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Full Length Research Paper

Chemical evaluation of protein quality and phenolic compound levels of some Cucurbitaceae oilseeds from Cameroon

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This work studies the chemical evaluation of protein quality and phenolic compound contents of some Cucurbitaceae (egusi) oilseeds from different areas in Cameroon. These seeds are *Cucumeropsis mannii*, *Cucurbita maxima*, *Cucurbita moschata*, *Lagenaria siceraria* and *Cucumis sativus*. The seeds were cleaned, dried, ground and part of the powder was defatted. The defatted cakes were analysed for total and soluble nitrogen, true proteins and amino acids, while the undefatted seeds were analysed for phenolic compound contents. The defatted cakes had high total protein contents. The trichloroacetic acid soluble fraction of these proteins ranged from 25% (*C. maxima* from North West) to 94% of total proteins (*C. sativus* from Adamawa and South West), due to the post harvest treatment of the seeds. They were rich in most essential amino acids, giving protein digestibility, corrected amino acid scores of 0.67 for *C. sativus* and 0.48 for *C. mannii* which was for lysine, indicating that in the absence of tryptophan and methionine, lysine was the limiting amino acid in these seeds. These seeds had low levels of phenolic compounds (0.34 to 0.43%). Defatted *C. mannii* could be good for preparing infant formula, especially when mixed with soybean, in order to increase its lysine content.

Key words: Cucurbitaceae (egusi) seeds, proteins, amino acids, phenolic compounds.

INTRODUCTION

There is an increasing prevalence of nutrition related illnesses especially in Africa due to poverty and insufficient knowledge on the nutritional and economic importance of locally available and easily accessible foods and foodstuffs. Studies on Cucurbitaceae seeds have shown that they contain high protein levels with high levels of most essential amino acids except lysine (Sharma et al., 1986; Nwokolo and Sim, 1987; Martin, 1998; El-Adawy and Taha, 2001). High protein levels in Cucurbit seeds have also been shown by other researchers from various countries, such as Lal et al. (1983) who studied the kernel oils of 15 species of Cucurbitaceae seeds from India and showed that the oil-free kernel meals of different Cucurbit species had 60 to 70% of proteins. Jacks (1986) reviewed the usefulness of Cucurbit seeds and showed that globulins account for 70 to 90% of the protein, they are rich in arginine, aspartic and glutamic acids, but deficient in lysine and sulfur-containing amino acids, and that supplementation with the limiting amino acids increases the values. Previous studies on the nutritive value of these Cucurbitaceae oilseeds from different regions in Cameroon by Achu et al. (2005) showed that these seeds and their defatted cakes are rich in proteins (28 to 40.49% and 61 to 73.59% respectively). Kanar et al. (2006) investigated some nutritional and antinutritional characteristics of *Cucumis sativus* and *Lagenaria vulgaris* seeds and showed that they contained 31.2 to 31.8% crude proteins and that heat treatment reduced the trypsin inhibitor and lectin activities in both samples to negligible levels. The essential amino acid profile compared well with the

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FAO/WHO scoring pattern except for a deficiency of lysine and isoleucine, with lysine being the first limiting amino acid in both seeds. Yanty et al. (2007) showed that the crude protein content of *Cucumis melo* seeds from Malysia was 25%. Loukou et al. (2007) also found similar results with Cucurbit seeds from Côte d'Ivoire where they found that these seeds have protein levels ranging from 29 to 36%. Ojieh et al. (2008) continued to show that *Citrullus lanatus* has a crude protein content of 23.4% with good quantities (g/100 g protein) of arginine (9.0), isoleucine (4.8), leucine (4.2), and phenylalanine (3.2) which are essential amino acids as well as glutamic acid (16.9) and aspartic acid (16.3) and that "egusi" melon compares favourably with the known protein rich foods such as soybean, cowpeas, pigeon peas and pumpkin. Mariod et al. (2009) found that the protein content from six Sudanese Cucurbit seeds ranged from 14 to 17.5%. These results show a great variability on the protein contents which depend on the specie and which also seem to depend on the regions, as seen from the low values obtained for Sudanese seeds.

