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Characteristics of Chinese bottle gourd (*Lagenaria siceraria*) seed flour

O.OLAOFE and E.I. ADEYEYE*

Department of Chemistry, University of Ado Ekiti, P.M.B. 5363, Ado-Ekiti (Nigeria).

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ABSTRACT

The Chinese bottle gourd (*Lagenaria siceraria*) seed flour was evaluated for physical dimensions/ properties, proximate composition, energy contributions by the proximate values, some anti-nutrients, fatty acids composition and the fatty acids levels in the flour sample. These proximate levels were high (g/100 g sample): crude fat, crude protein and total energy with calculated total fatty acids as 28.0 %. Proportion of total energy contributed by fat was 60.9 % and utilizable energy due to protein was 17.5 %. All the anti-nutrient values were low and percentage phosphorus due to phytic acid was just 0.84 %. The highest concentrated fatty acid was C18:2n-6 (67.7 %) and n-6/n-3 was 41.1. C18:2n-6 (67.7 %) was contained in 19.0 g of seed flour whereas C18: 3n-3 (1.65 %) was contained in 0.46 g of the seed flour.

Key words: Chinese bottle gourd seed flour, characteristics, chemical composition.

INTRODUCTION

Lagenaria siceraria and its fruits are widely cultivated in Nigeria from the savannah region of the North to the forest areas of the South. It belongs to the family Cucurbitaceae with the common name of bottle gourd, calabash gourd, etc. It is a herbaceous annual climber and trailer of about 4.5 m long. The seed is used as a substitute for melon. The seeds have a dark brownish testa with its endosperm oily but there is no industrial utilization in the country due to lack of information and non-extensive research in terms of food supplement as compared to soybean. There are many variants of the Chinese bottle gourd.

The Chinese bottle gourd has a round bulge at the top with a bowl or bulb shaped bottom separated by a chubby neck. Crafters favour this gourd, as they are shaped like a showman and can be crafted to resemble people and a variety of characters[1].

Ogungbenle *et al*[2]. have reported on the chemical and energy evaluation of some underutilized legumes flours. The chemical composition, proximate composition and mineral composition of some oil seeds have been reported by Olaofe *et al*. [3]

Ogungbenle [4] had also reported the proximate, amino acid compositions and functional properties of gourd seeds, white melon, yellow melon, benniseed and bulma cotton seed. The proximate composition and anti-nutrients in some oil and gourd seeds had been evaluated by Olaofe *et al*.[5,6] . Aremu and Olaofe[7] have reviewed the fats and fatty acid composition of some plant foods found in Nigeria. In all these reported results, there was no indication of any particular work carried out on the specie of *Lagenaria siceraria* . Also, none of the reported works precisely and strictly analyzed for the proximate, anti-nutrients and fatty acids compositions of the Chinese bottle gourd seed flour. This work reports on the physical characteristics and properties of *L .siceraria*; its proximate, anti-nutrients, fatty acids compositions and calculated levels of energy contributions by the proximate composition values, total fatty acids (g/100 g) and fatty acids content in given values of *L. siceraria* seed flour.

MATERIAL AND METHODS

Collection and treatment of samples

Matured samples of Chinese bottle were obtained from Are-Ekiti, Nigeria. The bottles were broken and the seeds removed and screened to select the good seeds. The seeds were divided into two groups (A and B). Group A was used for the determination of the physical properties whilst group B was used for the determination of the proximate composition, anti-nutrients and fatty acids composition. In the laboratory, the seeds were shelled, dry milled and stored in freezer in McCartney bottles pending analysis.

Proximate analysis

Moisture, ash, crude fat and crude fiber were determined by the AOAC methods[8], while nitrogen was determined by the micro-Kjeldahl method[9] and the percentage of nitrogen was converted to crude protein by multiplying by 6.25.The carbohydrate was determined by difference. The crude fat value was used to calculate the total fatty acids by multiplying with a conversion factor of 0.80 (for vegetables and fruits)[10]. The calorific values in kiloJoules were calculated by multiplying the crude fat, protein and carbohydrate by Atwater factor of 37, 17 and 17 respectively. Determinations were in triplicate.

