Chemical Composition of Some Defatted Gourd Melon (Egusi) Seed Flours

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ABSTRACT

Increase consumption of fatty foods may promote hypertension and obesity, which are well known risk factors of stroke. *Citrullus colocynthis, Citrullus vulgaris*, (*Lagenaria siceraria* I African Wine Kettle gourd (AWK), *Lagenaria siceraria* II Basket Ball Gourd (BBG) and *Lagenaria siceraria* III Bushel Giant Gourd (BGG) seeds flour samples were defatted using n-hexane. Proximate, energy and sugar compositions of the samples were determined on dry matter weight using standard methods. Oil extraction significantly increased the percentage protein from (24.37-34.64) % to (56.17-67.95) % and percentage carbohydrate from (3.23-10.88) % to (13.29-27.85) %. Gross energy of the full-fat samples (FFS) and defatted samples (DFS) ranged from (2,440.74 to 2,693.82) KJ/100g and (1,331.60 to 1,452.45) KJ/100g respectively. There were increase in the values of the sugar contents of the gourd seeds with oil extraction. Predominant sugars in the FFS was Lactose, D-Ribose and Maltose sugar; ranged from (65.90 to144.40) mg/100g, (48.93 to106.00) mg/100g and (60.71 to 93.69) mg/100g respectively. These three sugars were also predominant in DFS, ranging in the same order (82.81 to 168.89) mg/100g, (55.13 to 98.46) mg/100g and (49 to 85.88) mg/100g. High value of maltose will enhance the use of these gourd melon seeds in drinks and beverages. The relatively low values of glucose ranging from (31.71 to 49.13) mg/100g in the FFS and (36.67 to 57.18) mg/100g in the DFS makes them suitable for the consumption of people especially for those with diabetes and the low fat contents make them more heart friendly for patients with hypertension.

Keywords: *Melon (egusi) seeds, Oil extraction, Sugar content, Energy content*

INTRODUCTION

Plants are primary sources of medicines and food used by humans every day. Their roots, stems, leaves, flowers, fruits and seeds provide food for humans (Amaechi, 2009; Hemingsway, 2004). *Citrullus collocynthis, Citrullus vulgaris* and *Lagenaria siceraria* species are gourd melon (egusi) seeds grown in most parts of Nigeria. Gourd melon seeds belong to the family Cucurbitaceae. They are versatile and include hundreds of species of vine bearing coiled climbing tendrils and some of the most unusual fruits in the world. This plant family is known for its remarkable genetic diversity and prevalent adaptation that includes tropical and subtropical regions, arid deserts and temperate locations (Oluba et al., 2008). Seeds of cucurbits are sources of oils and protein with about 50% oil and up to 35% proteins (Oluba et al., 2008; Achu, et al., 2005). Egusi (*Colocynthis citrullus L*.) belongs to the species of the genus *Citrullus* of cucurbitaceae family, which usually consists of outsized amount of varieties that are commonly known as melons (Mabaleha, et al., 2007). *Colocynthis citrullus L*. is among the 300 species of melon found in tropical Africa and it is cultivated for its seeds, which are rich in oil (53%) and protein (28%) (Ntui, et

al., 2009). The regions of its cultivation include: Middle East, Nigeria, Ghana, Togo, Benin, Cameroon and some other countries in Africa for the foods in the seeds and as a crop inter-planted with maize, cassava and yam (Uruakpa and Aluko, 2004). Some *Lagenaria siceraria* gourds melon (egusi) seeds are grown in Yoruba land, Nigeria mostly for utility purposes. *Lagenaria siceraria* I (AWK), otherwise called Akeregbe in Yoruba land, *Lagenaria siceraria* II (BBG) is called Igbaademu and *Lagenaria siceraria* III (BGG) is known as Igba-je. However, some indigenous rural dwellers eat the seeds of these gourd plants as soup thickners and are called Melon or Egusi in Yoruba. Like some common egusi that have been worked on, they are very good sources of fats and protein, with fat content of about 50% (Ogundele and Oshodi, 2010; Ogundele et al., 2012). However, increased consumption of fatty foods may promote hypertension and obesity, which are well known risk factors of stroke (Nguemeni, et al., 2014). Myristic and palmitic acids have been established as the most important of the dietary risk factors in cholesterol high density (CHD) (Bender, 1992). High level of blood cholesterol is associated with the incidence of CHD which increases the LDL (low density lipoprotein in which 46% of the molecule is cholesterol) (Bender, 1992). Hence, it is important to see the effect of removal of the oil content of these melon seeds on the nutritional value of the seeds.

