ngoddy (1985). The ω6 and ω3 which are referred to as essential fatty acids have critical roles in the membrane structure (Lynch and Thompson, 1984; Kinsella, 1990) and as precursors of eicosanoids, which are potent and highly reactive compounds (Adeyeye *et al.*, 1999). However, *Anarcadium occidentale* contained enough essential fatty acids C18:2ω6 (2.5%) and C18:3ω3 (23.0%). Furthermore, the ω6 / ω3 ratio, which the WHO/FAO (1994) recommends should not be higher than 10 in the diet as a whole was 0.11 (due to low ω6 content) indicating that *Anarcadium occidentale* may be of use for reduction of ω6/ ω3 ratio (Mahan and Escott-Stump, 2000). The oleic acid/linoleic acid (O/L) ratio has been used as an indicator of peanut oil stability. High O/L associated with high stability and potentiality of the oil for deep frying fat (Branch *et al.*, 1990). The O/L level of *Anarcadium occidentale* (12.48) in the present study is

Table 1: Relative fatty acid content (%) of *Anarcadium occidentale* oils

Fatty acid	Concentration (%)
Myristic (C14:0)	00.6
Palmitic (C16:0)	25.0
Stearic (C18:0)	03.7
Oleic (C18:1 ω9)	30.7
Linoleic (C18:2 ω6)	02.5
α-Linolenic (C18:3 ω3)	23.0
Unknown	14.5
Saturated fatty acids (SFA)	29.3
Monounsaturated fatty acids (MUFA)	30.7
Polyunsaturated fatty acids (PUFA)	25.5
ω6/ ω3	00.11
O/L level	12.28

O/L = Oleic: Linoleic ratio

Table 2: Proximate composition (%) of *Anarcadium occidentale* protein concentrate

Parameter	AOC
Crude protein	69.6 (0.04)
Ether extract	ND
Ash	4.1 (0.20)
Crude fibre	0.9 (0.15)

Numbers in partheses are standard deviation for triplicate determinations; ND = not detected

Table 3: Amino acid composition (g/100g crude protein) *Anarcadium occidentale* protein concentrate

Amino acid	(g/100g protein)
Lysine (Lys)[a]	5.5
Histidine (His)[a]	2.2
Arginine (Arg)[a]	5.2
Aspartic acid (Asp)	10.2
Threonine (Thr)[a]	3.2
Serine (Ser)	2.5
Glutamic acid (Glu)	13.6
Proline (Pro)	2.3
Glycine (Gly)	2.8
Alanine (Ala)	3.5
Cystine (Cys)	1.4
Valine (Val)[a]	3.5
Methionine (Met)[a]	1.7
Isoleucine (Ile)[a]	3.5
Leucine (Leu)[a]	6.2
Tyrosine (Tyr)	3.2
Phenylalanine (Phe)[a]	4.3
Isoelectric point (pI)	4.25

[a]Essential amino acids;

much higher than peanut oil (1.48) (Branch *et al.*, 1990) hence *Anarcadium occidentale* oil may be more stable than peanut oil and may also be useful as frying oil. Table 1 also depicts fatty acid distribution according to saturation and unsaturation of components (%). The total unsaturated acid was higher than saturated ones. It has been established that relative to carbohydrates, the saturated fatty acids elevate serum cholesterol while the polyunsaturated fatty acids (PUFA) lower serum cholesterol (Keys *et al.*, 1957; Hegsted *et al.*, 1993). Linoleic and α-linolenic (PUFA) are the most important essential fatty acids required for growth, physiological functions and body maintenance (Salunkhe *et al.*, 1985). *Anarcadium occidentale* seed oils will participate well in these functions.

Proximate composition of *Anarcadium occidentale* protein concentrate is shown in Table 2. Crude protein value (69.6%) in this report is cower than crude protein values of protein concentrates of some Nigerian plant foods; liman bean, 70.2% and African yam bean, 78.4% (Akintayo *et al.*, 1998), liman bean, 72.48% (Oshodi *et al.*, 1998), mucuna bean, 78.3% (Lawal and Adebowale, 2004) and *Prosopis africana*, 73.4% (Aremu *et al.*, 2007) however it is higher than that of pigeon pea, 60% (Akintayo *et al.*, 1998). The percentage ash and crude fibre were low though lower in the protein concentrate than in its flour (Aremu *et al.*, 2006a) while ether extract was not detected.