Determination of anti-nutrients

Quantification of tannin and phytin

Finely milled flour (200 mg in 10 ml of 70 % aqueous acetone) were extracted for 2 h at 30^0 C in a water-bath using a Gallenkamp orbital shaker (Electro Ltd, Avon, UK) at 120 r.p.m. Pigments and fat were first removed from the sample by extracting with diethyl ether containing 1 % acetic acid. Therefore, the total polyphenols (as tannic equivalent) were determined in 0.05 ml aliquots in test tubes by the addition of distilled water to make it to 1.0 ml, followed by the addition of 0.5 ml of the Folin Ciocalteau reagent (Sigma, St Louis, MO, USA) and then 2.5 ml of sodium carbonate solution. The tubes were vortexed and the absorbance recorded at 725 nm after 40 min as described by Makkar and Goodchild[11]. The amount of total polyphenols (as tannic equivalent) was calculated from the standard curve.

For the quantification of phytin, 8 g of finely ground sample was soaked in 200 ml of 2 % hydrochloric acid and allowed to stand for 3 h. The extract was therefore filtered through two layers of hardened filter paper. Filtrate of 50 ml was pipetted in triplicate into 400 ml capacity beakers before the addition of 0.3 % ammonium thiocyanate solution as an indicator, and 107 ml of distilled water to obtain the proper acidity (pH 4.5). The solution was then titrated with a standard iron chloride ($FeCl_3$) solution containing 0.00195 g Fe/ml until a brownish yellow colour persisted for 5 min. A 4:6 Fe/P was used to calculate phytin-phosphorus and phytin content was calculated by multiplying the value of phytin-phosphorus by 3.55[12]. Each milligram of iron is equivalent to 1.19 mg of phytin –phosphorus. Determinations were in triplicate.

Determination of oxalate

One gram of the sample was weighed into 100 ml conical flask, 75 ml of 1.5 NH_2SO_4 added, stirred intermittently with magnetic stirrer for about 1 h and filtered using Whatman no.1 filter paper. 25 ml of sample extract was collected and titrated hot (80-90 ^{0}C) against 0.1 M KM_n0_4 solution to the point when a faint pink colour appeared that persisted for at least 30 sec[13]. Determination was in triplicate.

Determination of phosphorus

Phosphorus was determined by the Vanado-molybdate method[8].

Determination of fatty acid

Esterification procedure

The oil sample was sun-dried. 1.0 g of oil was weighed and heated in a borosilicate beaker container at 140 ^{0}F with pump running to allow homogeneity. Some drops of boron trifluoride were added to the oil in a fairly fast manner for proper distribution. The sample oil was homogenized. About 3 ml of methanol was added in a fast manner. The mixture was properly mixed whilst the fan blew the fumes away. The mixture was covered and temperature allowed to drop to ambient temperature (65 ^{0} F). The pump and fan were on occasionally for about 4 h. After cooling, the sweet fragrance ester was decanted into a clean borosilicate before injecting into the gas chromatography[8].

The GC conditions

The gas chromatography (GC) conditions for the analysis of fatty acid methyl esters were as follows:

GC	:	HP 6890 powered with HP ChemStation rev. A09.01 [1206] software
Initial temperature	:	60 ^{0}C for 3.0 min
First rate	:	8 ^{0}C/min to 140 ^{0}C for 10.0 min, constant at 140 ^{0}C for 5.0 min
Second rate	:	10 ^{0}C/min to 250 ^{0}C for 11 min, constant at 250 ^{0}C for 10.0 min
Detection temperature	:	275 ^{0}C
Injection temperature	:	230 ^{0}C
Detector	:	Flame ionization detector (FID)
Carrier gas	:	Nitrogen
Column type	:	HP-INNOWax (Cross-linked PEG)
Column length	:	30.0 m
Column i.d.	:	0.32 mm
Film thickness:		0.50 µm
Nitrogen pressure	:	30.0 psi
Hydrogen pressure	:	22 psi
Compressed air pressure	:	28 psi

The peaks were identified by comparison with standard fatty acid methyl esters.