MATERIALS AND METHOD

The melon seeds used for this research work are *Citrullus colocynthis*, *Citrullus vulgaris*, *Lagenaria siceraria* I (African wine kettle), *Lageneria siceraria* II (Basketball gourd) and *Lagenaria siceraria* III (Bushel giant gourd). The seeds were bought from Ilora in Oyo State and in Akure. The seeds were identified at Federal Research Institute, Ibadan, Oyo State, Nigeria. They were dehusked, dried, picked and milled in a blender into flour.

Defatting of melon (egusi) flour samples

Defatted samples of the five varieties of gourd melon seeds were prepared by putting some quantities of the flour sample in soxhlet apparatus and refluxing continuously under heat with n-hexane (b.p. 40-60 ^{o}C) for nine hours.

Determination of Proximate Composition

Proximate analysis of the samples were carried out on dry matter and expressed in percentages, using standard procedures recommended by Association of Official Analytical Chemists (AOAC, 1990). The fat content (FC) was determined using solvent extraction method with n-hexane (b.p. 40-60 ^{o}C) in a soxhlet extractor. The moisture content (MC) was determined using air oven as weight difference after oven-drying for 4-5 hours at 105 ^{o}C. Crude Protein (CP) was determined by Kjeldahal method to determine percentage nitrogen content and converted to protein content as percentage Nitrogen x 6.25. Total ash Content (TAC) was determined by weight difference after incinerating a known weight to ash in a muffle furnace. The Crude Fibre (CF) was determined according to Pearson, 1981. Carbohydrate was determined by difference 100 % - ∑(FC, MC, CP, TAC, and CF)). The Proximate analysis was carried out in triplicates and the results are in % dry matter weight of samples (Ogundele and Oshodi, 2010).

Determination of Defatting Efficiency (DE)

The defatting efficiency (DE) was carried out on the defatted samples as follows:

$$DE = \frac{FCRM - FCDM}{FCRM}$$

Where:

FCRM = Fat content of raw melon

FCDM = Fat content of defatted melon

2.4 Determination of Total Sugar

The sugar content was determined by the method of Shaffer Somogyi sugar-

thiosulphate (AOAC, 1990).

Preparation of the reagent solution used in sugar analysis

(a) Shaffer Somogyi Carbonate 50 reagent 5.0 g KI, 25 g each of anhydrous Na_2CO_3 and KNa tartrate. $4H_2O$ (Rochelle salt) was dissolved each in 500 mL H_2O in large beaker. Solution of $CuSO_4.5H_2O$ (75 mL) with concentration of 100 g/L was added through a funnel with tip under surface, with stirring occasionally and 20 g $NaHCO_3$ and 5 g KI were dissolved in the large beaker. The whole mixture was then transferred to 1 litre volumetric flask and 250 mL 0.10 M KIO_3 was added and later made up to the mark in another one litre volumetric flask. The mixture was covered and left over night before use.

(b) Iodine-oxalate solution: -2.5 g KI and 2.5 g $K_2C_2O_4$ were dissolved in distilled water and diluted to 100 mL. The solution was prepared fresh.

(c) Thiosulphate standard solution:- 0.005 M $Na_2S_2O_3$ solution was prepared daily from standardized stock of 0.1 M $Na_2S_2O_3$ solution.

(d) Starch indicator: 2.5 g starch and 10 g HgI_2 in little water and then dissolved in 500 mL boiling water.

