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DETERMINATION OF THE CHEMICAL COMPOSITION, THE PHYSICOCHEMICAL PROPERTIES OF THE OIL EXTRACT AND THE AMINO ACID PROFILES OF THE SEEDS OF *TELFAIRIA OCCIDENTALIS* (FLUTED PUMPKIN)

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ABSTRACT

The chemical composition of the seed of *Telfairia occidentalis* (fluted pumpkin), the physicochemical properties of the seed oil and the amino acids profiles of the seed protein have been determined. In proximate composition, the crude fat content of 58.41% indicates that the plant seed is an oil seed. Its protein content of 28.88% is high, it contains vitamins A and C and a number of nutritional minerals, it also contains tolerable amounts of toxicants (antinutrients). The physicochemical properties of the seed oil show that the oil has high saponification value, low free fatty acid, low peroxide value and specific gravity of 0.87, thus indicating that the oil is an edible one. The amino acids profiles of the seed protein show that the seed contains a good number of essential amino acids which compare well with FAO/WHO protein (standard) and the Hen's Egg protein. These results are discussed in terms of the nutritional values and the industrial potential of the seeds of *Telfairia occidentalis*.

KEYWORDS: Chemical composition, *Telfairia occidentalis* seed, physicochemical properties, amino acids profiles, oil seed.

INTRODUCTION

The plant, fluted pumpkin (*Telfairia occidentalis*) which belongs to the family cucurbitacae can grow as a creeping or climbing plant if given a support and its stem can grow up to a length of 10m. It is widely cultivated as garden and farm vegetable; it is a native of West Africa, growing in humid tropical climate in well drained soil. Although the farmers grow it in farms as annual crop because of the shifting cultivation system, the plant behaves like a perennial plant and can grow for many years if there is enough moisture in the soil (Enwere, 1995). The leaves of the fluted pumpkin are important vegetables for the people of the eastern and midwestern states of Nigeria (Oguntona, 1998). Mature leaves and the soft shoot can be harvested at intervals for food preparation one month after planting. The plant also bears 4 – 10 whitish fluted fruit pods with numerous brown seeds embedded in the soft pulp (Achinewhu, 1986). The leaves of the plant are important articles of trade throughout Nigeria and they are highly cherished vegetables in popular local traditional dishes such as Edikang Ikong soup of the Ibibios and the Efiks; on the other hand the seeds are only eaten occasionally or fermented and used as condiment to flavour soup; they are also sold mainly for planting. However, studies have shown that the fluted pumpkin seeds are rich in oil, protein and other nutritional materials which make them generally acceptable as food substances (Fetuga, et al., 1974; Longe, et al., 1983; Asiegbu, 1983; Okoli and Mgbeogu, 1983; Odemena and Onyeneke, 1988; Badifu and Ogunsua, 1991; Akwaowo et al., 2000). In this way, fluted pumpkin seeds are potential sources of oil and protein which if well exploited can be used in many industrial preparations such as food supplements and body cream. However sufficient information on the physicochemical properties of the oil extract and the amino acid profiles of the seeds of *Telfairia occidentalis* are inadequate. Therefore, in this study, the chemical composition of the seed of fluted pumpkin, the physicochemical properties of its oil extract and the amino acid profiles of the seed protein have been determined.

MATERIALS AND METHODS

The fluted pumpkin fruits (pods) were purchased from the farmers in Essien Udim Local Government Area of Akwa Ibom State, Nigeria. The analytical samples were treated according to the methods adopted by Joslyn (1970) and A.O.A.C. (1975, 1993). The pods were cut open and the seeds removed. They were dried in an air circulating oven at 40°C for 24 hours in the Central Laboratory of the University of Uyo. The dried seeds were crushed into powder and each sample stored in labeled air-tight plastic containers and preserved in a desiccator; but fresh, undried sample was used for moisture content determination.

Determination of the Chemical Composition

The conventional methods approved by the Association of Official Analytical Chemists were used for proximate analysis (A.O.A.C., 1984). The parameters determined were the moisture content by gravimetric method, crude fat by extraction with petroleum ether (Bp: 40-60°C) using Soxhlet extractor, crude fibre by consequential treatment with 1.25% $\left(\frac{V}{V}\right)$ H_2SO_4 and 1.25% $\left(\frac{W}{V}\right)$ NaOH solutions and thorough washing with hot water and 90% ethanol before drying at 100°C and igniting at 500°C in a furnace. The difference in weight of the dry residue and the ash constituted the crude fibre.