Table 3 presents amino acid profile of *Anarcadium occidentale* protein concentrate. Leu is the most highly concentrated essential amino acid. However the value obtained in this report is lower than Leu content of protein concentrate of some Nigerian leguimes; liman bean (7.59g/100g protein), pigeon pea (8.40g/100g protein) and African yam bean (7.45g/100g protein) reported by Oshodi *et al.* (1998). It is observed that glutamic and aspartic acids (together make up 23.8g/100g protein) are the most abundant amino acids in the plant food sample. Some workers (Olaofe *et al.*, 1994; Adeyeye, 2004; Aremu *et al.*, 2006b, 2006c; Oshodi *et al.*, 1998) had made similar observation. The least concentrated amino acid was Met (1.7g/100g protein). Tryptophan (Try) was not determined. The calculated isoelectric point (pI) was 4.25. This is useful in predicting the pI for protein in order to chance a quick precipitation of protein isolate from biological samples (Olaofe and Akintayo, 2000).

The nutritive value of a protein depends primarily on the capacity to satisfy the needs fro nitrogen and essential amino acids (Pellet and Young, 1980). Total essential amino acid (with His) of *Anarcadium occidentale* protein concentrate (Table 4) is greater than that of *Prosopis africana* concentrate (31.9g/100g protein) reported by Aremu *et al.* (2007). However, it is less than that of some Nigeria legume concentrates; liman bean (44.88g/100g protein), pigeon pea (48.11g/100g protein) and African yam bean (48.28g/100g protein) reported by Oshodi *et al.* (1998). Nevertheless protein concentrate of *Anarcadium occidentale* satisfied the FAO requirements (FAO/WHO/UNU, 1985) for the essential amino acids. Essential Alphatic Amino Acid (EAAA), Ile, Leu and Val, which constitute the hydrophobic regions of protein is more abundant in the protein concentrate (16.4g/100g protein) than the flour (14.6g/100g protein) (Aremu *et al.*, 2006a). The Total Acidic Amino Acid (TAAA) is found to be far greater than the total basic amino acid indicating that *Anarcadium occidentale* protein was probably acidic in nature (Aremu *et al.*, 2006d).

Table 4: Classification of amino acid composition (g/100g crude protein) of *Anarcadium occidentale* protein concentrate

Classification	Concentration (g/100g protein)
Total Amino Acids (TAA)	74.8
Total Essential Amino Acids (TEAA)	
*with Histidine	35.3
*without Histidine	33.1
% TEAA	
*with Histidine	47.19
*without Histidine	44.25
Total Non-Essential Amino Acids (TNEAA)	39.5
% TNEAA	52.81
Essential Aromatic Amino Acids (EArAA)	4.3
% EArAA	5.75
Essential Alphatic Amino Acids (EAAA)	16.4
% EAAA	21.93
Total Acidic Amino Acids (TAAA)	23.8
% TAAA	31.82
Total Basic Amino Acids (TBAA)	12.9
% TBAA	17.25
Total Neutral Amino Acids (TNAA)	38.1
% TNAA	50.94
Total Sulphur Amino Acids (TSAA)	3.1
% TSAA	4.14
% Cystine in TSAA	33.82

Table 5: Amino Acid Scores of *Anarcadium occidentale* protein concentrate

	Concentration (g/100g crude protein)		
AAC	PAAESP[a]	EAAC	AMSS
Ile	4.0	3.5	0.88
Leu	7.0	6.2	0.9
Lys	5.5	5.5	1.0
Met + Cys (TSAA)	3.5	3.1	0.89
Phe + Tyr	6.0	7.5	1.25
Thr	4.0	3.2	0.80
Try	1.0	nd	na
Val	5.0	3.5	0.7
Total	36.0	32.5	6.41

[a]Source: Belschant *et al.* (1975), AAC = Amino Acid Composition, PAAESP = Prosional Amino Acid (Egg) Scoring Pattern, EAAC = Essential Amino Acid Composition (see Table 3), AMSS = Amino Acid Score, nd = not determined, na = not available

Result of the amino acid scores is shown in Table 5. With exception of Phe + Tyr and Lys, the essential amino acid contents were lower than the FAO/WHO (1991) recommended pattern. Thus by implication, dietary formula based on the protein concentrate of *Anarcadium occidentale* will require some essential amino acids supplementation such as Val, Thr, Met and Cys. It has been reported that EAAs most often acting in a limiting capacity are Met (and Cys), Lys and Try (FAO/WHO/UNU, 1985). In this study, Val and Thr were the first and second limiting amino acids respectively.

Conclusions: There is an indication that *Anarcadium occidentale* oils contained a relatively high level of polyunsaturated fatty acids, making it a healthy low-fat food. Amino acid analysis revealed that the sample contained nutritionally useful quantities of most of the essential amino acids while the first limiting amino acid was valine.

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