Determination of individual sugars

Sample of the flour (2.5 g) was dissolved in 20 mL distilled water and hydrolysed by using 20 mL 0.1 M H_2SO_4. 5 mL of the resulting solution was pipetted into 25x200 mm test tube and then 5 mL reagent (a) was added and the mixed well by swirling. The test tube was placed in boiling water and heated for between 15 and 35 minutes. The test tube was thereafter removed carefully without agitation to a running water and allowed to cool for about 4 minutes. The cap on the tube was removed and 2 mL KI-$K_2C_2O_4$ was gently added. The mixture was mixed thoroughly to ensure that Cu_2O was dissolved and then allowed to stand in cool water bath for 5 minutes with mixing done twice during the period. The remaining mixture was later titrated against 0.005 M $Na_2S_2O_3$ using starch indicator. The blank was equally run as described above and then the test solution titre value subtracted from the blank. The titration was repeated until two concordant results were obtained. The amount of sugar present was calculated based on the equation of Somogyi sugar-thiosulphate equivalents given in Table 1.

Table 1: Shaffer-Somogyi sugar- thiosulphate equivalent for sugar calculation

Sugar	Heating Time (minutes)	Equation
L-Arabinose	30.00	y = 0.1234x +0.060
Fructose	15.00	y = 0.113x + 0.079
D-Galactose	30.00	y = 0.1332x + 0.033
Glucose	15.00	y = 0.1099x + 0.048
Lactose	25.00	y = 0.2031x + 0.030
Maltose	30.00	y = 0.2199x + 0.072
D- mannose	35.00	y = 0.1148 + 0.084
D-Ribose	25.00	y = 0.1381x + 0.098
L-sorbose	15.00	y = 0.1244x + 0.116
D-xylose	30.00	y = 0.1130x + 0.044

AOAC, (1990); (y = mg sugar in 5 mL; x = mL of 0.005 M $Na_2S_2O_3$)

Determination of Calorific energy values using bomb calorimeter

Ballistic bomb calorimeter (Gallenkamp CBB-330-030F) was used to ignite about 5 g of each sample electrically and burned in excess oxygen (with recommended oxygen pressure of 25 atmospheres) in the bomb. The maximum temperature rise of the bomb calorimeter was measured with the thermocouple and galvanometer system. The rise in temperature obtained was compared with that of benzoic acid to determine the calorific/energy values of the sample materials. Energy was also calculated using Atwater factor (FAO, 2002).

Statistical Analysis

One way analysis of variance (ANOVA) and least significance difference (LSD) were carried out on the replicate data generated using SPSS 18. The results are expressed as mean ± standard deviation. Duncan was also used to determine values that are significantly different with $p \leq 0.05$ (Ogundele and Oshodi, 2010).

RESULTS AND DISCUSSION

Proximate composition of full-fat seeds FFS and defatted flours samples DFS

The proximate composition values of the full-fat seed (FFS) flour samples and defatted flour samples (DFS) are relative to dry matter (mg/100 g dw) and are presented in Tables 2 and 3 respectively. The proximate composition of the five varieties of gourd melon (egusi) seeds in Table 2 (Ogundele and Oshodi, 2010; Ogundele et al., 2012) shows that the seeds are very high in crude fat content, ranging from 46.03±1.14 to 56.61±0.10 % with *Citrullus colocynthis* having the highest value and *Lagenaria siceraria* I (AWK) having the least value. On the average, the crude fat content of the five varieties of gourd seeds is 51.30 %. These values are consistent with the fat contents of some other melons in the same family like *Citrullus lanatus, C. mannii and C. melo* which are (56.67±4.90, 45.89±4.73, 42.67±3.43) respectively as reported by Loukou et al., (2007).

The fat contents of these varieties of melon seeds are however higher than the fat contents of seeds like chick pea (1.5 %) (Sanche-Vioquez et.al., 1998) and African yam bean (0.58 to 1.79 %) (Oshodi et al., 1995). Hence, *Citrullus colocynthis, Citrullus vulgaris, Lagenaria siceraria I (AWK), Lagenaria siceraria II (BBG) and Lagenaria siceraria III (BGG)* seeds have high vegetable oil content for human consumption and for industrial applications such as in the cosmetics and food industries.

