NUTRITIONAL AND CHEMICAL PROPERTIES OF FLUTED PUMPKIN (*Telfairia occidentalis*) SEED FLOURS, PROTEIN CONCENTRATES AND ISOLATES

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ABSTRACT

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The studied was conducted to assess the nutritional and chemical properties of fluted pumpkin seed flours, protein concentrates and isolates. Fluted pumpkin seeds were processed as raw, boiled and germinated seeds and then milled into flour. Protein concentrates and isolates were produced from the processed flours using two methods: alkaline extraction-isoelectric precipitation (IP) method and alkaline extraction-methanol precipitation (MP) method. Protein concentrates were produced from both methods whereas protein isolates were only produced from the IP method. The protein, fat, ash, crude fibre and carbohydrate contents of the raw, boiled and germinated full fat samples ranged between 27.78 – 32.21%, 40.67 – 45.91%, 2.11 – 4.56%, 2.22 – 3.79% and 9.81 – 13.28% respectively. The protein, fat, ash, crude fibre and carbohydrate contents of the raw, boiled and germinated defatted samples ranged between 61.74 – 65.30%, 5.64 – 6.77%, 5.96 – 7.98%, 4.12 – 5.16% and 10.49 – 17.54% respectively. The energy value ranged between 23.31 – 27.42 KJ per g. Ca, Mg, K, and P were all present in reasonable amounts in all the samples. Protein isolates produced from the boiled defatted flour had the lowest amounts of tannin and phytic acid (0.31g per Kg and 2.23g per Kg respectively) and also the highest protein digestibility (90.70%).

Keywords*: Nutritional, chemical Telfairia occidentalis, Seed Flours, protein concentrates*

INTRODUCTION

One of the major public health problems in some parts of the world including Nigeria and the West Africa sub region is protein malnutrition. This stems from the fact that starchy foods such as tubers and cereals are the major diets in these regions (Oluwamukomi *et al*., 2004; Aberoumand and Deokule, 2009). Due to insufficient supply of food in the world, it becomes necessary to collect food (especially proteins) from all available food sources in order to enhance food and nutritional stability. According to Padulosi *et al*., 2002, neglected and underutilized crops could be crucial in maintaining the poor rural African communities by raising their available food and protein sources. Replacing foods from animal sources with legumes and other foods from plant sources has been said to enhance the general well being of human beings (Guillion and Champ, 1996). Insufficient supply of animal proteins in most third world countries has continued to arouse research interest in plant proteins of suitable attributes. If the world is to have a shot at solving the problem of food shortages and protein malnutrition, novel and conventional sources of nutrients (especially protein) must be found. Protein products are becoming popular as ingredients in food systems all over the world. However, their use will depend on some favourable attributes they contribute to the food system.

Fluted Pumpkin (*Telfairia occidentalis*) seed which has an oil content of about 54% and can serve as a good source of protein (27%), can be easily obtained locally but is underutilized and wasted annually (Fagbemi and Oshodi, 1991; Oshodi and Fagbemi, 1992). It also contains some antinutrients such as tannin and phytic acid which may limit its use (Giami and Isichei, 1999; Moran *et al*., 1968). The seeds can be eaten cooked or ground into flour and used as a thickener or ingredient in some local foods (Achinewhu, 1987). There have been previous works on the chemical and nutritional composition of fluted pumpkin seed flours and concentrates (Fagbemi and Oshodi, 1991; Giami and Isichei, 1999; Fagbemi, 2007; Fagbemi *et al*, 2005). Most authors produced the protein concentrates using one method and analyzed the nutritional and chemical properties. Fernandez-Quintela *et al*, 1997 reported that different results were found for some properties depending on the method used for the preparation of protein isolates from some selected legumes. This points to the fact that the chemical and nutritional properties of protein concentrate and isolate may differ depending on the method of preparation used. There is limited information on the effects of different methods of production of fluted pumpkin seed protein concentrates and isolates on their nutritional and chemical properties.

The objectives of this study were to investigate the chemical and nutritional properties of fluted pumpkin seed (*Telfairia occidentalis*) flours, protein concentrates and isolates and the effects of processing and method of production of the protein concentrates and isolates on the flours, protein concentrates and isolates.

MATERIALS AND METHODS

Procurement of raw materials

Matured fluted pumpkin seeds were purchased from Bodija Market, Ibadan, Oyo State, Nigeria.