Phenolic compounds have been shown to have a lot of beneficial effects as antioxidants, antithrombotic and anti-inflammatory effects, and inhibit carcinogenesis (Kris-Ertherton et al., 2002). Siger et al. (2008) investigated the content and antioxidant activity of phenolic compounds in cold-pressed plant oils in Poland and showed that pumpkin (*Cucurbita pepo L.)* oils had the highest total phenolic content (2.5 mg/100 g) and phenolic acids (vanillic acid; 11.4 and protocatechuic acid; 3.1 mg/100 g) and also displayed high antioxidant activity (70%). Xanthopoulou et al. (2009) studied the antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts and showed that most extracts tested demonstrated radical scavenging activity, with fractions rich in phenolics showing the highest activity and that the presence of molecules being able to scavenge radicals and inhibit lipoxygenase in pumpkin seeds may in part explain the health benefits attributed to them. Peričin et al. (2009) showed that phenolic acids in pumpkins (*C. pepo*) were caffeic acid (in the skin, oil cake meal and dehulled kernel) and syringic acid (whole hull-less seed and dehulled kernel). The dominant phenolic compound was p-hydroxybenzoic acid amounting to 34.7% (hull-less seed), 52.0% (oil cake meal), 51.4% (skin), 67.4% (dehulled kernels) and 51.8% (hulls) of the total phenolic acid content. Most phenolic acids were present in bound (esterified and insoluble) form, from 50.6% (skin) to 84.1% (hull-less seed). Edeoga et al. (2010) investigated the pharmaceutical and therapeutic potential of some wild Cucurbitaceae species *(Lagenaria vulgaris, Trichosanthes cucumerina, Momordica charantia* and *Luffa cylindrical)* from South - East Nigeria and showed that the seeds generally contained more alkaloids than the other parts (0.03 to 0.07 mg/ml). They contained more tannins (0.484 to 0.9 mg/ml) than the other phytochemicals. Andjelkovic et al. (2010) studied the phenolic compounds and some quality parameters of pumpkin seed oil and showed that the total phenolics content ranged from 24 to 50.93 mg GAE/kg of oil. The individual phenolics were tyrosol, vanillic acid, vanillin, luteolin and sinapic acid. The maximum antioxidant capacity measured by the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was 62%, which was comparable to 0.16 mM Trolox equivalent (DPPH is a stable radical that is used to screen free-radical-scavenging ability of compounds). Parry et al. (2006) characterized cold- pressed onion, parsley, cardamom, mullein, roasted pumpkin, and milk thistle seed oils and found that the seed oils may serve as dietary sources of special fatty acids, tocopherols, carotenoids, phenolic compounds, and natural antioxidants.

Phenolic compounds and phytochemicals in food have been shown to have both adverse (antinutrients) and beneficial health effects (antioxidants) in humans. For example, tannins exhibit their toxicity effects, by forming protein-tannin complexes through multiple hydrogen binding between their hydroxyl groups and the carboxyl groups of protein peptide bonds of proteolytic enzymes in the gastrointestinal tract (Bressani et al., 1983). Although some saponins have been shown to be highly toxic under experimental conditions, acute poisoning is relatively rare both in animals and man (Osagie and Eka, 1988). Phytic acid, lectins, tannins, saponins, enzyme inhibitors, cyanogenic glycosides and glucosinolates reduce the availability of certain nutrients and impair growth. When used at low levels, phytic acid, lectins, enzyme inhibitors and saponins, reduce blood glucose and/or plasma cholesterol and triacylglycerols levels. Phytic acid, protease inhibitors, saponins, lignans and phytoestrogens have been shown to reduce cancer risks. Phenolic compounds found in foods also contribute to their astringency, and may also reduce the availability of certain minerals such as zinc. During thermal processing, phenolic compounds may undergo oxidation and the oxidized phenolics so formed, such as quinones, may combine with amino acids, thus making them nutritionally unavailable (Shahidi, 1997). The levels of some of these antinutrients are usually reduced during culinary transformations. A decline in phytic acid content was achieved by dehydration of lentil (44%), white beans and pink-mottled cream beans. Cooking and dehydration significantly reduced the levels of enzyme inhibitors and lectins in beans to negligible concentrations. Increased *in vitro* protein digestibility was produced by dehydration in these legumes from 12% to 15%. (Martín-Cabrejas et al., 2009).

In view of the beneficial effects of proteins and phenolic compounds to health, it is important to discover new sources of these compounds. There is still shortage of data on the protein quality and the content in phenolic compounds of these seeds grown in Cameroon, which is essential in order to exploit these seeds for better health, well-being and development. This work is therefore

aimed at evaluating the protein quality (nature of proteins and amino acid composition) and total phenolic compounds of five Cucurbitaceae seeds from Cameroon.

