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Properties of Proximate Composition and Elemental Analysis of *Citrullus Vulgaris* (Guna) Seed

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ABSTRACT

This work analysed proximate composition, mineral elements and anti-nutrients in Citrullus vulgaris (guna) seed. Proximate composition of guna showed by percent dry matter of moisture, fat/lipid, crude protein, ash, crude fibre, carbohydrate, for undefatted, defatted and protein concentrate. The mineral elements in mg/100g include; sodium, calcium, zinc, iron, copper, manganese and chromium, but chromium was not detected in any of the three samples. The anti-nutrients determined were phytate, oxalate and tannin, in undefatted, defatted and protein concentrate. Tannin was not detected in any of the flours samples. Thus, 'guna' compares with other protein rich foods like soybean, pumpkin, cowpea and pigeon pea.
Keywords: Protein concentrate, Anti-nutrients, Defatted flour, Analysis, Oilseed, *Citrulus vulgaris*

INTRODUCTION

The production of food protein in sufficient amounts posses many problems especially since they are more expensive to produce than carbohydrate or lipids. In order to satisfy this steady growing demand for protein, new protein sources must be explored [1].

The *citrullus* class is an effective potential source. Works done on many varieties of *C. vulgaris* seed, had been found to contain a high protein value that makes the seed valuable as a good source of protein. It contains lipid, carbohydrate and minerals, making it a complete food [2].

Citrullus vulgaris is a member of the cucurbitaceae or the cucurbit family, commonly referred to as the gourd, melon, cucumber or pumpkin. Cucurbitaceae are found in warmer regions of the world [3] It is a major family with various economically important species particularly those with edible fruits e.g. water melon, sweet melon etc. They have both medicinal and nutritional uses [4].

C. vulgaris is grown widely in the tropic and also in the temperate regions of the world where it requires a lot of heat [5]. Its cultivation is simple and the fruits can produce up to 200-300 seeds per gourd [6]. It is a monoecius plant grown from seeds and creeps with plant population density of 20000-40000 plants per hectare [5]. It is estimated that one plant can produce up to 10 gourds [7]. *C. vulgaris* plant bears a roundish fruit which matures 3-4 months after planting [5]. In Nigeria more than 66,000 metric tons of melon seeds are produced per year [7]. The seeds are used for oil production at subsistence level in Nigeria [8], in several other African countries and in the Middle East [9]. They are used as cooking oil in these countries because of the unique flavour of the oil [10].

C. vulgaris is a variety of water melon, but unlike the common water melon whose flesh is sweet and red in colour, this variety of melon's juicy flesh is pale yellow or light green and tastes bitter [2] (Appen. 1).

C. vulgaris (guna) is yet another variety resembling the popularly known 'egusi' melon. It is a creeping annual plant with tendrils. The fruit is a berry containing many seeds. The seeds are smaller than the normal 'egusi' melon. (Apendices 2 and 3) This variety of melon is called 'guna' in Hausa, 'egusi ito' in Yoruba, 'ugbogoro' in Igbo, 'jiri' in Mbula, 'bawe' in Bwatiye, 'mwa'a' in Gude and 'chil' in kilba.

Apart from their potential as a source of protein, melon seeds generally are reported to contain approximately 35% protein by weight of decorticated seeds that have a nutritionally adequate amino acid profile [11].

'Guna' seeds could be used as raw material for the production of protein concentrate which play an important role in human nutrition and as a functional ingredient to improve protein content of processed foods [12].

They have nutritive and calorific values which make them necessary in our diets. The protein product of 'guna' seed is used as additives to food, in the same way, as soy protein [7]. The seed is used for local soup, in breakfast cereals and as snacks among the Mbula, Bwatiye, Bura, Kilba, Marghi in Adamawa States and among other tribes in Nigeria.