The crude protein was determined as ammonia nitrogen using Kheldahl apparatus for digestion of the sample and subsequent treatment of the digest with appropriate reagents and titration with 0.1MHCl solution. The carbohydrate content, excluding the crude fibres was obtained as the difference after subtracting the total organic nitrogen, crude fat and crude fibre from the dry matter. The caloric value in Kcal/100g was obtained by multiplying the values of the crude protein, crude fat and carbohydrate by 4, 9 and 4 respectively and taking the sum. The antinutrients determined were: hydrogen cyanide (HCN), phytic acid, oxalate and tannins using standard methods (Vogel, 1962; Dye, 1956; McCance and Widdowson, 1953).

The mineral elements were determined using atomic absorption spectrophotometer (AAS) after digestion in the appropriate reagents (Vogel, 1962). They were Mg, Fe, Ca, Zn and Mn, the actual values were read from the calibration curves for each element determined at the appropriate wavelengths. Na and K were determined using flame

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photometer and measuring the intensity of Na and K emission at the wavelength of 587nm and 766nm respectively.

Phosphorus (P) was determined by colourimetric method described by James (1984) in which a reducing agent 1.0ml hydroquinone was mixed with 0.5ml of the sample mineral digest for the development of a blue colour after 30 minutes. The intensity of colour at 660nm was measured and the quantity of Phosphorus (P) was calculated by the use of blank titration reading.

Determine of Vitamins

Vitamin A was determined by approved method of A.O.A.C (1984) while vitamin C was determined using Reo and Kuether (1943) method and the value measured using colourimeter at a wavelength of 540nm.

Determination of Physicochemical Properties of the Oil Extract

The physicochemical properties of the seed oil were determined using the appropriate methods; these were the melting point (Mp), the boiling point (Bp), specific gravity, refractive index, iodine value, saponification value, acid value, free fatty acid and peroxide value (Woollatt, 1985).

Amino Acid Profiles

Amino acids profiles were determined by Pearson (1991) method through extraction of the oil with petroleum ether (Bp. 40-60°C) in Soxhlet apparatus for six hours. The defatted residue was mixed with 6MHCl in an ampoule, sealed and heated at 105°C for 22 hours.

The content in the ampoule was cooled and filtered; the solvent was evaporated to dryness at 40°C and the dry extract was used for amino acid profile determination using Technicon Sequential Multi Sample (TSM) amino acid analyzer (DNA0209) made by Technicon (Ireland) Ltd. (Spackmann, et al., 1958). The concentration of amino acid in the sample was obtained by comparing the integrated area of its peak with that of the standard.

RESULTS AND DISCUSSION

The results of the analysis for the proximate composition, mineral element composition, vitamins A and C, and the amino acid profiles of the seeds of *Telfairia occidentalis* and the physicochemical parameters of the seed oil extract are shown in Tables 1, 2, 3 and 4 below.

The moisture content of the fluted pumpkin seeds was 36.18% based on fresh weight; this value is comparable to 33.37% reported by Akwaowo et al. (2000). It is known that the moisture content of fresh seeds depends on many factors such as the harvest season (dry or wet), humidity of the air and the presence of adsorbed or occluded moisture in the seed (Frazier and WestHoff, 1978). Therefore, the use of moisture content as an index for nutritional value of any seed should be treated with reservation. However, it has been observed by Aurand and Wood (1975) that the inherent moisture content in food affects the shelf life and perishability of food materials, thus to preserve the seeds of the fluted pumpkin they must be properly dried at suitable temperature; this is the practice adopted by the local farmers to preserve the seeds for eating purposes only as they boil the seeds first and then hang them by the fire place to dry gradually for about a week.

The protein content of fluted pumpkin seeds was 28.88% and this value is quite high and is comparable to the values earlier reported by Giami and Bekebain (1992) : 28.1%, 29.60% and 28.80% for the raw, germinated and fermented pumpkin seeds respectively, but it is lower than 47.85% for fermented and 47.57% for unfermented seeds reported by Achinewhu (1998). The high protein content emphasizes the fact that the seeds are very useful in food preparations, hence should form one of the components for preparing baby foods. The high lipid (oil) content of 58.41% of the pumpkin seed compares well with the results of other researchers namely, Longe et al. (1983) and Akwaowo et al. (2000) whose results were 53% and 56.24% respectively. This value places pumpkin seed in the class of oilseed hence a potential source of industrial vegetable oil.