MATERIALS AND METHODS

Collection and treatment of samples

The "egusi" samples were collected from different bio-climatic areas in Cameroon. These regions are Sahel, High Savanna, Rain forest and Swamp forest regions. The seeds; *Cucumeropsis mannii,* (egusi melon), *C. maxima,* (pumpkin or squash gourd), *Cucurbita moschata,* (musk melon), *Lagenaria siceraria* (bottle gourd or calabash) *and C. sativus,* ("Ibo" egusi); were bought already dried under local conditions by the farmers. Where the seeds could not be found, the fruits were bought from the farmers during periods of harvest and the seeds extracted, washed, sun-dried and decorticated.

They were then transported in polythene bags to the laboratory, where they were cleaned with filter paper to remove all traces of dust and insects, dried in an air-convection oven at 70°C to constant weight. They were ground in an electric grinder (Blender mill/grater 3), placed in airtight bottles and stored in a dessicator for analysis.

Extraction of the oils and collection of the defatted flour

Part of the seeds were defatted while the rest were analysed undefatted. Oils were extracted from the ground seeds by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent (AOAC, 1980). The hexane was evaporated on a rotatory evaporator (Laborata 4003-digital Heidalph) and the defatted cake obtained was dried in an air-convection oven at 60°C for 24 h to remove all traces of solvent. This cake was then ground into flour in a grinder, put in airtight bottles and used for analyses.

Total protein content of defatted seeds: This was calculated from the following formula:

Total protein content of defatted flour = B x 100/100-A (g proteins/100 g defatted cake)

Where, A = lipid content of whole seed and B = protein content of whole seed (Achu et al., 2005).

Soluble protein content of defatted seeds: Most of the soluble nitrogen (non-protein nitrogen) concerns the products of degradation of proteins such as small peptides and free amino acids (Laure et al., 1971). Soluble nitrogen was extracted from the defatted samples following the method described by Nilo Rivas et al. (1981), which was done by precipitating the proteins with a solution of trichloroacetic acid (TCA) and filtration, to obtain a solution containing non-protein nitrogen. The quantity of nitrogen in this solution was determined through digestion of this solution and assay by a spectrophotometric method described by Devani et al. (1989). Ammonium sulphate solution was used as standard and the absorbance was read at 412 nm against the reagent blank. The soluble protein (non-protein nitrogen) content was obtained by multiplying the soluble nitrogen content by the coefficient 6.25.

True protein content of defatted seeds: This was calculated from the following formula:

True protein content = total proteins - soluble proteins (g proteins/100 g defatted flour).

The amino acid composition: This was analysed in *C. mannii* and *C. sativus* seeds, for they are the most widely distributed (found in most markets) and widely consumed seeds. 20 mg of defatted sample were weighed into a dry tube and 5 ml of HCl (6n) added. Norleucine was added to the sample at a final concentration of 0.25 mM as internal standard. The tubes were tightly corked (Teflon cork) and put in an oven at 110°C for 24 h. This hydrolysed the peptide bonds, liberating amino acids, but oxidized sulphur-containing amino acids, cysteine and methionine. Tryptophan was also destroyed by acid hydrolysis and so was not detected by this method. Asparagine and glutamine were also transformed into aspartic and glutamic acids respectively. After 24 h of hydrolysis, 50 µl of the hydrolysate was collected (in duplicate) and dried under vacuum. The samples were derived by phenylisothiocyanate (PITC or Edman's reagent) in order for the amino acids to be detected spectrophotometrically at 254 nm. Since the reaction with PITC occurred at basic pH, the samples were first neutralized in a mixture of ethanol/water/triethylamine (TEA) (2/2/1 v/v/v). 50 µl of this mixture was added in the tube and dried under vacuum. For derivation, 40 µl of a mixture of ethanol/water/TEA/PITC (7/1/1/1 v/v/v/v) were added into the tube. After 10 min at room temperature, the tube was dried under vacuum again (about 1 h) and the sample was dissolved in a solution containing 95% (v/v) 2 mM Na_2HPO_4, pH 7.4 and 5% (v/v) acetonitrile.