Preparation of flours from raw, boiled and germinated fluted pumpkin seeds

The fluted pumpkin seeds were extracted from the fruits. Part of the extracted seeds were dehulled, cleaned, freed from foreign materials and sliced into small pieces. Part of the sliced seeds were boiled for one hour, drained and allowed to cool according to the method of Giami and Bakebain (1992). Part of the extracted seeds was germinated using the method as described by Giami and Bakebain (1992). The seeds were arranged in layers of sawdust in a locally woven basket and wetted daily for 7 days. Sprouted seeds were picked, dehulled, cleaned, freed from foreign materials and sliced into small pieces. The differently processed seeds were dried in an oven at 50 °C. The dried seeds were ground using a Waring commercial blender (Smart Grind, Black and Decker, Towson, MA, USA) and sieved through 500 µm sieve. The flours were defatted in Soxhlet apparatus, using n-hexane for 8 hours at room temperature. The defatted flours were oven dried at 50°C to remove all traces of the solvent, milled and stored in airtight polyethylene bags.

Preparation of protein isolates and concentrates from defatted flour

The protein isolates and concentrates were prepared using two (2) different methods including alkaline extraction-isoelectric precipitation method (IP) and alkaline extraction-methanol precipitation method (MP).

Alkaline Extraction-Isoelectric Precipitation Method (IP), the procedure of Wagner *et al*. (2000) was used for the production of proteins via the IP method. Extraction of the defatted fluted pumpkin seed flour was done at room temperature (about 20°C) by using fluted pumpkin seed flour to water ratio of 1:10. The pH of the distilled water used was adjusted to 9.0 (Mettler Toledo, 320, pH meter) using 0.1N NaOH. The solution was stirred using a high-speed stirrer for one hour, and then cold-centrifuged at 1000 x G for 30 minutes at 4°C. The extraction was repeated as above and cold-centrifuged again. The supernatants were combined and pH value adjusted to 4.5 by addition of 0.1N HCl and allowed to stand for 2 hours at 4°C before being centrifuged again for 30 minutes at 4°C and 1000 x G to recover the protein. This was followed by decantation of the supernatant. The slurry was again suspended in water of pH 4.5 and the extraction carried out again as above to obtain fluted pumpkin seed slurry. The fluted pumpkin seed protein slurry was freeze-dried (Alpha 2-4 Freeze-Dryer, Martins Christ, Germany), pulverized, packaged in polyethylene bags and stored.

Alkaline extraction-methanol precipitation method (MP), the procedure described by Hanson (1974) was employed for the MP production of fluted pumpkin seed protein. Extraction of the defatted fluted pumpkin seed flour was done at room temperature (about 20°C) by using fluted pumpkin seed flour to water ratio of 1:10. The pH of the distilled water used was adjusted to 9.0 (Mettler Toledo, 320, pH meter) using 0.1N NaOH. Stirring of the suspension was done for one hour using a high-speed stirrer. Insoluble material was separated from the filtrate by means of filtration. The extraction was repeated as above and filtered again. The filtrates were combined together and a methanol concentration of 80% was added to the mixture. This was allowed to stand for 2 hours at 4°C and then cold-centrifuged at 1000 x G for 30 minutes. This was followed by decantation of the supernatant leaving behind the fluted pumpkin seed protein slurry. The slurry was again suspended with 80% methanol and the extraction carried out again as above. All traces of alcohol were removed using a vacuum desolventiser. The fluted pumpkin seed protein slurry was freeze-dried (Alpha 2-4 Freeze-Dryer, Martins Christ, Germany), pulverized, packaged in polyethylene bags and stored.

Analytical methods

Proximate analysis (moisture, protein, fat, ash and crude fiber) was carried out on the raw, boiled and germinated fluted pumpkin seed flours according to the methods described in AOAC (2000). Carbohydrate content was determined by difference.

Determination of energy value

The energy value of the full fat flour samples was determined using a bomb calorimeter. Each sample was weighed into the bomb calorimeter. The bomb was closed and charged in with oxygen up to 30atm. The bomb was fixed up by depressing the ignite switch to burn the sample in an excess oxygen. The maximum temperature rise in the bomb was measured with the thermocouple and galvanometer system.

Mineral elements analysis

Iron, calcium, magnesium and potassium contents of the samples were determined using Atomic Absorption Spectrophotometer (AAS), after wet oxidation of the samples according to the method as described by AOAC (1990).