RESULTS AND DISCUSSION

Physical dimensions of the seeds

The present results on the physical dimensions of the seed are shown in Table-1. Whilst the whole seed had a dark brown colour, the cotyledon had a cream colour. The cotyledon had a sharply pointed shape at one end like in the melon seed (*Citrullus vulgaris*) while the whole seed was generally oblong and pointed at one end. The dimensions of the whole seed were (cm): 1.29 (length), 0.62 (breadth), 0.23 (thickness) and 0.23 g/cm^3 (loosed density). The dimensions are important in the design of machine for industrial processing (shelling and milling) of the seeds. An area of interest is the percentage shell, which revealed an average of 42.3 % and 57.7 % shell and cotyledon respectively. Thus, in purchasing seeds, one would get a little above half of the seeds as cotyledon. This is a useful tool in trading of the whole seed which is expected to be competitive in the years ahead due to its nutritive importance and importance of its products industrially. Both the bulk and loose densities were lower than in other variety of *L. siceraria* whose respective values were 0.851 and 0.928 g/cc [14]. Also the % mass of kernel was 56.8 whilst it was 57.7 % in the present report.

Proximate composition

The proximate values of *L. siceraria* are shown in Table-2. The moisture content (6.52 g/100 g) was close to the value of 6.15 g/100 g for Chinese gourd kernel[14] but lower than 8.12 g/100 g in gourd seed reported by Badifu and Ogunsua[15]; melon seed (8.32 g/100 g), pumpkin seed (6.93 g/100 g)[3] but higher than 3.48 g/100 g in gourd seed[3]. The low

Table 1: Physical dimensions and properties of Chinese bottle gourd seed

Dimension	Whole seed	Cotyledon
Length (cm)	1.29	1.12
Breadth (cm)	0.62	0.43
Thickness (cm)	0.23	0.14
Colour	Brown	Cream
Shape	Oblong	Oblong
Bulk density (packed) (g/cm^3)	0.31	0.53
Loosed density (g/cm^3)	0.23	0.39
Percentage (%) mass	42.3(shell)	57.7

moisture content makes the Chinese kernel less susceptible to microbial attack, hence affording a long shelf- life for the seeds. The ash content was moderate but lower than 4.97 in reference [14,15]; 4.66 g/100 g in fluted pumpkin and 4.14 g/100 g in melon kernel[3]. This suggests that the kernel would provide essential minerals for body mechanism and development.

Table 2: Proximate composition of the Chinese bottle gourd seed flour (g/100 g)

Parameter	Concentration
Moisture content	6.52
Total ash	3.91
Crude fiber	4.29
Crude fat	35.5
Crude protein	37.1
Carbohydrate (by difference)	12.5
Total energy (kJ/100 g)	2157
Total fatty acids (0.8×35.5)	28.0

Table 3: Energy values as contributed by protein, fat and carbohydrate in the Chinese bottle gourd seed flour

Parameter	Seed flour
Total energy	2157
[a]Proportion of total energy due to protein %	29.2
[b]Proportion of total energy due to fat %	60.9
[c]Proportion of total energy due to carbohydrate %	9.85
[d]Utilizable energy due to protein %	17.5

[a]PEP ; [b]PEF ; [c]PEC ; [d]UEDP.

Table 4: Some anti-nutrients (mg/100 g) in the Chinese bottle gourd seed flour

Anti- nutrient	Mean value
Tannin	9.20
Phytin-phosphorus (Pp)	3.81
Phytic acid	13.5
Phosphorus (P)	451
Oxalate	1.80
Pp/P %	0.84

The kernel had high fat content of 35.5 g/100 g although lower than in gourd seed (50.6 g/100 g)[3], *C. vulgaris* (47.9 g/100 g)[16], pumpkin seed (47.0 g/100 g)[17], *Cucumeroplis edulis* (43.7 g/100 g)[16], and Chinese bottle kernel (46.1 g/100 g)[14]. However, the value was higher when compared with 23.5 g/100 g reported for soybean by Paul and Southgate[18]. This indicates that the Chinese bottle seed kernel is a better source of oil than soybean seed. Chinese bottle seed kernel could be used as a source of vegetable oil for industrial and domestic purposes.