The analysis was done by reverse phase high performance liquid chromatography (HPLC) waters (Millennium), equipped with a photodiode array detector (Waters model 596), on a column Picotag C18 (4 mm x 15 cm, Waters). The HPLC column was equilibrated with buffer A (94% (v/v) 0.14 M CH_3COONa containing 3.6 mM triethylamine, pH 6.4, 6% (v/v) acetonitrile) and the elution was performed with a convex gradient from 100% buffer A to 46% buffer B (40% H_2O/60% acetonitrile, v/v) for 10 min, at a flow rate of 1 ml/min. Both the column and buffers were maintained at 38°C and the absorbance was recorded at 254 nm. An amino acid mixture (Pierce) of known concentration, derived under the same conditions was used to identify and to calculate the amino acid concentration. Different concentrations of standard were injected to make a calibration curve (250, 500, 750 and 1000 picomoles).

Uncorrected amino acid scores (AAS): This was calculated from the following formula:

AAS = Essential amino acid in test protein / essential amino acid in reference pattern

The reference pattern was the 1985 FAO/WHO 2 to 5 year old requirement pattern.

Protein digestibility corrected amino acid score (PDCAAS): This was calculated from the following formula:

PDCAAS = AAS x true digestibility (TD) (Achu, 2006)

Content in total phenolic compounds of whole seeds

The content in total phenolic compounds was determined by the spectrophotometric method of Marigo (1973). This method uses the Folin-Coicalteu reagent, which is a mixture of phosphotungstic acid and phosphomolybdic acid. Through reduction during the oxidation of phenols, a mixture of tungstene blue and molybdenum oxides is formed. Gallic acid solution was used as standard and the absorbance of the blue colour was read at 745 nm against the reagent blank.

Statistical analysis

The data was analyzed using the SPSS 9.0 software. Analysis of variance (ANOVA) was used to find the correlation between the

parameters measured and the different ecological regions of cultivation of the seeds, and between these parameters in the different species of seeds. Where the ANOVA test indicated significant differences, the Student-Newman-Keuls (S-N-K) test was used to locate these differences. The tests were done at the 5% level of significance.

RESULTS AND DISCUSSION

Total, soluble and true protein contents of defatted seeds

The total, soluble and true protein contents of the defatted seeds are shown in Table 1. The total protein contents range from 61.91 (*C. sativus*) to 73.59% (*C. mannii*), soluble proteins from 29.19 (*C. maxima*) to 53.76% (*C. sativus*) and true proteins from 8.15 (*C. sativus*) to 39.53% (*C. maxima*). There is a significant difference between the total, soluble and true protein contents of the various species of seeds. The total protein level of *C. mannii* is similar to that of *C. maxima* (68.72%) and *L. siceraria* (68.52%) but significantly higher ($p < 0.05$) than that of the other seeds, which have similar levels. These seeds contain more soluble proteins than true proteins, except *C. maxima* where the quantity of true proteins is more than that of soluble proteins. *C. sativus* has the highest amount of soluble proteins (53.76%) and the least amount of true proteins (8.15%). This soluble protein level of *C. sativus* is significantly higher ($p < 0.05$) and the true protein levels of *C. mannii* and *C. maxima* are also significantly higher than those of the other seeds.

A view of the various regions of collection of these seeds shows that in all the species, the samples from the West have higher proportions of soluble proteins than those from the North West. Samples from the Adamawa region also have high soluble protein values similar to those from the West. For *C. mannii*, the total proteins of this specie from Adamawa have 67% of soluble proteins, against 38% from the South. The West and Adamawa regions are in the High Savanna area with similar climate. This area is further away from the sea (inland) than the Rain and Swamp forest areas and it receives less rain than the coastal belt. It has good drainage and lacks enough moisture. Rainfall varies depending on the location of the area to the rain-bearing winds. The climate is the equatorial type. In Adamawa, the high elevations in this region lend it a relatively cool climate, between 22 to 25°C. Rainfall is within 150 to 200 cm with a long dry period followed by a long wet period. The West region has moderate to high humidity, the temperature is about 22°C, and rainfall is moderated by the mountains and averages 100 to 200 cm per year (Fokou et al., 2009). If the climate difference could explain this variation in values, the high temperature of the Adamawa should be more favourable for the activity of proteases, contributing to this rise in soluble protein values during drying of the seeds. *C. mannii* from these two regions has comparable soluble protein proportions (67 and 64%) as well as *C. sativus* (94 and 85%). Also for *C. sativus,* the South West and the Littoral regions which are located in the Swamp Forest with about the same climate have comparable proportions (94 and 86%). However, even if the total protein contents do not vary considerably according to the region (Achu et al., 2005), the profile of these proteins indicates that they depend on the region: Hot and humid regions have higher values of soluble proteins than the other regions. In this respect, the lower value of 49% for *L. siceraria* in the far North might be due to the hot and dry climate of this region. However, the comparable value for this sample from the Littoral (46%) with a hot and humid climate explains the fact that not only heat and humidity are responsible for the hydrolysis of proteins in the seeds after harvest, but the modes of treatment can also influence proteolysis.