Determination of in vitro protein digestibility

This was carried out according to the method described by Hsu *et al*. (1977). 3.1 mg of each sample was dissolved in 2 ml distilled water and the pH was adjusted to 8.0 with 0.1 N NaOH while stirring at 37 °C. A multi-enzyme solution consisting of 0.8mg trypsin, 6.2 mg chrymotrypsin and 2.6 mg peptidase per 2ml of distilled water, was maintained in an ice bath and adjusted to pH 8.0 as earlier described above, 200µl aliquot of the multi-enzyme solution was added to the sample solution and was constantly stirred at 37 °C. The pH of the solution was recorded 10 minutes after adding the enzyme solution. The *in vitro* digestibility was then calculated using the equation below:

$Y = 210.46 - 18.1X$

Where:

Y= *In vitro* digestibility (%)

X= pH of the sample suspension after 10 minutes digestion with multi enzyme solution.

Determination of tannin content

Quantitative determination of tannin content for each sample was carried out using Vanillin-HCl in methanol method according to the procedure described by Price *et al.* (1978). Catechin was used as standard. The color developed was read with a spectrophotometer at 500Nm wavelengths. Concentration of the standard was then plotted against their measured wavelengths, and the regression equation obtained was used to calculate the concentration of tannin as catechin equivalent.

Determination of phytic acid

Phytic acid content of the samples was determined by modification of the method described by Gullberg *et al*. (2004). About 100 mg of each sample was extracted using an extraction buffer [chloroform: methanol: water, in the ratio 1:3:1 (v/v/v)] in a ratio of 20:60:20. The sample was vortex at 4°C for 10 minutes. The supernatants were poured into an eppendorf tube, 2 equal aliquots of each sample was prepared and dried in speed vacuum (Refrigerated vapour trap, model RVT 400 by SAVANT Company, USA) at 35 °C for 18 hours. Dried pellets were re-suspended in 200 µl each of HPLC-water, the aliquots combined. 200 µl of each sample was purified using microtiterplate containing a 10KD filter (micron, 10KD, Millipore). 20µl HPLC-water was added to each filter before loading the samples. The filtrates of the samples were then centrifuged at 4°C and 4000 rpm for 60-80 minutes. The sample was then transferred into HPLC-MS vials. Phytic acid hydrate with calcium from rice was used as standard, 10Mm of the standard was prepared, 20 µl of each of the samples and standard was then transferred (using pipette) into vials, and run in HPLC-MS.

Statistical analysis

Each sample was analyzed in duplicate. Statistical analysis was carried out using Analysis of Variance (ANOVA) using SPSS version 16.0 statistical pages (SPSS Inc., Chicago, IL, USA) and the means separated by Duncan method. Significant differences were taken at 5% confidence limit.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of full fat and defatted fluted pumpkin seed flours are presented in Tables 1 and 2 respectively.

Moisture content

The moisture content of the full fat samples ranged between 4.68% - 5.94%. The moisture content of the defatted samples ranged between 4.31% - 5.39%. These values were within the range reported by Hamed *et al*. (2008), 5.47%; Fagbemi (2007), 1.89% - 4.59%. The values reported by Giami and Isichei (1999): raw defatted flour 4.7% and germinated defatted flour 9.6%; were higher than those observed in this work. The different moisture content values obtained for each sample could have been due to the effects of the different processing methods used. Lower moisture values were indicative of a longer shelf life.

Ash content

The ash content of the full fat fluted pumpkin seed flours ranged between 2.11% – 4.56%. This was close to the range of 2.50% - 5.08% reported by Fagbemi (2007). For the defatted samples, the ash contents were noticed to have increased and ranged between 5.96% – 7.98%. These values were within the range reported by Hamed *et al*. (2008), 9.04% and Fagbemi (2007), 6.01 – 8.98%. Ash content was indicative of the presence of minerals. This justifies the high amount of some minerals present in fluted pumpkin seed flour. The significant differences obtained for ash values in all the samples could be due to the effects of the different processing methods employed.

Crude fibre

For the full fat samples, the crude fibre content was found to range between 2.22% - 3.79% which was very close to the range of 2.49% - 3.98% reported by Fagbemi (2007). For the defatted samples, the crude fibre content ranged between 4.12% - 5.16% which was higher than the value of 2.98% reported by Hamed *et al*. (2008).

