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Research Paper

Mineral, anti-nutritional and amino acid composition of *Citrullus vulgaris* (*guna*) seed protein concentrate

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Abstract. The amino acid composition, proximate composition, mineral composition and some anti nutrients of *Citrullus vulgaris* (*guna*) seed protein concentrate were investigated. The protein concentrate contained all the essential and semi essential amino acids (EAA) with respect to FAO/WHO requirements. The proximate composition gave a high percentage of crude protein (83.56%). Ash, crude fiber, ether extract (lipid), and carbohydrate were at low levels of concentration. The minerals content analysis indicated both macro and micro minerals which are essential in metabolic processes, while certain heavy metals were below detection levels. The anti nutrients, phytate and oxalate were detected but within acceptable safe limits in diets, while tannins were below the detectable levels.

Keywords: Amino acids, '*guna*' seed, protein concentrate, proximate analysis.

INTRODUCTION

The fast demographic growth, coupled with the low economic resources in developing countries create the necessity to look for protein sources that can substitute animal protein, compliment the nutritional value of cereal-based foods and prevent malnutrition (Gonzelez-Quijada et al., 2002). Many plant proteins usually in the form of protein extracts or seed flour are being investigated and tested for new products which are nutritionally viable and acceptable to consumers just like conventional protein sources from plants and animals, via soy beans, meat, fish and dairy products (Ojieh et al., 2008).

Citrullus vulgaris (*guna*) plant belongs to the family of the *Cucumbitaceae* or *Cucurbit*. This family is a major family with various economically important species particularly those with edible fruits e.g. watermelon. This family has both medicinal as well as nutritional uses (Gurcharan-Singh, 2004). *C. vulgaris* is a variety of water melon, unlike other common watermelon whose flesh is sweet and red in colour, this variety of melon has a pale yellow or light green juicy flesh and tastes bitter (Ojieh et al., 2008). The 'guna' seed resembles the popular 'egusi' seed, but this seed is smaller than the 'egusi' melon seed. The seed is called 'guna' in Hausa, 'egusiito' in Yoruba, 'ugbogoro' in Igbo. The '*guna*' seed is used for soup, in breakfast cereals and as snacks among certain tribes such as the "Mbula, Bwatiye, Kilba, and Bura' in Adamawa State among others tribes in Nigeria (Penuel et al., 2013).

Several research works on some other oil seeds like soya beans, sun flower, sesame, castor and cotton had been carried out and their protein by products: the grits, flours, and concentrates have been extensively used to fortify bakery products, cereal products etc (Ige et al., 1984). Guna seed can be exploited as raw material for the production of protein concentrate which could be used as additives to foods in the same way as soya protein is used to improve the content of processed foods (Achinewhu, 1984; Penuel et al., 2013).

Object or aim of study

Previous studies with some other varieties of *C. vulgaris* had been documented by Eka and Osagie (1998). Most of these works focused on nutritional properties of the seed and some aspects of their physical and chemical properties, but so far, no work has been documented on amino acid profile of 'guna' seed.

The aim of this work is to prepare a protein concentrate of 'guna' seed, determine its mineral composition/proximate composition and the amino acid profile of the seed. Also, to compare its content with that of WHO and FAO nutrient standard as a basis for the wider/ worldwide utilization.

MATERIALS AND METHOD

Collection, preparation and treatment of sample

The 'guna' seed was purchased from Borrong market in Demsa Local Government Area of Adamawa State, Nigeria. The seeds were screened to identify good seeds and shelled manually. The shelled seeds were air dried, ground into, powder using an electric blender, stored in polyethylene bags and kept in desiccators for subsequent analysis.

Protein concentrate

The method described by Aremu et al. (2007) was modified and adopted for the preparation of protein concentrate. The sample flour was defatted by a soxhlet extractor using n-hexane at 35 to 37°C for 8 h. The defatted flour was dried at room temperature and sieved using mesh size number of 70. One part of the flour was dispersed in six parts of warm (50°C) distilled water by stirring for 30mins. The $_{P}$H of the solution was adjusted to 4.5 by drop wise addition of 0.1 M HCl. The extraction was continued by occasional stirring for 24 h. The solution was centrifuged at 4000 rpm for 1 h. The solid phase protein concentrate was air dried, sieved and kept in polyethylene bags for chemical analysis.

Proximate analysis

The moisture content, crude fat, crude protein, ash, crude fibre and carbohydrate contents of the protein concentrate were determined following the method as described by AOAC (2000) and Ibitoye (2005).

Amino acid analysis

Amino acid was determined adopting the method by Erkan et al. (2010), using Isocratic high performance liquid chromatography (HPLC Buck Scientific BLC 10/11 model).