Looking at the various postharvest modes of treatment up to the end of drying, it was observed that for some of these seeds, the fruits are usually opened and left for about 2 to 3 days for the pulp to slightly ferment before the seeds are extracted from the fruit. This is in order to reduce the sticky and slimy nature of the content of the fruits for easy washing of the seeds. After washing, the seeds are dried under the sun. If the sun is not hot enough, this drying can take a number of days before the seeds are completely dried. This slow drying causes the seeds to remain damp, leading to slow fermentation. Fermentation favours the action of lipolytic enzymes, which hydrolyzes triglycerides in the seeds, liberating free fatty acids (Fokou et al., 2009). This acidity favours the action of proteases in the seeds which hydrolyze proteins into small peptides and free amino acids, leading to an increase in soluble proteins and a decrease in the true protein values. On the other hand, *C. maxima* whose edible fruit (the pumpkin) serves as a delicacy, is not allowed to ferment before seed extraction. This reduces the activity of proteases. The seeds are extracted once the fruit is mature and the fruit cooked and consumed both by man and animals. This could partly explain the high true protein values observed in this specie. The fruit of *C. moschata* is also edible, but the seeds once extracted are usually stored for further planting during the next farming season. Sometimes, they are used for consumption, but this is only done during periods of famine, for the seeds are very small in size and so very laborious to decorticate, since it is done manually. It is also the case with *L. siceraria* seeds. They are bigger in size, but the cork is hard, and so difficult to decorticate. Consequently, these seeds are usually stored for long, and at room temperature. This may favour the action of proteases, thereby leading to high soluble protein values. Depending on the water activity (Aw) of the seeds during storage, (if it is ≥ 0.6), microbes can set in. Umoh and fields (1981) and Collar et al. (1991) showed that during fermentation, proteins can be hydrolyzed into volatile

Table 1. Total, soluble, true protein and phenolic compound contents of seeds.

Sample	Area	Region	*Total proteins (%)	Soluble proteins (%)	[1]Percentage of soluble proteins	True proteins (%)	[0]Phenolic compounds (%)
C. mannii	High Savanna	North West	78.38	39.56	50	38.82	0.41
	High Savanna	West	68.60	43.64	64	24.96	0.30
	High Savanna	Adamawa	70.0	47.05	67	22.94	0.46
	Rain Forest	South	69.41	26.18	38	43.23	0.49
	Rain Forest	East	74.50	31.27	42	43.24	0.32
	Swamp Forest	South West	80.71	38.19	47	42.52	0.34
		Average±SD	73.59± 4.65[a]	37.65± 7.75[c]	51 ± 12	35.95 ± 9.46[a]	0.39 ± 0.08
C. maxima	High Savanna	North West	71.65	18.08	25	53.57	0.46
	High Savanna	West	69.08	42.14	61	26.94	0.33
	Rain Forest	Centre	65.42	27.34	42	38.08	0.46
		Average±SD	68.72 ± 2.56[ab]	29.19 ± 12.14[d]	43 ± 18	39.53 ± 13.37[a]	0.42 ± 0.08
C. moschata	High Savanna	North West	71.46	31.89	45	39.57	0.44
	High Savanna	West	62.48	33.14	53	29.34	0.52
	Rain Forest	Centre	61.76	58.33	94	3.43	0.44
	Swamp Forest	South West	65.66	37.75	57	27.91	0.27
		Average±SD	65.34 ± 3.83[b]	40.28 ± 12.3[bc]	62 ± 22	25.06± 15.33[b]	0.42± 0.11
L. siceraria	Sahel Savanna	Far North	68.18	33.11	49	35.07	0.29
	High Savanna	North West	65.31	46.77	72	18.54	0.39
	High Savanna	West	67.98	57.27	84	10.71	0.37
	Swamp Forest	Littoral	72.59	33.35	46	39.24	0.32
		Average±SD	68.52± 2.61[ab]	42.63 ± 11.67[b]	63 ± 18	25.89 ± 13.50[b]	0.34 ± 0.05
C. sativus	High Savanna	West	58.73	49.82	85	8.91	0.54
	High Savanna	Adamawa	61.20	57.25	94	3.95	0.30
	Rain Forest	Centre	63.94	49.12	77	14.82	0.53
	Swamp Forest	South West	59.74	56.03	94	3.71	0.41
	Swamp Forest	Littoral	65.94	56.56	86	9.38	0.37
		Average±SD	61.91 ±2.67[b]	53.76 ± 3.94[a]	86± 7	8.15 ± 4.58[c]	0.43± 0.10