Table 2: Proximate composition (%) of full-fat gourd seed flours

Parameter	Sample				
	Ogundele et al., 2012		Ogundele and Oshodi, 2010		
	C.colocynthis	C.vulgaris	LSI(AWK)	LSII(BBG)	LSIII(BGG)
Protein	24.37^{a}±2.13	32.96^{c}±2.53	34.64^{c}±0.08	27.71^{b}±0.41	32.70^{c}±0.35
Fat	56.61^{d}±0.10	49.59^{b}±1.40	46.03^{a}±1.72	53.35^{c}±0.24	50.91^{b}±1.57
Moisture	3.08^{b}±0.80	2.75^{a}±0.27	5.67^{d}±0.06	5.13^{c}±0.04	5.67^{d}±0.11
Ash	3.15^{a}±0.30	3.53^{ab}±0.32	3.75^{bc}±0.17	4.07^{cd}±0.22	4.50^{d}±0.22
Fibre	1.91^{a}±1.00	2.00^{a} ±1.00	1.62^{a}±0.25	0.75^{a}±0.15	2.99^{a}±0.50
Carbohydrate	10.88^{b}±3.03	9.17^{b}±2.88	8.75^{b}±1594	8.99^{b}±0.76	3.23^{a}±1.90

Values with different superscriptions on the same row are significantly different at $p \leq 0.05$ (Ogundele and Oshodi, 2010; Ogundele et al., 2012)

Defatting these oil seeds flour samples with n-hexane was very effective as presented in Table 3.

The defatting efficiency ranges from 95.56 to 99.17 %, showing that virtually all the seed oil was effectively removed with only a negligible fraction left during oil extraction. The fat content of the defatted seeds ranges from 0.47±0.35 % *(C. colocynthis*) to 2.20±0.79 % for *C. vulgaris*. These results are similar to the report given for the fat content of some defatted samples like defatted *Cassia fisula* seed (0.39) % (Akinyede and Amoo, 2009).

Table 3: Proximate composition (%) of some de-fatted gourd melon seed flours

Parameter	Sample				
	C.colocynthis	C.vulgaris	LSI(AWK)	LSII(BBG)	LSIII(BGG)
Protein	56.17^{a}±4.91	59.76^{ab}±0.13	67.95^{b}±0.28	62.04^{ab}±2.17	58.78^{ab}±4.26
Fat	0.47^{a} ±0.35	2.20^{c}±0.79	0.77^{ab}±0.73	0.96^{ab}±0.36	1.64^{bc}±0.69
Moisture	4.64^{ab}±0.76	5.73^{ab}±0.13	3.72^{a}±0.76	9.13^{c}±2.17	7.07^{bc}±1.48
Total Ash	6.46^{a}±1.19	6.42^{a}±1.28	8.75^{b}±0.72	8.31^{ab}±1.38	11.96^{c}±0.25
Fibre	4.39^{bc}±2.32	5.00^{bc}±1.00	5.52^{c}±0.77	3.42^{abc}±0.85	4.59^{bc}±0.91
Carbohydrate	27.85^{b}±5.16	20.89^{ab}±9.25	13.29^{a}±1.41	16.12^{a}±2.80	15.98^{a}±6.16
DE	99.17	95.56	98.33	98.20	96.78

Values with different superscriptions on the same row are significantly different at $p \leq 0.05$.
DE is the % Deffatning Effectiveness

The values of the percentage crude protein (dw) for *Citrullus colocynthis*, *C. vulgaris*, *Lagenaria siceraria I (AWK), L. siceraria II (BBG)* and *L. siceraria III (BGG*) seed flours in Table 2 are 24.37±2.13, 32.96±2.53, 34.64±0.08, 27.71±0.41 and 32.70±0.35 % respectively with an average of 30.48 %. These values are higher than the protein contents of five cultivated African yam bean ranging from (20.18 to 25.78) % (Adeyeye, 1997); whole *Adenopus breviflorus* seed (28.60 %) (Oshodi, 1992) and chickpea (24.70 %) (Sanchez-Vioque, 1998). These melon (egusi) seed flour varieties are good sources of protein and can compete favourably with some other good sources of protein like Conophor nut (29.09 %), Jatropha curcas seeds (29.40 %) and Cucumeropsis edulis which is another variety of "egusi", that was reported to contain 31.85 % protein (Akpabange et al., 2008). Hence *C. colocynthis, C. vulgaris, L. siceraria I (AWK), L. siceraria II (BBG) and L. siceraria III (BGG)* are essentially good sources of protein for human consumption and can be good substitutes for animal protein for the fast growing need of protein for children and the fast growing world population. In addition, they can also serve as good sources of protein concentrate for human and animal food formulation.