The crude protein obtained (37.1 g/100 g) was close to the value of 39.5 g/100 g in Chinese bottle gourd kernel[14], a value which is high compared with crude proteins in protein-rich foods such as soybeans, cowpeas and pigeon peas[19], and some oil seeds such as melon seed (23.7 g/100 g), pumpkin seed (33.0 g/100 g) and gourd seed kernel (30.8 g/100 g)[3] and also higher than that of white melon (36.1 g/100 g)[15]. Therefore, Chinese bottle seed kernel could be an alternative source of dietary protein and would be a better supplement in enhancing the amino acid profile in highly malnourished areas, especially in developing countries like Nigeria where the majority of the populace live on starchy foods. The crude fiber of Chinese bottle seed kernel was 4.29 g/100 g which was higher than that of gourd seed kernel values of (g/100 g): 1.86[14] and 2.80 [4] respectively. The carbohydrate had a value of 12.5 g/100 g which was better than in gourd values in literature (g/100 g): 3.26 [14], 9.89 [4] and pumpkin seeds, 6.93 g/100 g[3] respectively. The calculated metabolizable energy value of 2157 kJ/100 g (2.16 MJ) was close to the value of 2.43 MJ[14], showing the sample to be a concentrated source of energy within the recommended energy dietary allowance for children[20]. The value was higher than those of legumes (1.59-1.69 MJ/100 g)[21], some vegetables (1.67-1.70 MJ/100 g) and 1.3-1.6 MJ/100 g in cereals [18].

Table 3 shows the various energy values as contributed by protein, fat and carbohydrate. The daily energy requirement for an adult man is between 2500–3000 kCal (10455–12548 kJ) depending on his physiological state whilst that of infants is 740 kCal, about 3095 kJ [22,23]. This implies that an adult man would require 485-582 g *L. siceraria* kernel to meet his energy requirement while infants would require 143 g. The utilizable energy due to protein (UEDP %) for the sample was 17.5. This value was greater than the recommended safe level of 8 % for an adult man who requires about 55 g protein per day with 60 % utilization. This shows that the protein concentration in the sample in terms of energy would be more than enough to prevent energy malnutrition in children (probably) and adult fed solely on the Chinese bottle seed kernel as a main source of protein. The PEF % was more than the recommended level 30 % [24] and 35 % [25] for total energy intake; this is useful for people wishing to adopt the guidelines for a healthy diet. The PEF % was however not bad for children under two years old who need fat for energy and nervous system development.

Table 5: Fatty acids composition of the Chinese bottle gourd seed flour

Fatty acid	Concentration (g/100 g)
Lauric acid (C12:0)	0.038
Myristic acid (C14:0)	0.016
Palmitic acid (C16:0)	0.069
Stearic acid (C18:0)	0.061
Palmitoleic acid (16:1n-7)	0.002
Oleic acid (C18:1n-9)	29.8
Linoleic acid (C18:2n-6)	67.7
Linolenic acid (C18:3n-3)	1.65
Unidentified peaks	0.695
Total saturated fatty acid (SFA)	0.184
Total unsaturated fatty acid (TUFA)	99.2
Monounsaturated fatty acid (MUFA)	29.8
Polyunsaturated fatty acid (PUFA)	69.3
n-6/n-3	41.1
PUFA/SFA	377

Anti-nutrients composition

Table 4 contains the concentration of the various anti-nutrients and the phosphorus levels. The tannin value was 9.20 mg/100 g. The nutritional significance of dietary tannins (especially in non-ruminants) derives, largely, from their ability to bind dietary proteins and digestive enzymes into complexes that are not readily digestible [26]. The poor palatability generally associated with high tannin diets is ascribed to their astringent properties which are a consequence of their ability to bind with proteins of saliva and mucosal membrane [27]. However, the tannin content in our sample can be said to be of very little nutritional significance. Our tannin result (9.20 mg/100 g) was very much lower than the values in the variously treated samples of *Glyciridia sepium* (1.7 g/100 g, leaf meal, LM) and (0.3 g/100 g leaf protein concentrate); and in *Leucaenia leucocephala* (5.5 g/100 g, leaf meal) and (2.5 g/100 g leaf protein concentrate, LPC)[28]; *Canavalia ensiformis* (0.3-0.9 g/100 g) and *Mucuna pruriens* (0.8-7.8 g/100 g) seed flours[29].