[0] = There is no significant difference between the phenolic compound levels of the same specie of seeds from different ecological regions and between these values in the different species of seeds. *= values are from Achu et al. (2005). [1] = Soluble Proteins/Total Proteins x100.SD = Standard deviation. Each value is a mean of 3 replications.

compounds such as ammonia into the environment as a result of proteolytic microorganisms such as *Clostridia* spp. Hence, the various treatments applied to the seeds, the duration of storage and drying of the seeds can influence the soluble protein values.

On the whole, the results show that these “egusi” seeds are very rich in proteins especially those of *C. mannii*. The total protein levels are similar to those obtained for defatted Cucurbit seeds by other researchers such as Lal et al. (1983) who showed values of 60 to 70% for 15 species of Cucurbitaceae seeds from India; Sharma et al. (1986) who found 62.1% for defatted *C. melo* (musk melon), 76.1% for *Citrullus vulgaris* (water melon) and 73.3% for *C. moschata (*pumpkin). Lazos (1992) also showed that the defatted seed flours of *C. pepo* and *C. maxima* have potential food use because of their protein content of 61.4%. This value is within our range of values. The total nitrogen content of the defatted cake of *Coula edulis* is 2.1 to 2.4 (Tchiégang et al., 1998), giving a protein level of 13.12 to 15%, which is much lower than the values for “egusi” seeds.

Phenolic compounds

The content in the total phenolic compounds of the

Table 2. Amino acid composition of defatted *C. sativus* and *C. mannii* flours compared to that of defatted soybean meal and casein.

Amino acid	*C. sativus*		*C. mannii*		Soybean meal		Casein
	(mg/g of flour)	(mg/g of protein)	(mg/g of flour)	(mg/g of protein)	[1](mg/g of flour)	[2](mg/g of protein)	[2](mg/g of protein)
*Histidine	23.6	36.91	27.8	**37.32**	15.8	**26**	**16.7**
Threonine	28.9	45.2	36.6	**49.13**	18.3	**37**	**47.7**
Valine	30	46.92	34.7	**46.58**	21.7	**50**	**35.5**
Isoleucine	29.9	46.76	33.7	**45.23**	21.2	**49**	**36.8**
Leucine	49.2	76.95	55.9	**75.03**	37.1	**82**	**59.8**
**Phenylalanine and Tyrosine	66.9	104.63	70.6	**94.77**	39.4	**90**	**67.8**
Lysine	25.7	40.19	23.4	**31.41**	32.5	**63**	**-**
Total essential amino acids	254.2	397.56	282.7	**379.46**	186	**397**	**264.3**
Aspartic acid	67.3	105.25	80.6	108.19	51.5	116	130
Glutamic acid	133.9	209.42	154.0	206.71	81	191	253.9
Serine	39.2	61.31	44.3	59.46	24.2	52	48.5
Glycine	44.5	69.6	46	61.74	21.4	42	100.8
Arginine	122.4	191.43	128.1	171.95	40	76	20.9
Alanine	36.4	56.93	42.5	57.05	20.8	43	38.9
Proline	25.9	40.51	29.6	39.73	25.2	51	14.7
Total non essential amino acids	469.60	734.45	525.10	704.83	264.10	571.00	607.70
Total amino acids	723.8	1132.01	807.8	1084.3	450.1	968	872
Essential amino acids/ Total amino acids (A_1)	0.35	0.35	0.35	0.35	0.41	0.41	0.3

Essential amino acids are shown in bold. [1] = Values are from Nwokolo and Sim (1987); [2] = Values are from Pc Priya Chemicals; * = Necessary only for infants, ** Necessary for synthesis of Tyrosine; - = Not found.