More importantly, the percentage protein content of the seeds is reasonably increased by defatting as presented in Table 3. The protein content of the defatted gourd seed flours ranges from (56.17±4.91) % for *(C.colocynthis*) to (67.95±0.28) % for *(L. siceraria I*). Defatting therefore increased the protein content of the seeds under study by 130.49, 81.31, 91.13, 123.89 and 79.76 % *for C. colocynthis, C. vulgaris, L. siceraria I (AWK), L. siceraria II (BBG) and L. siceraria III (BGG)* respectively.

The crude fibre contents of these seed flours are considerably low with values ranging from (0.75±0.15) % (*L. siceraria* II) to (2.99±0.50) % (*L. siceraria* III) as seen in Table 2. There is however no significant difference in these values at $p \leq 0.05$. These values are comparable with the crude fibre reported for several indigenous cucurbits like the varieties of African yam beans (ranging 1.61 to 2.38) % (Adeyeye, 1997), peal millet (1.8±0.30 %) (Oshodi et al., 1999), *Cucurbita lanatus* (1.33 %) and for raw *Jatropha catharica* (1.60 %) (Oladele, 2008). On the other hand, the crude fibres of the defatted samples are higher with values ranging from 3.42±0.85 % for *L. siceraria II* to 5.52±0.77 % for *L .siceraria I.* The increase in the fibre content of the defatted samples is possibly due to the relative availability of the fibre that was increased after removing almost 100 % fat from the samples. The high values of the fibre of the defatted gourd seed flours will aid the rate of digestion and absorption compared with the FFS. Hence, defatting makes the fibre in the gourd melon seed samples to be available more as roughages. Fibre consists of cellulose and hemicellulose, a heterogeneous group in which pentosan usually dominate over lignin and pectin substances. There is now evidence that dietary fibre has a number of beneficial effects related to its indigestibility in the small intestine (Asp, 1996). Due to physical properties, dietary fibre and polysaccharides also influence digestion and absorption processes in the small intestine (Cherbut et al., 1995).

Sugar content of FFS and DFS

The sugar contents of the FFS and DFS are seen in Figures 1 and 2. There is general increase in the values of the sugar contents of the gourd seeds with defatting Figure 3. This is consistent with the increase in carbohydrate values of the seeds with defatting as in Tables 2 and 3.

The predominant sugars in the FFS are Lactose, D-Ribose and Maltose sugar; ranging from (65.90 to144.40) mg/100g, (48.93 to106.00) mg/100 g and (60.71 to 93.69) mg/100 g respectively; while in the DFS, these three sugars are still predominating. The range of these sugars in DFS are: Maltose (82.81 to 168.89) mg/100g, Lactose (55.13 to 98.46) mg/100g and D-Galactose (49 to 85.88) mg/100g. Lactose and Maltose are both disaccharides, hence on the average, the gourd melon seeds have high disaccharide content. The high Lactose value is likely to account for the milky appearance of the FFS and DFS in solution, making the melon seeds of possible quality for infant mixes formulation. Maltose on the other hand will enhance the use of these gourd melon seeds in drinks and beverages. Xylose content in both the FFS and DFS arerelatedly low. Xylose is classified as a monosaccharide of the aldopentose type, which means that it contains five carbon atoms and includes an aldehyde functional group. It is the precursor to hemicellulose, one of the main constituents of biomass.