Food contains a variety of nutrients, but there is less awareness that many foods contain small amounts of potentially harmful substances. These are toxins or commonly referred to as anti-nutrients [13]. Since anti-nutrients are found in some levels in almost all foods for a variety of reasons, it is necessary to test for their presence in the 'guna' seeds, because Balogun and Fetuga (1988) reported that the seed is normally the most concentrated source of the toxic factors in plants [14].
Taking cognizance of the known nutritive value of *C. vulgaris* (guna), its medicinal and cosmetic importance [2], this work is going to analyse the raw (undefatted) flour, defatted flour and protein concentrate of 'guna' for proximate composition, anti-nutrient and elemental analysis.

MATERIALS AND METHODS

Seed Collection and Preparation

'Guna' seeds were collected from Borrong town in Demsa Local Government of Adamawa State, Nigeria, and a specimen of the plant kept in their herbarium.
The seed was screened to remove the bad ones and shelled manually. The shelled seeds were dried under shade, powdered and placed in polythene bags and stored in a desiccator for analysis.

Preparation of Protein Concentrate

This was prepared by the methods described by Ihekoronye [15] and Aremu *et al.,* [16]. One part of the raw flour was dispersed in six parts of warm water (50^0C), by stirring for 30mins. The pH of the dispersion was adjusted to pH 4.5 by drop wise addition of 0.1M HCl. The extraction was continued by occasional stirring for 1hr. The dispersion was centrifuged at 400rpm for 1hr to separate the solid, aqueous and oil/emulsion phases. The solid phase, which is the protein concentrate was air dried, sieved and kept in polythene bag for analysis.

Defatted Flour

The defatted flour was obtained by soxhlet extraction according to AOAC [17]. 100g of the raw flour was weighed in a thimble and placed in the inner chamber of the soxhlet apparatus. This was fitted to a round bottom flask containing the hexane and boiling chip with a reflux condenser fitted and extracted for 8hrs. The sample was removed from the thimble, air dried sieved and stored in sample bottle for further analysis.

Proximate composition

Proximate analysis was carried out on the raw flour (undefatted), defatted flour, and the protein concentrate according to the methods described by AOAC and Ibitoye [17, 18]

Mineral analysis

Determination of mineral elements was done according to the method of AOAC [17]. 2.0g of the sample was ashed in a furnace at 550^0C for 18hrs and the ash dissolved in 10ml of 0.1M HCl, filtered into a 100ml volumetric flask and made up to mark with distilled H_20. This was used to determine the mineral content by the use of Atomic Absorption Spectrophotometer (AAS) using prepared standards of the different mineral elements to be analyzed

Anti-nutrient Determination

Tannin

Tannin was determined according to the method by Trease and Evans [19]. 0.5g of the dry sample was boiled with 20ml of water. 0.1% $FeCl_3$ was added to observe for brownish green or blue-black colouration.

Oxalate

Oxalate was determined according to the method by Day and Underwood [20] 1.0g of the sample was dissolved in 100ml of 0.75M H_2S0_4. The solution was then carefully stirred with a magnetic stirrer for 1hr and filtered. 25ml of the filterate was pipetted and titrated hot (80—90^0C) against 0.1M $KMn0_4$ to an end point of a faint pink colour that persisted for more than 30 seconds. Result was calculated as follows:
T x constant (0.225)
T = Titre value

Phytate

The method by Reddy and Kove [21] was adopted for the determination of phytate. 4.0g of sample as soaked in 100ml of 2% HCl for 5hrs and filtered. 25ml of the filterate was pipetted into a conical flask and 5ml of 0.3% ammonium thiocyanate (NH_4SCN) solution was added. The mixture was titrated against 0.1M $FeCl_3$ until a brownish yellow colour end point that persisted for 5mins was obtained. The result was calculated as:
T x constant (0.1635)

T = litter value

RESULT AND DISCUSSIONS

The proximate composition of the different 'Guna' flour preparations are as shown in table 1.