Table 1: Chemical Composition of the fluted pumpkin seed. (a) Proximate composition, (b) Mineral Elements (c) Toxicants (antinutrients)

(a) PROXIMATE COMPOSITION		(b) MINERAL ELEMENT		(c) *TOXICANTS	
Parameters	% Composition*	Elements	*Concentration mg/100gDM	Toxicants	*Concentration mg/100gDM
Moisture content	36.18 ± 2.65	Mg	11.94 ± 0.08	HCN	10.80 ± 0.52
Crude Protein	28.88 ± 2.14	Fe	0.33 ± 0.05	Phytic Acid	1.96 ± 0.08
Lipid (oil)	58.41 ± 0.49	Ca	135.40 ± 1.06	Total oxalate	246.40 ± 1.05
Ash Content	5.20 ± 0.27	Zn	1.75 ± 0.03	Soluble oxalate	167.20 ± 0.66
Crude Fibre	3.45 ± 0.47	Na	1.48 ± 0.01	Tannins	4.88 ± 0.62
Carbohydrate	4.06 ± 0.50	K	57.30 ± 0.29		
Caloric Value	657.45	Mn	<0.002		
(Kcal/100g)		P	73.50 ± 2.37		

*average of three determinations

The other proximate parameters: ash content, 5.20%; the crude fibre, 3.45% and the carbohydrate, 4.06% are within the acceptable levels for food substances (Eka, 1980; Osagie and Eka, 1998), the low level of crude fibre, suggests that the pumpkin seeds are suitable for formulating baby's food, for they do not need excessive intake of dietary fibre (Eneobong, 2001). The caloric value calculated from the protein, lipid and carbohydrate contents (James, 1984; Lee, 1983) was 657.45 Kcal/100g and if the seeds of the fluted pumpkin are eaten with other foodstuffs one can meet the recommended daily caloric allowance of 2,404 Kcal/100g weight of an adult (FAO/WHO, 1973).

The mineral elements present in the seeds of the pumpkin are listed in Table 1(b) and are within the limits of such minerals in other edible seeds (Onyenuga, 1968; Food Basket Foundation, 1995). The concentration of vitamins A and C per 100g pumpkin seed (DM) were 1.86mg and 2.12mg respectively. These vitamins could help in the proper functioning of the body systems when the seeds are eaten by man.

The toxicants (antinutrients) levels in mg/100g (DM), shown in Table 1(c) are HCN (10.80 ± 0.52), phytic acid (1.96 ± 0.08), total oxalate (246.40 ± 1.05), soluble oxalate (167.20 ± 0.66), tannins (4.88 ± 0.62). Although these are within the levels of acceptable edible seeds, these toxicants may have adverse effects in the human body system if the seeds are eaten raw, for example HCN may interfere with metabolic processes; reduction in bioavailability of Ca and Fe by oxalates, while tannins are associated with carcinogenic

Table 2: Physicochemical Properties of pumpkin seed oil and the amino acid profile of the pumpkin seed protein

(a) Physicochemical Properties of pumpkin seed oil		(b) Amino Acid Profiles of pumpkin seeds	
Properties		Amino Acid	
Bp (°C)	58.90 ± 0.21	Lysin	5.26*
Mp (°C)	18.50 ± 0.15	Histidine	2.33
Refractive Index	1.462 ± 0.20	Arginine	5.14
		Aspartic	8.25
Sp. G.	0.87 ± 0.05	Threonine	2.33
Saponification value (mgKOH/g)	91.16 ± 3.63	Serine	3.26
Iodine value (gI_2/100g)	51.52 ± 1.12	Glutamic Acid	15.26
		Proline	1.25
Acid Value (mgKOH/g)	0.76 ± 0.09	Glycine	3.20
Ester Value (mgKOH/g)	90.40 ± 2.83	Alanine	4.01
% FFA (as oleic acid)	0.38 ± 0.08	Cysteine	0.69
Peroxide value mEq/Kg	11.75 ± 0.69	Valine	3.51
		Methionine	0.80
		Isoleucine	2.93
		Leucine	6.24
		Tyrosine	6.26
		Phenylalanine	3.63
		Trypphan	ND
		Total	74.35

* Mean of three determination
ND = not determined

effects in man such as poor protein utilization and liver and kidney toxicity (Munro and Bassir, 1969). Therefore proper processing before consumption is necessary. Dye (1956) has shown that boiling reduces oxalate toxicity levels in food; Lee (1983), Ekop and Eddy (2006) reported that cooking process eliminates most of these toxicants from seeds.

PHYSICOCHEMICAL PROPERTIES OF EXTRACTED OIL

Table 2(a) shows the physicochemical properties of the oil (lipid) extracted from the fluted pumpkin seeds. The melting point (Mp), 18.50°C and the boiling point (Bp), 58.9°C show that the oil is liquid at ambient conditions. The low Mp makes the oil suitable for making body creams in cold weather within the tropics because at a temperature just above the Mp the oil still flows and spreads evenly on the body to cover the skin, thereby cushioning the skin from cold air, even at temperatures hot enough to be a discomfort to the body the oil may evaporate from the skin thereby cooling the skin because of its low Bp. This is characteristic of cold cream (British Standard Method, 1958). Its low specific gravity of 0.87 also makes the oil flow and spread easily on the skin; its specific gravity is also within the limits recommended by CODEX (1995) for edible oils.