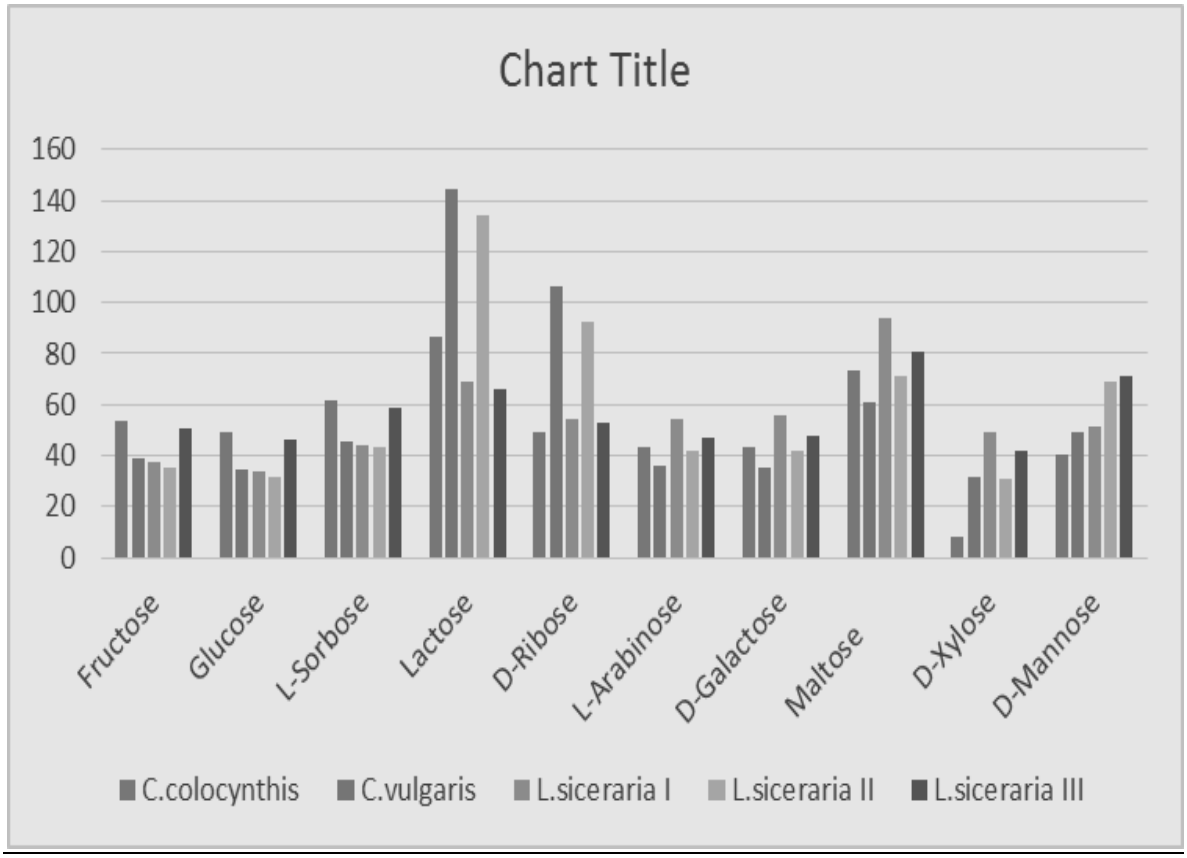

Figure 1: Varieties of sugar contents of full-fat gourd seed flour samples (mg/100g)