Crude protein

For the full fat fluted pumpkin seed flours, the highest crude protein value of 35.21% was observed in the germinated sample while the lowest value of 27.78% was observed in the boiled sample. Results within the ranges observed in this study were similarly reported for the protein content in full fat fluted pumpkin seed flours as follows: 29.92 – 33.53% (Fagbemi, 2007), 30.1% (Asiegbu, 1987), 26.6% (Longe *et al.*, 1983), 30% (Maduewesi, 1977). These values were higher than the values reported for some legumes and oil seeds such as cashew nut 21.70% (Ogunwolu *et al*., 2010); bambara groundnut 17.70% (Eltayeb *et al*., 2011); African walnut (*Tetracarpidium conophorum*) 24.01% (Ihemeje *et al*., 2012) and bambara bean 24.78 (Mune *et al*., 2011). The protein content of fluted pumpkin seed was observed to increase after defatting. For the defatted fluted pumpkin seed flours, the highest value for crude protein content (65.30%) was also observed in the germinated sample while the lowest value (61.74%) was observed in the boiled sample. This increase in the protein content of the germinated samples could be due to the liberation of bound proteins during germination. According to Hsu *et al*. (1980), the protein content of legumes usually increased as germination occurred because of biochemical changes

brought about by sprouting which resulted in an increase in free amino acids. Other authors also observed similar increase in protein content during germination (Akpapunam and Achinewhu, 1985; Giami, 1993). These values for the defatted fluted pumpkin seed flours were in the range of values reported by Oshodi and Fagbemi (1992), 67.5%; Fagbemi (2007), 65.50% – 66.87%; Hamed *et al*., (2008), 65.05% and Longe *et al*., (1983), 69.7%. These values were however higher than the value (52.51%) reported for defatted walnut flour by Mao and Hua (2012). Thus, pumpkin seeds are more proteinaceous than most legumes and oil seeds consumed locally in Nigeria and could contribute significantly to the recommended human daily protein requirement reported to range from 23% - 56% (NRC, 1980). The protein contents in the three samples were significantly different from each other probably due to the effects of the different processing methods used.

Crude fat

Fat content of the full fat samples ranged between 40.67% – 45.91%. These values were within the range (42.23% – 50.49%) reported by Fagbemi (2007). There were significant differences in the oil contents of the three samples. The oil contents in the defatted samples were found to range between 5.64% – 6.77%. These values however were higher than the 1.37% oil content reported by Hamed *et al*. (2008) for the defatted raw sample. The defatted germinated sample had the highest oil content. This could have been due to the activity of lipolytic enzymes which produce freer fatty acid as sprouting occurs.

Carbohydrate content

The carbohydrate content of the full fat samples ranged between 9.81% - 13.28% while that of the defatted samples ranged between 10.49% - 17.54%. Fagbemi (2007) also reported values close to these. The defatted germinated sample had the least carbohydrate content. The decrease in carbohydrate as germination occurred agreed with the observations of Inyang and Zakari (2008) and Yaboub *et al*. (2008).

Table 1: Proximate composition (%) of full fat fluted pumpkin

Sample	Moisture	Ash	Fiber	Fat	Protein	CHO
FRF	5.22^{b}±0.00	4.56^{a}±0.01	3.79^{a}±0.00	45.91^{a}±0.01	30.72^{b}±0.00	9.81^{c}±0.01
FBF	4.68^{c}±0.01	2.11^{c}±0.00	2.56^{b}±0.01	40.67^{c}±0.00	27.78^{c}±0.01	22.21^{a}±0.03
FGF	5.94^{a}±0.01	2.47^{b}±0.00	2.22^{c}±0.14	43.90^{b}±0.00	32.21^{a}±0.00	13.28^{b}±0.02

Values within a column with different superscripts are significantly different ($p<0.05$). FRF = Full fat raw flour; FBF = Full fat boiled flour; FGF = Full fat germinated flour.
*CHO = Carbohydrate

Table 2: Proximate Composition (%) of defatted fluted pumpkin

Sample	Moisture	Ash	Fiber	Fat	Protein	CHO
DRF	5.39^{a}±0.01	7.23^{b}±0.00	4.29^{b}±0.01	6.11^{b}±0.01	63.21^{b}±0.00	13.78^{b}±0.01
DBF	5.01^{b}±0.01	5.96^{c}±0.01	4.12^{c}±0.01	5.64^{c}±0.01	61.74^{c}±0.01	17.54^{a}±0.00
DGF	4.31^{c}±0.01	7.98^{a}±0.00	5.16^{a}±0.01	6.77^{a}±0.00	65.30^{a}±0.01	10.49^{c}±0.03