"egusi" seeds ranges from 0.34 (*L. siceraria*) to 0.43% (*C. sativus*) (Table 1). The phenolic compound levels of the seeds do not depend on the region of cultivation. There is also no significant difference between the phenolic compound levels of the different species of seeds. These values are higher than that of pumpkin seed cake (*C. pepo*, 0.26%) (Zdunczyk et al., 1999). They are lower than those of *Canarium schweinfurthii* (0.47 to 0.77%) (Kapchie Noutchogoué, 2000) and berries (contain much higher levels of antioxidants than other commonly consumed dietary plants), which range from 0.3 to 0.7% of anthocyanins (Törrönen, 2003). Phenolic compounds are natural antioxidants with heart protecting properties. Those found in nuts and red wine (resveratrol) have antioxidant, antithrombotic and anti-inflammatory effects, and inhibit carcinogenesis (Kris-Ertherton et al., 2002). The total phenolic compounds in these "egusi" seeds can therefore have beneficial effects on health, though at lower levels than those of walnut, pomegranates and berries with high levels of phenolic compounds (Törrönen, 2003).

These phenolic compounds can thus help to reduce the atherogenic risk in "egusi" seeds. On the other hand, these phenolic compounds (tannins) can bind proteins, carbohydrates, fats, and minerals, making them unavailable (Ganora, 2005). However, a study of the composition of the different types of phenolic compounds present in "egusi" seeds is needed in order to confirm these suggestions.

Amino acid composition of defatted seeds

The amino acid composition of defatted *C. sativus* and *C. mannii* flours are compared to those of defatted soybean meal and casein as shown in Table 2. The values are given in terms of mg/g of flour and protein. The content of non essential amino acids in these flours is more than that of essential amino acids, almost 2 times more. Apart from the combination of phenylalanine and tyrosine, leucine is the most abundant essential amino acid while glutamic acid is the most abundant non essential amino acid in these flours, followed by arginine and aspartic acid (in mg/g of protein). These results are similar to those of Jacks (1986) who showed that Cucurbit seeds are rich in arginine, aspartic and glutamic acids; and to those of Ojieh et al. (2008) who showed that *C. lanatus* has good quantities (mg/g of protein) of arginine (90), isoleucine (48), leucine (42), and phenylalanine (32) which are essential amino acids as well as glutamic acid (169) and aspartic acid (163) and that "egusi" melon compares favourably with the known protein rich foods such as soybean, cowpeas, pigeon peas and pumpkin.

Table 3. Essential amino acids and uncorrected and corrected amino acid scores.

Amino Acid	*C. sativus*			*C. mannii*			[3]Reference protein (mg/g of protein)
	(mg/g of protein)	[1]AAS	[2]PDCAAS	(mg/g of protein)	AAS	PDCAAS	
Histidine	36.91	1.94	1.87	37.32	1.96	1.74	19
Threonine	45.2	1.33	1.28	49.13	1.44	1.28	34
Valine	46.92	1.34	1.29	46.58	1.33	1.18	35
Isoleucine	46.76	1.67	1.61	45.23	1.62	1.43	28
Leucine	76.95	1.17	1.12	75.03	1.14	1.01	66
Phenylalanine and Tyrosine	104.63	1.03	0.99	94.77	0.87	0.77	63
Lysine	40.19	0.69	0.67	31.41	0.54	0.48	58
Total essential amino acids	397.56			379.46			303

[1]AAS: Amino acid score (uncorrected) = essential amino acid in test protein / essential amino acid in reference protein; [2]PDCAAS: protein digestibility corrected amino acid score = AAS x true digestibility (TD); [3]1985 FAO/WHO 2 to 5-year old requirement pattern; [4]True digestibility (TD): TD = 96.19% (*C. sativus*) and 88.7% (*C. mannii*) (Achu, 2006).

C. sativus has higher levels of essential amino acids than *C. mannii*. These "egusi" seeds also have higher levels of total essential amino acids than casein. The amino acid composition of the egusi seeds therefore shows that they have an excellent protein configuration. They have higher levels of histidine, threonine, phenylalanine and tyrosine than soybean, which has higher levels of lysine, leucine, isoleucine and valine. The total level of amino acids in terms of mg/g of protein (essential and non essential amino acids) is higher in *C. sativus* than in *C. mannii*, soybean and casein, while the A_1 ratio (A_1 = sum of essential amino acids/total amino acids) is higher in soybean (0.41) than in *C. sativus* (0.35), *C. mannii* (0.35) and casein (0.3). The essential amino acid values of these seeds are generally higher than those of melon and fluted pumpkin seed meals. These seed flours are superior to soybean in their content of all amino acids except lysine, when the amino acids are measured in terms of mg/g of flour (Nwokolo and Sim, 1987). This means that in the absence of tryptophan, lysine is the limiting amino acid in these seeds. However, looking at the amino acid values in terms of mg/g of protein, these "egusi" flours are inferior to soybean in their essential amino acid levels except histidine (essential for infants) threonine, phenylalanine and tyrosine. These seeds also have higher levels of valine, isoleucine, leucine, phenylalanine and tyrosine than casein. This shows that they can be good for infants. They have higher levels of most non essential amino acids than soybean except aspartic acid and proline. These give "egusi" meals a lower ratio of A_1 (0.35) than soybean (0.41). The higher A_1 ratio in soybean than these seeds means that soybean generally has higher levels of essential amino acids than these "egusi" seed meals. However, the levels of essential and other amino acids of these seeds are high enough (for some of their essential amino acid levels are higher than that of the 2 to 5 years old reference protein (Table 3) to enable them to adequately supplement (even at low levels of inclusion), the high carbohydrate-low protein diets of people in the tropics). However, a blend of "egusi" and soybean meal will greatly improve the nutritional value of these seeds when used in preparing sauces.