The phytin–phosphorus (Pp) level was 3.81 mg/100 g. Literature values of Pp were (mg/100 g): 0.2 and 1.9 *L. leucophala* LM and LPC respectively, 2.8 and 2.8 *G. sepium* LM and LPC respectively [28]; 1.4-5.22 in *C. ensiformis* and 1.7 -4.3 in *M. pruriens*[29]; however in some wild legumes (17 in number) it varied between 0.06-0.29 g/100 g [30].

The phytic acid (phytin) was 13.5 mg/100 g. In *L .leucocephala* it was 0.6 (LM) and 6.9 (LPC)

Table 6: Fatty acid levels in the Chinese bottle gourd seed flour (g)

Fatty acid	Fatty acid value	Levels of seed flour
C18:1n-9	29.8	8.34
C18:2n-6	67.7	18.96
C18:3n-3	1.65	0.46
Others	0.186	0.05
Unknown	0.695	0.20

in *G. sepium* [28]; in *C. ensiformis* it was 5.1-14.0 and 6.0-15.3 in *M.pruriens*[29]. The anti-nutritional nature of phytin, especially in non-ruminants, lies in its ability to chelate certain divalent metals such as Ca, Mg, Fe and Zn [31, 32], thereby rendering them metabolically unavailable. Our phytin level is low to cause any nutritional concern.

The total phosphorus was 451 mg/100 g which was lower than in 17 wild leguminous crop seeds with values of 0.18-0.56 g/100 g and the Pp as percentage of total P range was 11.4-84.1[30] whereas our Pp/P % was 0.84. This meant that only 0.84 % of the total phosphorus was linked to phytin. The nutritional implication of high phytin phosphorus rests on the fact that monogastric animals lack phytase, which can break down the phytin to release phosphorus for utilization; our sample does not have this problem.

The oxalate content was low at 1.80 mg/100 g. Oxalic acid has the ability to bind some divalent metals such as Ca and Mg and has therefore been suspected of interfering with the metabolism of these minerals. According to Blood and Henderson[33], the ingestion of an excessive amount of oxalate could cause gastrointestinal irritation, blockage of the renal tubules by calcium oxalate crystals, muscular weakness or paralysis. Plants generally tend to accumulate high oxalate levels during the early stages of growth[34]. We are not expecting any nutritional discomfort with the level of oxalate in our sample.

Fatty acids composition

Fat provides a major portion of man's energy supplies, giving weight-for –weight more than twice as much energy as proteins or carbohydrates[35]. Just as the proportion of carbohydrate in the human diet is influenced by ecological and economic factors, so also is the level of fat which varies considerably from 6-10 % in underdeveloped and overpopulated areas to 35-45 % in the more prosperous countries[35]. Table 5 shows the fatty acid composition of the oil from Chinese bottle gourd kernel. In our result, the saturated fatty acids (SFA) were (%): C12:0, C14:0, C16:0 and C18:0; the monounsaturated fatty acids (MUFA) include C18:1n-7cis and C18:1n-9 cis while the polyunsaturated fatty acid (PUFA) include C18: 2n-6 cis and C18:3n-3 cis. The total lipid content values of legumes vary with variety, origin, location and climate as well as seasonal and environmental conditions, and type of soil in which they are grown [36]. The saturated fatty acids (SFA) C12:0, C14:0 and C16:0 are the primary contributors to elevated blood cholesterol, and so contribute to cardiovascular disease. C14:0 is the main culprit. C18:0 is also thought to increase the risk of cardiovascular disease. The negative effect on the heart is probably due in part to an increase in blood clotting[37]. Both C12:0 and C14:0 were less than 0.06 % total fatty acids each, hence they are regarded as been present in trace amount[10]; on the whole, the SFA was only 0.184 % showing that the sample was very poor in SFA.