Mineral analysis

Determination of minerals was done according to AOAC (2000). The sample was ashed at 550°C in an furnace to constant weight and dissolved in 10 ml of 0.1 MHCl, filtered into a 100 ml volumetric flask and made up to mark with distilled water. This was used to determine the mineral content by the use of atomic absorption spectrophotometer (AAS) (Buck Scientific model 210 VGP) using prepared standards of the different mineral elements to be analyzed.

Anti-nutrient

Tannin was determined according to the method by Trease and Evans (1989). Exactly 0.5 g of the dry sample was boiled with 20 ml of H_2O, 0.1% $FeCl_3$ was added to observe for brownish green or blue-black coloration.

Oxalate was determined by the method of Day and Underwood (1986), wherein, 1.0 g sample was dissolved in 100 ml of 0.75 M H_2SO_4 and the solution was carefully stirred with a magnetic stirrer for 1 h. The sample was then filtered and 25 ml of the filtrate was pipetted and titrated hot (80 to 90°C) against 0.1 M $KMnO_4$ to an end point of a faint pink colour that persisted for more than 30 s.

Phytate was determined according to the method of Reddy and Love (1999). 4.0 g of sample was soaked in 100 ml of 2% HCl for 5 h and filtered. 25 ml of the filtrate was pipetted in to a conical flask and 5 ml of 0.3% NH_4SCN solution was added. The mixture was titrated against 0.1 M $FeCl_3$ until a brownish yellow colour end point that persisted for 5 min was obtained.

RESULTS AND DISCUSSION

Based on the results from Table 1, the crude protein level in the protein concentrate of *C. vulgaris,* was 83.56% is lower as compared to the protein concentrate of red skin groundnut given as 88.90% (Ihekoronye, 1986), however, this value is above that of some Nigerian legumes such as lima bean, 70.20%; African yam, 78.40% (Akintayo et al., 1998) and mucuna bean, 78.30% (Lawal and Adebowale, 2004). The moisture content of 4.46% is comparable with that of ebony seed, 4.46% (Gonzalez et al., 2002) and 'red skin' groundnut, 4.30% (Ihekoronye, 1986).

The protein concentrate of 'guna' has a low fat content of 2.67%. The process of defatting of the raw flour with hexane to obtain defatted flour and finally preparing the protein concentrate decreases the fat content in protein concentrate (Gonzalez-Quijada et al., 2002). Crude fibre content of 4.00% falls within the values reported by Eka and Osagie (1998) for some varieties of melon seeds ranging from 2.0 to 8.2%.

The ash content of 3.50%, was almost similar to the value obtained for 'egusi' melon 3.70% (Ojieh et al., 2008), but it is above the values recommended for seeds used in animal feed formulation (1.5 to 2.5%) (Pormeranz

Table 1: Proximate compositions of protein concentrate, (% by weight).

Composition	%
Moisture	4.50 ± 0.3
Crude Fat/lipid	2.67 ± 0.1
Crude protein	83.56 ± 0.4
Crude Ash	3.30 ± 0.2
Crude fibre	4.00 ± 0.3
Carbohydrate (NFE)	1.77 ± 0.4

Values are mean of three replicates ± standard error of the mean.

Table 2. Mineral composition mg/100g.

Mineral	Concentration (mg/100 g)
Na	191.50
Ca	36.50
Zn	35.50
Fe	111.50
Cu	2.61
Mn	11.00
Mg	71.00
Cr	BDL

BDL = below detectable leve

Table 3. Some anti-nutrient in the protein concentrates (mg/100 g).

Anti-nutrient	Concentration (mg/100g)
Tannin	BDL*
Oxalate	0.383
Phytate	0.115

*BDL = below detectable level.

and Clifton, 1981).

'Guna' is rich in dietary minerals that are required to support human biochemical processes. As shown in Table 2, sodium, magnesium and calcium are the main elements analyzed; sodium has the highest value of 191 mg/100 g, followed by magnesium 71 mg/100 g and calcium 36.50 mg/100 g. These values however fall below the World Health Organization (WHO, 1973) and National Research Council (NRC, 1989) where daily intake of 500 mg sodium and 400 mg were recommended for adults and children respectively. Magnesium content of 350 mg for adults, 170 mg for children and 800 mg of calcium is recommended for both adult and children. Copper has the least value detected, while chromium was below detectable level.