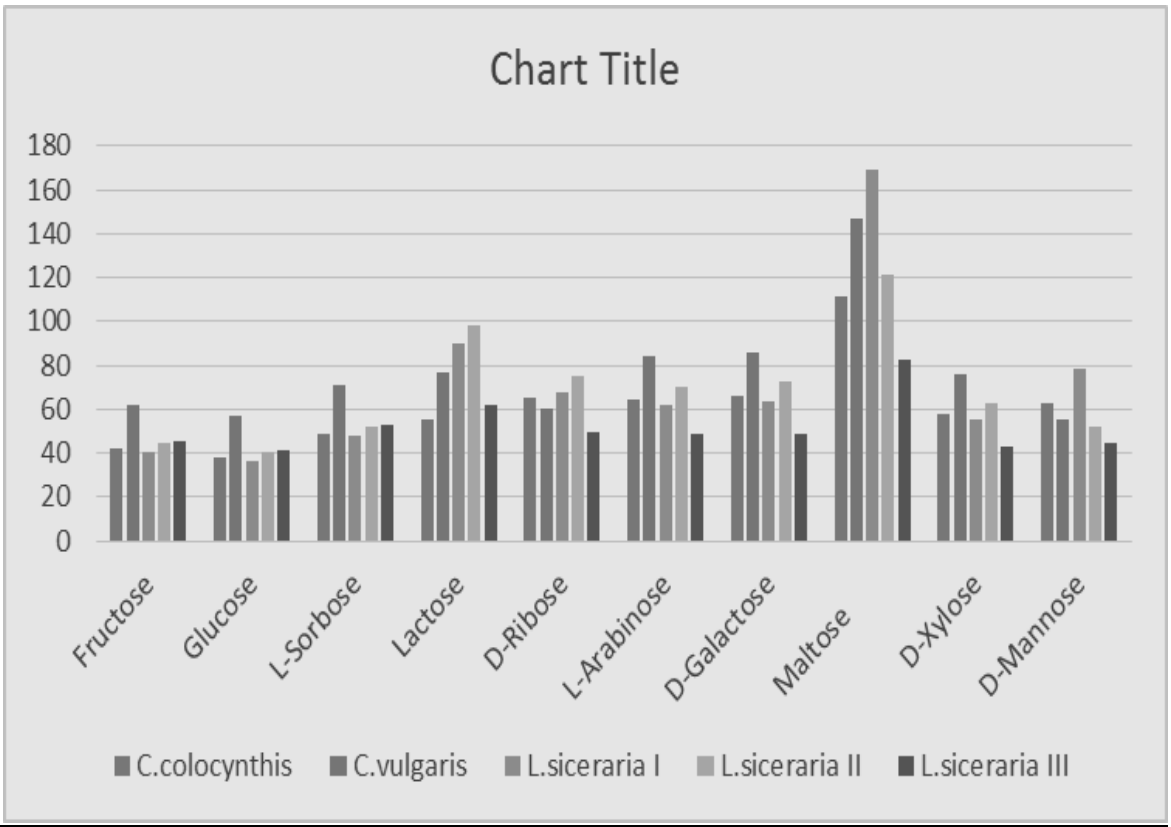

Figure 2: Varieties of sugar contents of de-fatted gourd seed flour samples (mg/100g)

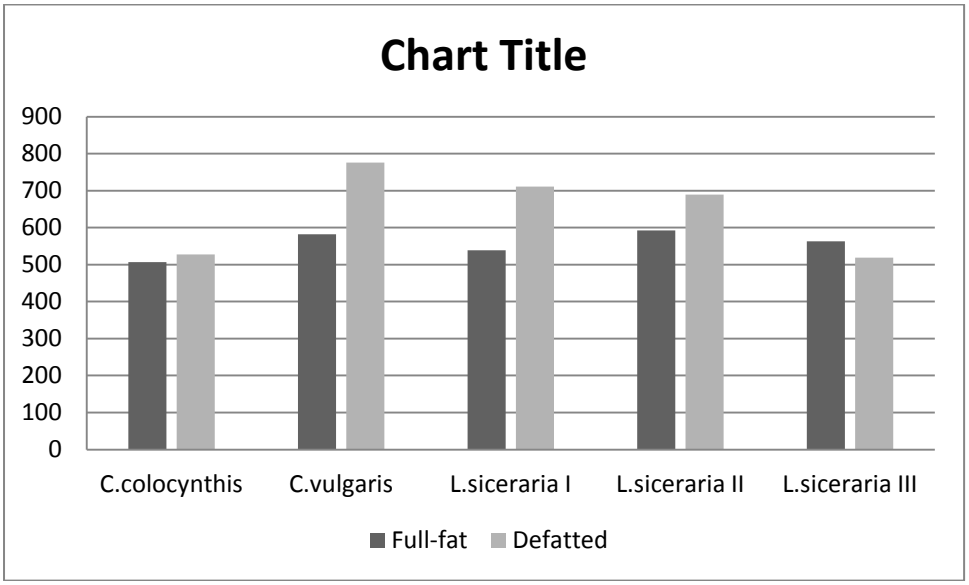

Figure 3: Comparison of the total sugar content of full fat and de-fatted gourd seed flour samples (mg/100g)