The saponification value (SV) of the oil, 191.16 ± 3.63 mgKOH/g compares well with that of palm oil, 195 – 205 mgKOH/g (Tewari et al., 1976, Woollatt, 1985); palm oil is generally acceptable as edible vegetable oil and has wide industrial applications as in soap making. The low acid value or percentage free fatty acid, low iodine value (IV) < 90g I_2/100g oil and low peroxide value further recommend the pumpkin seed oil suitable for human consumption but unsuitable for paint production because it is a non-drying oil. These physicochemical properties together with the high yield (crude fat 58%), strongly support industrial production of fluted pumpkin seed oil for commercial purposes.

AMINO ACID PROFILES OF FLUTED PUMPKIN PROTEIN

Table 2(b) shows the amino acid profiles of the fluted pumpkin protein prepared as described earlier in the text. Table 3 compares the essential amino acids composition of the seeds with FAO/WHO (1973) provisional protein and Hen's Egg protein. The seed protein contains a good number of essential amino acids as well as the non-essential amino acids at useful level of concentration; the essential amino acids are listed in Table 3 and the following are the non-essential amino acids: glutamic acid, arginine, aspartic, serine, proline, tyrosine and histidine (Table 2(b)).

The Essential Amino Acids

The amino acids help in regulating specific functions in the human body for the well being of an individual. Thus leucine is responsible for regulating the body sugar concentration, growth and repair of muscles/tissues and wound healing; its deficiency causes dizziness, headaches, fatigue, depression, irritability and hypoglyceamia in infants (Reference Guide, 1995); isoleucine helps in development of haemoglobin, repair of muscles and regulation of energy while lysine is to ensure adequate absorption of Ca and formation of antibodies, hormones and enzymes. These essential amino acids need be replenished adequately regularly. Therefore any seed that contains these essential amino acids at adequate levels should be recommended as edible seeds and should be incorporated as a component of some commercial foodstuffs, example: non-alcoholic beverage similar to the popular bournvita which is made from cocoa seeds.

Table 3: Comparison of Essential Amino Acids Composition (g/100g Protein) of the Fluted Pumpkin Seed with FAO/WHO Provisional protein and Hen's Egg Protein

Essential Amino Acids	*FAO/WHO Protein	Fluted Pumpkin Seed Protein	Hens Egg Protein	As Percentage of FAO/WHO Protein
				Fluted Pumpkin Seed
Lysine	4.20	5.26	4.36	125.24
Threonine	-	2.33	1.60	
Cysteine	4.20	0.69	3.62	17.00
Valine	4.20	3.51	2.60	83.57
Methionine	-	0.80	-	-
Tyrosine	2.80	6.26	7.20	223.57
Isoleucine	4.20	2.93	3.93	69.76
Leucine	4.80	6.24	5.51	130
Phenylalanine	-	3.63	-	-
Tryptophan	1.40	-	-	-
Total	25.8		28.82	

Table 4: Limiting Amino Acid Index based on FAO/WHO Protein and Hen's Egg Protein

	LAA	Pumpkin Seed	Protein Score
FAO/WHO	4.20	0.35	35
Hen's Egg	3.62	0.41	41

Limiting Amino Acid (LAA) = methionine + cysteine

In Table 3 the essential amino acids of the fluted pumpkin seeds are compared with the FAO/WHO (1973) provisional protein and Hen's Egg protein, the seed samples showed a deficiency in methionine. The calculated percentage of essential amino acids in terms of the total amino acids determined in the fluted pumpkin seeds was 42.56. The limiting Amino Acid (LAA) index based on FAO/WHO (1973) protein and Hen's Egg protein is shown in Table 4; the most limiting amino acids are the sulphur containing methionine and cysteine, while the seed protein score based on FAO/WHO provisional protein was 35% and based on Hen's Egg protein was 41%. High quality proteins contain the essential amino acids in proportions capable of promoting growth when they are the sole source of protein in the diet, they usually contain 33% or more essential amino acids (Gulthrie, 1972), and therefore fluted pumpkin seeds contain high quality protein.

CONCLUSION

The proximate composition of the fluted pumpkin seeds shows that the seeds contain adequate amounts of nutritional substances (crude protein, lipid oil), carbohydrate and mineral elements); have high caloric value and some toxicants (antinutrients) at acceptable levels and which are usually removed by boiling before consumption. The physicochemical properties of the oil extract show that the seed contains edible oil while the amino acids profiles confirm that the seeds contain high quality protein hence the use of the seeds as food substance.

Its high oil content suggests that it will be economical to extract the oil at industrial scale to be used as any vegetable oil either for food preparation or for other industrial uses. In view of the fact that presently fluted pumpkin plants are cultivated specifically for their leaves for food preparation, it is suggested that large commercial gardens/farms should be developed for the main purpose of producing fluting pumpkin pods from which the seeds will be obtained for use in the infant food formulation or for oil extraction.

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