Linoleic acid (C18: 2n-6) was the most concentrated fatty acid (FA) in the sample and followed by oleic acid (C18:1n-9). C18: 2n-6 is the most concentrated fatty acid in pigeon pea (54.8 %)[38], soybean (52.0 %)[18], corn oil (55.7 %) and safflower oil (72.6 %) [39]. C18:2n-6 and C18:1n-9 are the major FA in peanut, soybean, chickpea, garden pea, broad bean and lentil. On the other hand, cowpea, black-eyed pea, kidney and California small white bean have linoleic and linolenic acids as the major fatty acids. Many lipids from legume seeds contain substantial acid amounts of SFA, especially palmitic acid (C16:0)[40,41]. A higher proportion of either C18:2n-6 or C18:3n-3 is associated with legumes containing insignificant amounts of lipids[42]. This may not be the case in all legumes as shown by the present report. The value of the unknown was 0.695 %; it is 9.83±2.30 % in the African yam bean (AYB) on the average[41], while it is 5.2 % and 0.9 % in pigeon pea (*Cajanus cajan*) and soybean, respectively[38].

The n-6 and n-3 fatty acids have critical roles in the membrane structure[43,44] and as precursors of eicosanoids, which are potent and highly reactive compounds, since they compete for the same enzymes and have different biological roles, the balance between the n-6 and the n-3 FA in the diet can be of considerable importance [45]. The ratio of n-6/n-3 in the diet should be between 5:1 and 10:1 [45]. Our present report put the n-6/n-3 as 41:1. This result suggests that consumers of Chinese bottle gourd seed kernel (as main oil source) should specifically include a regular intake of C18: 3n-3 or one of its related n-3 FAs, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). This would almost certainly require consumption of fatty fish-salmon, tuna, sardines, herring, mackerel, white fish, wild trout, swordfish and halibut at least twice a week[37]. This recommendation, for consuming n-3 FAs stems from the observation that compounds made from 3n-3 FAs tend to decrease blood clotting and inflammatory processes in the body, whereas the n-6 FAs (particularly arachidonic acid) generally increase those processes. Blood clots are part of the heart attack process. 3n-3 has a favourable effect on heart rhythm, thereby decreasing the risk of heart attack[37]. 3n-3 FA was low in the sample. The US Food and Nutrition Board recommendations per day were (g/day): omega-6, 17 (men) and 12 (women) (see Table-6, sample satisfied this); omega -3, 1.6 (men) and 1.1(women)[46]. A deficiency of n-6 FAs in the diet leads to skin lesions. A deficiency of n-3 FAs leads to subtle neurological and visual problems. Deficiency in PUFA produces growth retardation, reproductive failure, skin abnormalities and kidney and liver disorders. However, people are rarely deficient in this essential fatty acids.[47].

The PUFA/SFA (P/S) was 377. This is an extremely high result showing that virtually all the oil had no SFA. The P/S in AYB ranged from 0.54-0.72 with an average of 0.52 [41]; it was 0.52 in the kernel of *Terminalia catapa* (Tropical almond)[48]. The relative amounts of PUFA and SFA in oils is important in nutrition and health. The P/S is therefore important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is oil. This is because the severity of arteriosclerosis is closely associated with the proportion of the total energy supplied by SFA and PUFA [49,50].

Holland *et al*. [51] have shown that SFA in soybean is 12.4 %, MUFA is 18.8 % and PUFA is 48.9 %, whilst our results showed that SFA was 0.184 %, MUFA was 29.8 % and PUFA was 69.3 %. Table 6 shows the levels of some FAs as well as the quantity of flour sample that would produce them. This type of information is good at the user database level when values per 100 g of food are required[10].

CONCLUSION

The Chinese bottle gourd seed flour was high in fat , protein and metabolizable energy, both the PEF % and UEDP % were high, it was low in anti–nutrients, had insignificant levels of SFA but high MUFA and n-6 resulting in very high level of both n-6/n-3 and PUFA/SFA. These good characteristics of *Lagenaria siceraria* seed flour would make it a good food source. Overall, the oil is classified as polyunsaturated.

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