Xylose is also the first saccharide added to the serine or threonine in the proteoglycan type O-glycosylation, and, so, it is the first saccharide in biosynthetic pathways of most anionic polysaccharides such as heparin sulphate and chondroitin sulphate (Wikipedia, 2015). The values of the total sugar of the raw gourd melon seed flours range from 506.47 mg/100g (*C. colocynthis*) to 592.27 mg/100g (*L. siceraria* II BBG). There is corresponding increase noticed in the total sugar values of the defatted gourd seeds with values (mg/100g) ranging from 527.62 (*C. colocynthis*) to 775.96 (*C. vulgaris*). This is possibly as a result of corresponding increase in the Carbohydrate values of the defatted gourd melon seeds compared to the lower Carbohydrate values of the raw gourd melon seed. Fructose ranges from 35.40 mg/100g to 53.48 mg/100g in the raw samples and 40.67 mg/100g to 61.73 mg/100g in the defatted flour samples. Glucose, on the other hand ranges from 31.71 mg/100g to 49.13 mg/100g in the raw samples and 36.67 mg/100g to 57.18 mg/100g in the defatted samples. The relatively low values of the monosaccharide especially glucose and fructose is an importance factor. The importance of blood glucose response after a meal is often expressed as the glycaemic index (Asp, 1996). The low glucose content of these melon seeds is a factor that enhances their suitability for the consumption of adults especially the hypertensive ones.

Energy Content (EC)

Tables 4 shows the energy contents of the full-fat and defatted gourd melon (egusi) seeds. The gross energy of the raw melon samples determined using bomb calorimeter ranges from (2685.77 to 1275.08) KJ/100g for *L. siceraria (III)* and *L. siceraria (I)* respectively. The calculated gross energy for the raw melon samples ranges from (2440.74 to 2693.82) KJ/100g. The calculated gross energy values got from the result of the proximate analysis of protein, carbohydrate and fat, using Atwater factors are higher than the calorimeter determined energy for *L. siceraria* II *(BBG) and L. siceraria III (BGG)*

Table 4: Energy content of full-fat and defatted gourd seed flours (KJ/100g)

Energy of full fat melon (egusi) seed			Energy of defatted melon (egusi) seed flours
Sample	*Gross Energy1	**Gross Energy2	**Gross Energy2
C.colocynthis	2674.90	2693.82	1445.73
C.vulgaris	2685.45	2551.04	1452.45
L.siceraria I	2685.77	2440.74	1409.57
L.siceraria II	1751.08	2583.23	1331.24
L.siceraria III	1275.46	2507.23	1331.60

*Energy determined using Bomb Calorimeter; **Energy calculated using Atwater factor

The lower calorimeter determined energy for *L. siceraria* II *(BBG) and L. siceraria III (BGG)* may be due to energy lost to the environment and calorimeter during experiment. Defatting of the melon seeds however led to reduction in the calculated energy values, ranging from (1331.60 to 1452.45) KJ/100g for *L. siceraria II and C. vulgaries* respectively. The percentage energy due to fat in the raw sample ranges from (61.11 to 77.75) % and this was reduced to (2.02-5.06) % in the defatted melon flour samples. Obviously, the fat extracted is most likely responsible for this reduction since fat has the greatest energy production according to Atwater factors. These values are however close to the values of energy determined for some seeds like pumpkin seeds 27.0 to 27.20 KJ/g (Achinewhu and Isichei, 1990). The energy value of the seeds under study are higher than the total metabolisable energy for *C. africanum* fruit that was observed to be 420.42 KCal (Edem and Dosunmu, 2011), 448.83 KCal reported for *Gnetum africanum* seeds (Ekpo, 2007), 403.54 KCal reported for *Solanum nigrum* seeds (Edem et al., 2009) and 384.33 KCal reported for *B. coricea* seeds (Amaechi, 2009).

CONCLUSION

In conclusion, this research shows that oil extraction from these five varieties of gourd melon seeds significantly reduced the fat content of the seeds effectively by values ranging from 95.56 to 99.17 % (i.e. almost 100 % efficiency), increased the protein, ash, fibre, carbohydrate and eventually the sugar contents of the samples. The predominant sugars in the FFS are Lactose, D-Ribose and Maltose while glucose values are relatively low in both FFS and DFS. On the other hand, defatting led to increase in Maltose and decrease in Lactose level of the gourd melon seeds. The low glucose content of these melon seeds is a factor that enhances their suitability for the consumption of adults especially the hypertensive ones while reduction of the fat contents makes it more nutritious, elimination the risk for hypertension and obesity.

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