Values within a column with different superscripts are significantly different ($p<0.05$). DRF = Defatted raw flour; DBF = Defatted boiled flour; DGF = Defatted germinated flour.
*CHO = Carbohydrate

Energy values

The energy values for the full fat samples are shown in Table 3. The energy values ranged between 23.31 – 27.42 KJ/g. The energy values obtained were close to the value reported by Achinewhu and Isichei (1990), 27 – 27.20KJ/g; and Fagbemi (2007), 26.55 – 29.54 KJ/g. Similar observations were made by Longe *et al*. (1983), Padmashree *et al*. (1987) and Agbede (2001) on fluted pumpkin, cowpea and some underutilized legume seeds respectively. According to (FAO/WHO/UNO, 1985), the daily energy requirement of an adult is 10,500 – 12,600 KJ. Hence, consumption of fluted pumpkin seed flour can help supplement this requirement. The low energy value observed in the boiled sample may be as a result of leaching (especially of fat) during the boiling process.

Table 3: Energy Value of full fat fluted pumpkin seed

Sample	Energy Value (KJ per g)
FRF	27.42±0.01
FBF	23.31±0.01
FGF	25.90±0.00

FRF = Full fat raw flour; FBF = Full fat boiled flour; FGF = Full fat germinated flour.
Calcium (Ca), Magnesium (Mg), Potassium (K); Iron (Fe) Contents of Fluted Pumpkin Seed Flour

Table 4 shows some nutritionally significant minerals present in fluted pumpkin seed flours. The potassium content of fluted pumpkin seed flour was seen to range between 845.96 mg per 100g - 1285.67 mg per 100g. The calcium content ranged between 150.25 mg per 100g - 170.65 mg per 100g. The Mg content ranged between 5.54

mg per 100g - 12.86 mg per 100g. The Fe content ranged between 70.34 mg per 100g - 80.91 mg per 100g. K was observed to be the most abundant mineral in fluted pumpkin seed flour. The values for these minerals were close to the values obtained by Longe *et al*. (1983). The values obtained for calcium, iron and potassium in fluted pumpkin seed flour were higher than those obtained in African walnut while the value obtained for magnesium was lower than that observed in African walnut as reported by Ihemeje *et al*. (2012). The iron content observed in fluted pumpkin seed flour was also higher than that observed in bambara bean flour, which was 16.64% (Mune *et al*., 2011). The values obtained for iron, calcium and magnesium in fluted pumpkin seed were also higher than those observed in germinated, and cooked African yam bean flour, 1.14-5.71mg, 0.33-0.53mg and 1.67-7.67mg respectively (Uwaegbute *et al*., 2012). These high values showed that fluted pumpkin seed flour was a good source of these minerals needed for body metabolism and proper functioning of body cells. The significant differences observed in the various samples might be due to the various processing methods used.

Table 4: Composition of some essential elements (mg per 100g) in full fat fluted pumpkin seed flour

Sample	Ca	Mg	K	Fe
FRF	150.25^{a}± 0.00	8.43^{b}±0.00	1001.67^{a}±0.00	80.91^{a}±0.00
FBF	170.65^{c}±0.23	5.54^{c}±0.10	845.96^{b}±0.01	0.34^{c}±0.04
FGF	165.85^{b}±0.07	12.86^{a}±0.07	1285.87^{c}±1.41	74.86^{b}± 0.02

Values within a column with different superscripts are significantly different ($p<0.05$). FRF = Full fat raw flour; FBF = Full fat boiled flour; FGF = Full fat germinated flour; Ca = Calcium; Mg = Magnesium; K = Potassium; Fe = Iron.