Table (1.), Proximate Composition of Raw (Undefatted) Flour, Defatted Flour and Protein Concentrate of 'Guna'

Composition	Undefatted (raw)	Defatted	Protein Concentrate
Moisture	5.05 ± 0.1	5.50 ± 0.2	4.50± 0.3
Fat/Lipid	49.00 ± 0.2	5.50 ± 0.3	2.67± 0.1
Crude protein	36.58 ± 0.2	50.93 ± 0.3	83.56± 0.4
Ash	4.83 ± 0.4	4.85 ± 0.1	3.30± 0.2
Crude Fibre	4.00 ± 0.3	6.00 ± 0.3	4.00± 0.3
Carbohydrate	0.59 ± 0.4	27.22 ± 0.2	1.77± 0.4

Values are mean of three trails ± standard error
All the results are expressed as % by weight.

From table 1, the moisture content of guna flour (raw undefatted is 5.05% which is low compared to those reported for legumes by Arkroyed and Doughty [22] which range between 7.0 - 10%. However this value agrees with that reported by Osagie and Eka [23] for white melon, pumpkin and water melon seeds which were between 5 - 10%.

The defatted flour moisture content of 5.50% is above the values reported for defatted ebony seed (4.59%) Gonzalez *et al* [12] and lower than the 'red skin' groundnut (6.35%) as reported by Ihekoronye [15] while the protein concentrate moisture content of 'guna' (4.50%) is comparable with that of ebony seed (4.46%) Gonzalez *et al.,* [12] and 'red skin' groundnut 4.30% Ihekoronye [15]

Raw (undefattted) flour showed high fat/lipid content. The value of 49.00% in guna falls within the range of oil seeds (43 - 51%) as reported by Ige *et al,* [24]. The lipid/fat content of 'guna' is however higher than egusi melon (45.7%) as reported by Ojieh *et al.,* [2]. The defatted flour and protein concentrate have low fat/lipid content of 5.50% and 2.67% respectively. The defatting process with hexane of the raw flour decreased the lipid content in the defatted and protein concentrate [12].

The crude protein in the raw (undefatted) flour is lower than the defatted (50.93%) and the protein concentrate (83.56%) values. The crude protein value (36.53%) of the raw flour competes favourably with those of protein rich food such as ebony seed (36.10%) [12], pumpkin, water melon and other melon varieties which range between 25.8 – 38.1% [23], cowpea, lima bean, pigeon pea with protein values ranging between 23.1 – 33.0% [25] are below that in 'guna' while the 'guna' value is lower than soya bean with 45.83% [26].

The defatted flour recorded crude protein content of 50.93% which is lower than in ebony seed (52.45%) [12] and the 'red skin' ground nut (66.17%) as reported by the Ihekoronye [15]. While protein concentrate has crude protein content as high as 83.56%, which is below the protein concentrate of 'red skin' groundnut (88.90%), but the value is above that of some Nigerian legumes such as Lima bean (70.20%), African yam (78.40%) [27] and mucuna bean 78.30% [28].

The crude fibre content of guna for undefatted, defatted and protein concentrate are 4.00%, 6.00% and 4.00% respectively. These values are above that obtained by Gonzalez et al., [12] in ebony seed (3.64%, 3.82% and 1.62%). Ojieh *et al.,* [2] reported crude fibre content as high as 12.0% in egusi melon, while Aremu *et al.,* [16] reported as low as 0.9% in cashew nuts. Osagie and Eka [23] reported crude fibre content of some varieties of melon seeds ranging between 2.0 – 8.2%. 'Guna' falls within this range.