Jacks (1986) and Martin (1998) showed that the proteins of Cucurbit seeds are mostly of globulin type. They are deficient in lysine and in sulphur-containing amino acids. These are similar to the results obtained in these seeds. The results of these "egusi" seeds are also similar to those of *C. Pepo* seed cake with respect to lysine. The proteins of pumpkin seed cake (*C. pepo*) contain low levels of lysine (3.21 g/16 g N or 32.1 mg/g of protein) and isoleucine (38.3 mg/g of protein) (Zdunczyk et al., 1999). These "egusi" seeds have higher levels of isoleucine than those of pumpkin seed cake (Zdunczyk et al., 1999). The results are similar to those of *C. sativus* and *L. vulgaris* seeds, in which lysine was shown to be the first limiting amino acid (Kanar et al., 2006).

All these results together with those of these "egusi" seeds showed that lysine is the limiting amino acid in these seeds. The results are different from those of Mansour et al. (1993a) who showed that isoleucine and valine were the first limiting amino acids for *C. pepo* seed meal, and those of the African pear cake (*Dacryodes edulis)* by Omoti and Okiy (1987) who showed that this fruit contains high contents of the essential amino acids, lysine, leucine and threonine. The "egusi" seeds studied also contain high contents of leucine.

Essential amino acids, uncorrected and corrected amino acid scores of "egusi" seed flours

The uncorrected AAS and PDCAAS of *C. sativus* and *C. mannii* seed flours are shown in Table 3. The results show that the PDCAAS of *C. sativus* is 0.67 and that of *C. mannii* is 0.48. These values are those of lysine,

indicating that in the absence of methionine and tryptophan, lysine is the limiting amino acid in these seeds.

CONCLUSIONS AND RECOMMENDATION

This work which was aimed at evaluating the protein quality (nature of proteins and amino acid composition) and total phenolic compounds of some Cucurbitaceae seeds from Cameroon shows that the defatted seeds are very rich in proteins (61 to 74%). They generally contain more soluble than true proteins, according to the regions and the various post harvest treatments applied to the seeds. The amino acid composition of *C. sativus* and *C. mannii* seeds shows that *C. sativus* has higher levels of essential amino acids than *C. mannii.* Both "egusi" seeds have higher levels of the essential amino acids, histidine (essential for infants), threonine, phenylalanine and tyrosine than soybean and higher levels of valine, isoleucine, leucine, phenylalanine and tyrosine than casein. They also have higher levels of most essential amino acids than the amount required by the 1985 FAO/WHO pattern for 2 to 5 years old children. *C. sativus* has a higher PDCAAS value (0.67) than *C. mannii* (0.48), indicating that *C. sativus* protein is more complete than *C. mannii* protein. However, both *C. sativus* and *C. mannii* seeds have lower protein quality than casein, for their PDCAAS values are less than that of casein (1.0), showing that casein is a complete protein. These seeds have low levels of phenolic compounds which can help to reduce atherogenic risk. This can also influence the digestibility and bioavailability of the nutrients in "egusi" seeds. On the whole, in the absence of tryptophan and methionine, these "egusi" seed proteins show that lysine is the limiting amino acid. The nutritive value of these seeds can be improved when the meals are mixed with soy flour or supplemental lysine. This will make these "egusi" meals good for the preparation of infant formula.

Further studies are underway to analyse the amino acid composition of *C. maxima, C. moschata* and *L. siceraria* seeds, and the various phenolic compounds present in these seeds.

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