Antinutritional factors

The tannin content of the full fat fluted pumpkin seed flour ranged between 6.09 g per kg (in the boiled sample) to 18.06 g per kg (in the raw sample). This was within the range of 7.5 – 19.1 g per kg reported by Fagbemi *et al*. (2005) for raw, boiled, fermented, germinated and toasted fluted pumpkin seed flours. It was however lower than the value (228.3 mg per 100g) reported by Hamed *et al*. (2008). This high reduction in tannin content in the boiled flour might be due to the effect of heat treatment. According to Hassan *et al*. (2005), processing methods such as cooking greatly reduced antinutritional factors of lupin seed. According to Reddy and Pierson (1994), over 90% of the tannin content in soybean was removed by dehulling and cooking because they were mostly concentrated in the seed coats. The tannin content of the protein concentrates from fluted pumpkin seed flour produced using the IP method ranged between 1.08 g per kg (in the concentrate from boiled flour) to 3.25 g per kg (in the concentrate from raw flour). On the other hand, the tannin content of the protein concentrates from fluted pumpkin seed flour produced using the MP method ranged between 1.80 g per kg (in the concentrate from the boiled flour) to 4.59g/Kg (in the concentrate from the raw flour). The concentrate from the raw flour showed 82% reduction in the tannin content compared to the raw flour. Variations in the tannin content in the protein concentrates from the two methods could be due to differences in the methods used. The tannin content of the protein isolates from fluted pumpkin seed flour ranged between 0.31 g per kg (in the isolate produced from the boiled seed flour) to 0.90g/Kg (in the isolate produced from the raw seed flour). The isolate produced from the raw flour showed a 97% reduction in the tannin content compared to the raw flour. This was comparable to the 95% reduction in tannin content of protein isolate produced from raw faba bean reported by Fernandez-Quintela *et al*. (1997). It was however higher than the 69% value reported by the same author for percentage reduction in tannin content of protein isolate produced from soybean seeds. This could be as a result of variations in the chemical composition of the different plants or variations in the processing methods used for the production of the isolates.

The phytic acid content of full fat fluted pumpkin seed flours ranged between 4.07 g per kg (in the boiled sample) to 15.28 g per kg (in the raw sample). This was within the range of 2.8% - 13.8 g per kg reported by Fagbemi *et al*. (2005) for raw, boiled, fermented, germinated and toasted fluted pumpkin seed flour. The high reduction in the phytic acid content in the boiled sample might be due to the effect of the heat treatment. The phytic acid content of the protein concentrates from fluted pumpkin seed flour produced using the IP method ranged between 3.03 g per kg (in the concentrate from boiled flour) to 10.07 g per kg (in the concentrate from raw flour). The phytic acid content of the protein concentrates from fluted pumpkin seed flour produced using the MP method ranged between 4.65 g per kg (in the concentrate from the germinated flour) to 9.62 g per kg (in the concentrate from the raw flour). The concentrate from the raw flour showed about a 35% reduction in the phytic acid content compared to the raw flour. This percentage reduction was lower than the 55% reduction reported by Giami and Isichei (1999). Reductions in phytic acid content of protein concentrates from raw dry beans ranging from 38% to 59%, compared to whole beans had earlier been reported (Deshpende and Cheryan, 1984). None of the processing methods used in this work could completely eliminate phytic acid from the seed flours and protein concentrates. The phytic acid content of the protein isolates from fluted pumpkin seed flour ranged between 2.23 g per kg (in the isolate from the boiled seed flour) to 8.28 g per kg (in the isolate from the raw seed flour). The isolate from the raw flour showed a 44% reduction in the phytic acid content compared to the raw flour. This was comparable to the 46% reduction reported by Fernandez-Quintela *et al*. (1997) for protein isolates produced from raw pea

seeds. This value was however higher than the 36% reduction in phytic acid content of protein isolate produced from raw soybean seeds as reported by Fernandez-Quintela *et al*. (1997).

***In Vitro* protein digestibility**

The *in vitro* protein digestibility of fluted pumpkin seed flours, concentrates and isolates are shown in Table 6. The *in vitro* protein digestibility of the seed flours ranged between 65.36% (for the raw seed flour) to 84.21% (for the boiled seed flour). These values were within the range reported by Giami and Isichei (1999), (58.4 and 65.1% for raw and germinated samples respectively). The IVPD of fluted pumpkin seed flour compared favorably with the digestibility of cashew nut flour (74.3% – 82.8%) reported by Fagbemi *et al*. (2005) and also that of some oil seeds like cotton meal (85.3%) Hsu *et al*. (1977). Bradbury *et al*. (1984) noted the relationship between IVPD and antinutrients. This may explain why the boiled sample showed the highest digestibility due to the fact that it contained the least amount of antinutrients. The relatively low protein digestibility (65.36%) of the raw flour may be partly attributed to the presence of high amounts of phytic acid and tannins. The IVPD of the protein concentrates from fluted pumpkin seed produced using the IP method ranged between 71% (for the concentrate from the raw flour) to 88.65% (for the concentrate from the boiled flour). The IVPD of the protein concentrates from fluted pumpkin seed produced using the MP method ranged between 75% (for the concentrate from the raw flour) to 84% (for the concentrate from the boiled flour). These values were within the range of 69% (for the concentrate produced from raw flour) and 72.4% (for the concentrate produced from the germinated flour) reported by Giami and Isichei (1999). It could be deduced that the method of production affected the IVPD of protein concentrates. The IVPD of the protein isolates from fluted pumpkin seed flour ranged between 79.15% (in the isolate produced from the raw seed flour) to 90.70% (in the isolate produced from the boiled seed flour). This was within the range reported by Mugendi *et al*. (2010) had reported IVPD of 81.39% and 87.67% for protein isolates produced from raw and processed mucuna bean respectively.