The ash content of 'guna' 4.83% in undefatted flour and 3.50% in protein concentrate are above the recommended range for seeds (1.5 – 2.5%) for animal feed formulation [29]. 'Guna' may not therefore be recommended in feed formulation. Some reported ash values are 3.7% in egusi melon [2] and 9.0% in *Cucumeropsis edulis* [24]

The carbohydrate content of undefatted flour is negligible (0.59%) compared to the value obtained in the defatted and protein concentrate flours, which are 27.22% and 1.77% respectively. This is because the hexane treatment of the raw flour concentrates the carbohydrate and protein. [12]. The carbohydrate value of the undefatted flour is far below the values reported for other varieties of melon seeds e.g. egusi melon (10.6%), *Coloncynthis citrullus (5.1%)* [23,2]. From the results, it is only the carbohydrate value of the defatted flour (27.22%) that falls within the value of other legumes which are as high as 20.0 – 60.0% [2]

The anti-nutrients analysed in 'guna' flour (undefatted, defatted and protein concentrate) are shown in Table 2. Tannin was not detected in any of the samples. This therefore implies no interference with digestion and absorption in monogastric animals [30].

Table 2: Some Anti-Nutrients in Guna Seed (mg/100g

Sample	Tannin	Oxalate	Phytate
Raw (undefatted) flour	ND	0.158	0.180
Defatted flour	ND	0.315	0.229
Protein Concentrate	ND	0.383	0.115

ND = Not detected

The value of phytate and oxalate obtained in the samples are very low 0.158mg/100g, 0.315mg/100g, 0.383mg/100g of oxalate, and 0.180mg/100g, 0.229mg/100g and 0.115mg/100g in raw (undefatted) flour, defatted and protein concentrates respectively. These values are low compared to values reported in other melon seeds e.g. water melon seed (2012.12mg/100g) phytate, and 40.65mg/100g oxalate [31]. Ebony seed was reported to have 0.725mg/100g phytate [12]. Pigeon pea has oxalate content of 310.50mg/100g [32]. Oladele *et al.,* [33] reported 21.42mg/100g phytate and 1.12mg/100g oxalate content in tiger nuts. Eka [34] reported that phytate and oxalate levels in traditional foods of the Northern Nigeria were below toxic level, which is in line with the values gotten from 'guna'

An oxalate diet limits the ingestion of oxalate to 40 – 50mg a day. Higher oxalate content contains more than 10mg per serving, while low content has less than 2mg per serving [35].

The minimum amounts of phytic acid to cause negative effect on iron and zinc absorptions are 10 – 50mg per meal [36]. In view of the aforementioned, the phytate and oxalate of 'guna' pose no danger in diet, as Siddhuraju and Becker [37] reported a safe or normal range of 4 – 9mg/100g for phytate and oxalate.

The result obtained for mineral values of 'guna' is shown in Fig.1. From the analysis, sodium has the highest value with 207mg/100g, 209mg/100g and 191mg/100g in the raw (undefatted) flour, defatted flour and protein concentrate respectively though the values fall below the World Health Organization (WHO) recommended daily intake of 500mg for adults and 400mg for children [37]. Copper has the least value of 13.0mg/100g, 1.31mg/100g and 2.61mg/100g for raw (undefatted), defatted and protein concentrate flours. Chromium was not detected in any of the samples.

The value of sodium is higher than in egusi melon (13.0mg/100g) [2] and some legume seeds like bambara nuts (0.05mg/100g), Jack bean (0.07mg/100g) pigeon pea (0.05mg/100g) [39].

Among the macro elements, it was only sodium and calcium that were analysed in 'guna' for reasons of their importance to metabolic activities. Calcium is an important mineral required for bone formation and neurological function of the body. The calcium content of raw (undefatted) (33.00mg/100g), defatted (42.00mg/100g), and protein concentrate (36.50mg/100g) are below the calcium values obtained in other legume seeds, e.g. lima bean (68mg/100g), pigeon pea (124mg/100g), jack bean (132mg/100g) [39] but is higher than in egusi melon (28.2mg/100g) [2]. The calcium values of 'guna' falls below the WHO daily required intake of 800mg /day for both adults and children.