Essential trace element like iron, copper, zinc and manganese are found in 'guna'. Iron which is important in blood formulation has a value of 115.50 mg/100 g which is below the values reported by Eka and Osagie (1998) in some melon seeds, viz fluted pumpkin and soya bean meals, but higher than the values reported for some under exploited leguminous seeds in Nigeria (Balogun and Fetuga, 1986). Copper which facilitates absorption of iron and enzymes production (Clifford, 1971) has a value of 2.61 mg/100 g which agrees with values reported by Dunu et al. (1986) for *Lagenaria siceraria* (2.7 mg/100 g). However the values obtained for 'guna' falls within the recommended dietary allowance of 3 mg for adult and 2 mg for children (Adeyeye and Fagbohon, 2005).

Magnesium helps in the prevention of circulatory diseases; it also helps in regulating blood pressure and insulin release (Onyiriuka et al., 1997). The magnesium content of some legumes ranges from 140 to 190 mg/100 g (Apata and Ologhobo, 1994). These values are quite high compared to values obtained for 'guna' 71 mg/100 g. 'Egusi' melon however was reported as having Mg content of 31.4 mg/100 g (Ojieh et al., 2008.) Manganese is required for enzyme functions for many redox enzymes. 'Guna' has a manganese content of 11.0 mg/100 g, which is higher than the 'egusi' melon 1.7 mg/100 g, but far lower than in some legumes like soya bean, pigeon pea, and jack bean with manganese values ranging between 1500 to 3210 mg/100 g (Eka and Osagie, 1998). Zinc content of 35.50 mg/100 g was obtained in '*guna*'. This value is higherthan that obtained for 'egusi' melon by Ojieh et al. (2008); 1.2 and 7.4 mg/100 g for *Lageneria siceraria* by Dunu et al. (1986). Zinc is important in diet for many protein enzymes e.g.hemoglobin which prevent anemia (Lippard and Jeremy, 1994).

The anti-nutrients analyzed in 'guna' are shown in Table 3. Tannin was below detectable level, while oxalate and phytate were 0.383 and 0.115 mg/100 g, respectively. The values detected at a safe level that pose no danger in diets. Siddhuraju P, Beeker (2001) reported a safe or normal range of 4-9mg/100g for phytate and oxalate. Eka (1977) also reported that phytate and oxalate levels in traditional foods of the Northern Nigeria were below toxic levels. The levels of phytate and oxalate in 'guna' did not cause any interference with absorption of vitamins, minerals and other nutrients as anti-nutrients (Oxford Dictionary of Biochemistry and Molecular Biology 2006). The amino acid profile of 'guna' in Table 4 shows that 'guna' has all the essential and non essential amino acids and is considered a "complete protein" for human nutrition (en.wikipedia.org/wiki/soy-protein) (Pellet and Young, 1980).

Glutamic acid, arginine and aspartic acid with 22.20, 15.20 and 9.33 g/100 g protein respectively are the most abundant in 'guna' seeds. These closely agree with the values obtained by Ojieh et al. (2008), in 'egusi' melon and Aremu et al. (2006) in cashew nuts. Tryptophan has the least concentration in 'guna'. The essential amino acids in 'guna' seeds compares well with soya bean, and FAO/WHO as shown in Table 5. FAO source: Ene-obong

Table 4. Amino acid compositions of the protein concentrate (g/100g) protein of *Citrillus vulgaris* (*guna*) seed.

Amino acid	Concentration (g/100 g protein)
#Lysine	4.11
#Methionine	1.76
#Threonine	3.00
#Isoleucine	3.61
#Leucine	5.66
#Phenylalanine	5.26
#Valine	4.41
#Tryptophan	1.10
#Histidine	2.06
Arginine	15.20
Serine	5.62
#Cysteine	2.33
Tyrosine	3.22
Alanine	5.10
Aspartic acid	9.33
Glutamic acid	22.20
Glycine	7.30
Proline	2.68
Asparagine	7.11
Glutamine	3.60

Essential amino acids.

Table 5. Essential amino acids g/100 g protein of '*guna*' seed compared with soya bean and WHO/FAO.

Amino acid	Soya bean	Guna	WHO	FAO
Isolencine	4.8	3.61	4.2	4.32
Lencine	8.0	5.66	4.2	4.90
Lysine	6.4	4.11	4.2	4.32
Phenylalanine	4.8	5.26	2.8	2.88
Tyrosine	3.2	3.22	2.8	
Cysteine	0.8	2.33	2.0	2.03
Methionine	0.9	1.76	2.2	2.30
Threonine	4.0	3.00	2.8	2.88
Tryphophan	1.3	1.10	1.4	1.44
Valine	4.8	4.41	4.2	4.32

Source: WHO and soya bean source: Temple et al. (1991

and Carnovale (1992).

CONCULSION

The nutritive value of 'Guna' can be harnessed as additives in food to supplement the protein content of our diets. It is rich in minerals and has negligible amounts of anti-nutrients. The amino acid profile of 'guna' shows that this particular seed is rich both in essential and non essential amino acids.

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