Table 5: Tannin and phytic acid content of fluted pumpkin seed flours, protein concentrates and isolates

Sample	Phytic acid (g per kg)	Tannin (g per kg)
FRF	15.28^{a}±0.40	18.06^{a}±0.01
FBF	4.07^{h}±0.00	6.09^{c}±0.01
FGF	8.92^{d}±0.02	13.38^{b}±0.00
RC (IP)	10.07^{b}±0.00	3.25^{e}±0.00
BC (IP)	3.03^{i}±0.01	1.08^{i}±0.01
GC (IP)	5.08^{f}±0.01	*2.41^{f}±0.01*
RC (MP)	9.62^{c}±0.00	4.59^{d}±0.01
BC (MP)	5.00^{f}±0.00	1.80^{h}±0.00
GC (MP)	4.65^{g}±0.01	2.37^{g}±0.02
RI	8.28^{e}±0.00	0.90^{j}±0.00
BI	2.23^{j}±0.00	0.31^{l}±0.01
GI	4.90^{f}±0.01	0.66^{k}±0.00

Values within a column with different superscripts are significantly different ($p<0.05$). FRF = Full fat raw flour; FBF = Full fat boiled flour; FGF = Full fat germinated flour; DRF = Defatted raw flour; DBF = Defatted boiled flour; DGF = Defatted germinated flour; RC (IP) = Concentrate from raw flour (IP method); BC (IP) = Concentrate from boiled flour (IP method); GC (IP) = Concentrate from germinated flour (IP method); RC (MP) = Concentrate from raw flour (MP method); BC (MP) = Concentrate from boiled flour (MP method); GC (MP) = Concentrate from germinated flour (MP method); RI = Isolate from raw flour; BI = Isolate from boiled flour; GI = Isolate from germinated flour; IP method = Alkaline extraction – isoelectric precipitation method; MP method =Alkaline extraction – methanol precipitation method.

Table 6 *In vitro* protein digestibility of fluted pumpkin seed flours, protein concentrates and isolates

Sample	*In viro* Protein Digestibility (%)
DRF	65.18^{h}±0.25
DBF	82.21^{c}±2.84
DGF	68.55^{g}±0.00
RC (IP)	71.00^{f}±0.00
BC (IP)	88.65^{a}±0.64
GC (IP)	74.00^{e}±0.00
RC (MP)	75.00^{e}±0.00
BC (MP)	84.00^{b}±1.41
GC (MP)	78.60^{d}±0.57
RI	79.15^{d}±0.21
BI	90.70^{a}±0.00
GI	81.40^{c}±1.90

Values within a column with different superscripts are significantly different ($p<0.05$). DRF = Defatted raw flour; DBF = Defatted boiled flour; DGF = Defatted germinated flour; RC (IP) = Concentrate from raw flour (IP method); BC (IP) = Concentrate from boiled flour (IP method); GC (IP) = Concentrate from germinated flour (IP method); RC (MP) = Concentrate from raw flour (MP method); BC (MP) = Concentrate from boiled flour (MP method); GC (MP) = Concentrate from germinated flour (MP method); RI = Isolate from raw flour; BI = Isolate from boiled flour; GI = Isolate from germinated flour; IP method = Alkaline extraction – isoelectric precipitation method; MP method = Alkaline extraction – methanol precipitation method.

CONCLUSION

In conclusion, the results obtained in this study showed that fluted pumpkin seed flours, concentrates and isolates were rich in nutrients especially protein and hence, can be used as a food source. This can help reduce protein malnutrition in Nigeria and Africa as a whole.

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