Iron is one of the elements with high content in 'guna' 136.00mg/100g in undefatted flour, 180.00mg/100g in defatted flour, 115.50mg/100g in protein concentrate. These values are higher than value report of *Lagenaria siceraria (43.5mg/100g)* [40] and in some under-exploited leguminous seeds in Nigeria as reported by Balogun and Fetuga [41] which range between 20.0 – 31.0mg/100g. However, Osagie and Eka [23] reported that melon seed, fluted pumpkin and soya bean meals have as high as 48510mg/100g, 54830mg/100g and 50800mg/100g iron respectively. The recommended dietary allowance for iron in adult and children is 10mg/day while female adult is 15mg/day. The results indicate that 'guna' is rich in iron and its value is far above the recommended standard. Iron is required for blood haem formation [42].

The copper content of the samples is the least with 3.27mg/100g in undefatted flour, to 1.31mg/100g in defatted and 2.61mg/100g in protein concentrate. These values are higher than in egusi melon 0.4mg/100g [2]. The copper content of 'guna' falls within the values reported by Ilelaboye and Pikuda [31] for *Jatropha curcas* (1.6mg/100g) and *Trichosanthes cucumerina* (2.9mg/100g). It is also within the range reported by Duna *et al.,* [40] for *Lagenaria siceraria* (2.6mg/100g). Copper is a mineral that facilitates the absorption of iron and its value may account for the value of iron in the samples [43].

Copper is also required in the body for enzyme production and biological electron transfer. The value of copper in 'guna' falls within the recommended dietary allowance of 3mg/day for adult and 2mg for children.

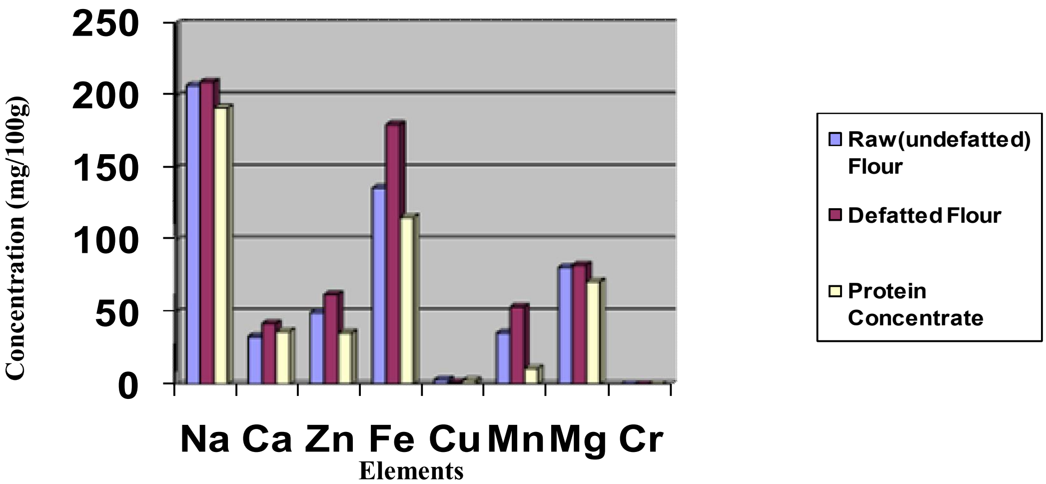

Fig. 1: Mineral Element Composition mg/100g

Magnesium has 81.00mg/100g in undefatted flour, 82.00mg/100g in defatted and 71.00mg/100g in protein concentrate. The magnesium contents of some legumes range from 140mg/100g - 190mg/100g [39]. These values are quite high compared to values obtained for 'guna'. Egusi melon was reported to be lower than 'guna' with magnesium content of 31.4mg/100g [2]. Magnesium is very important in calcium metabolism in bones and also involved in prevention of circulatory diseases. It helps in regulating blood pressure and insulin release [44,45]. Recommended dietary allowance of magnesium in adult is 350mg /day, while in children is 170mg/day [46] The result for 'guna' is far below the recommended values.

Manganese content of guna 35.50mg/100g in undefatted, 53.50mg/100g in defatted and 11.0mg/100g in protein concentrate are found to be higher than that in egusi melon 1.7mg/100g, but far lower than in legumes like soybean, pigeon pea, jack bean, with manganese ranging between 1500mg/100g - 3210 mg/100g [23]. The required daily intake of manganese is 2.5mg [47] which shows 'guna' to have more than the daily required value.

Zinc content of guna was 49.50mg/100g in undefatted flour, 62.50mg/100g in defatted flour and 35.50mg/100g in protein concentrate. The zinc content of guna is higher than that obtained for egusi melon by Ojieh *et al.,* [2] 1.2mg/100g and for *Lagenaria siceraria* with a value of 7.4mg/100g [40]. Zinc is important in diet for many protein and enzymes e.g. haemoglobin to prevent anaemia [47]. WHO recommended standard for zinc in adult and children are 15mg/day and 10mg/day respectively. The 'guna' value of zinc is above the recommended standard.

Comparing the results of the proximate composition for the raw (undefatted) flour, defatted flour and protein concentrate, the parameters have close values except for fat and crude protein contents. Fat/lipid has the highest value in the raw (undefatted) flour (49.0%) and the protein concentrate with the least fat/lipid content of 2.76%. The protein concentrate on the other hand has the highest crude protein content with a value of 83.56%. This results show that 'guna' is a good source of protein and oil in diet. Defatted flour has the highest carbohydrate content of 27.22% compared to .059% in undefatted.

The anti-nutrient content of guna did not decrease with defatting or protein concentrate preparation though the values seem negligible, they are increased rather. Soaking, cooking, fermenting, dehulling and germinating processes have been reported to reduce the anti-nutrient content of food. [48]. The processes of defatting and the protein concentrates have little increase on the anti-nutrient content though the values pose no threat. The mineral values increased with defatted flour. This could be due to lack of freedom of expression of the minerals in the raw (undefatted) because of the fat, but with the removal of the binder could be freely expressed in the defatted flour. The protein concentrate processes allowed salts to leach out from the 'guna' flour [49], this might have accounted for the lower values of minerals compared to defatted and undefatted flours.

CONCLUSION

Comparing the result of the proximate analysis of the guna flours: undefatted, defatted and protein concentrate with those required in other food minerals, it is noted that the guna has a considerable amount of protein which enhances enzymes activity in the body. The fat/lipid content makes it a good source of oil, and an energy source in the body.

The mineral analysis in table 3 shows 'guna' as a good source of minerals in the diet and hence can contribute to the maintenance of ion balance, acid balance, teeth and bone development in young children, regulation of muscles and nerve irritability in human health [50].

The low content of phytate and oxalate in 'guna' makes it safe for mineral utilization in the body and also for safe consumption in diet.

From this research, 'guna' seed flour can be used in any of the three categories depending on what is desired from the 'guna' seed.

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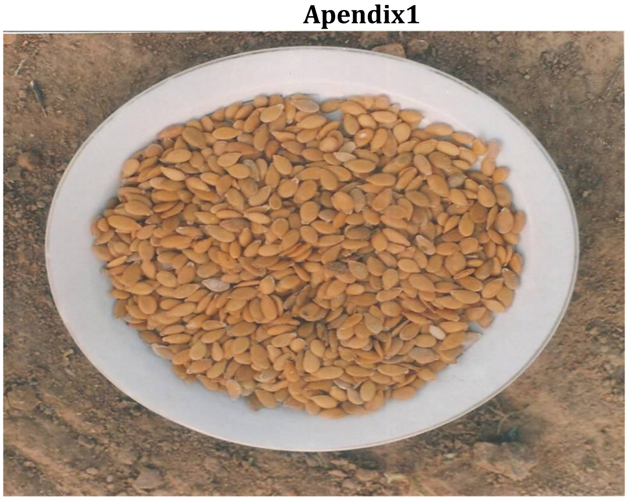
Apendix1



Apendix2

Apendix3