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Effects of processing on the nutritional composition of fluted pumpkin (*Telfairia occidentalis)* seed flour

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ABSTRACT

Fluted pumpkin seeds were processed into the raw, boiled, fermented, germinated and roasted seeds, dried at 50^{0}C, milled and sieved. The seed flours were analyzed for nutritional composition, energy, amino acids and fatty acids of the oils. Processing affected the levels of nutrients in the seed. The energy values ranged between 26.55 ± 0.7 – 30.06 ± 0.8 KJ/g and the seed is a good source of some essential minerals. Deleterious elements are very low and significantly reduced by processing. The fatty acids consist of oleic acid, 34.52 ± 0.03 – 46.39 ± 0.06%; linoleic acid, 11.0 ± 0.06 – 30.94 ± 0.2%; palmitic acid, 13.61 ± 0.1 – 19.56 ± 0.04% and stearic acid, 11.84 ± 0.06 – 18.91± 0.3%. Predominant amino acids in the seed are glutamic acid, 132.04 ± 0.02-152.30±0.7mg/g cp.; and aspartic acid, 124.61±0.03-130.78±0.07mg/g cp. The limiting amino acids are methionine, 6.02 – 8.11mg/g cp. and tryptophan, 0.08 – 13.78mg/g cp. Fermentation and germination improved the protein quality while boiling and roasting reduced them. Processing reduced deleterious metals and improved some of its nutrients.

Key words: Processing, nutrition, fluted pumpkin seed flours

INTRODUCTION

Studies on the utilization of vegetable proteins continue to gain attention due to the worldwide increasing demand for cheap and acceptable dietary protein, particularly for the low-income groups. The need to search for unconventional legumes and oil seeds therefore attract research in this direction (Onweluzo *et al.*, 1994; Chau and Cheung, 1998). Past studies on the nutritional composition and functional properties of vegetable proteins were carried out on the raw seed flours, benniseed, (Oshodi 1985), Pigeon Pea, (Oshodi and Ekperigin, 1989) and fluted Pumpkin seed (Fagbemi and Oshodi, 1991). There is limited information on the effects of processing on the nutritional properties of seed flours (del. Rosario and Flores, 1981; Abbey and Ibeh, 1988; Bakebain and Glami, 1992; and Nwanekezi *et al.*, 1994). Those who worked on processing effects only focused on heat processing without considering other traditional processes.

Some of the plant seeds contain antinutritional factors, which can be removed by Processing (Moran *et al.*, 1968; Wu and Inglett, 1974). Sprouting or germination has been reported to improve vitamins and Protein quality of some cereals and legumes (Kylen and McCready, 1975; Padmashree *et al.*, 1987; Asiedu *et al.*, 1992) with reduction in antinutritional factors. Traditional fermentation improves food nutrients, preserve and detoxify them (Steinkraus, 1975). Fluted Pumpkin is a widely consumed vegetable in Nigeria, (Akoroda, 1990). The Seeds though high in protein, (Fagbemi and Oshodi 1991), (Oshodi and

Fagbemi 1992) are wasted annually. Fagbemi *et al.*, (2005), reported that processing significantly reduced antinutritional factors of fluted pumpkin seed. This study investigates the effects of traditional Processing techniques on the nutritional composition of the seed in order to improve its utilization in food system.

MATERIALS AND METHODS

Sample Preparation

The fluted pumpkin fruits were obtained from the Federal University of Technology Akure teaching and research farm. The seeds were extracted, dehulled and sliced into small pieces. Parts of the sliced seeds were oven dried at 50^0 C. Some part of the sliced seeds were boiled for 1hour as described by Bakebain and Giami (1992), drained and allowed to cool. Parts of the boiled seeds were oven dried at 50^0C (Gallenkamp, England). The other part of the boiled seeds were wrapped in blanched banana leaves and allowed to ferment naturally (Bakebain and Giami, 1992; Achinewhu, 1982). The fermented seeds were oven dried at 50^0C. Parts of the seeds extracted from the fruit were germinated as described by Bakebain and Giami (1992) using saw dust in a locally woven reed basket. The seeds were arranged in layers of the sawdust, wetted daily and observed for sprouting. Sprouted seeds (6-8 days) were picked, washed, dehulled, sliced and oven dried. Part of the oven dried raw seeds were roasted in hot cast iron pan at 75 – 85^0C and allowed to cool. The differently processed seeds were pulverized using coffee grinder and sieved through 500mm sieves, packaged in plastic containers, labeled and kept in cool dry place. Parts of the seed flours were defatted continuously for 8 h using n – hexane as solvent. Defatted flours were oven dried at 50^0C to drive off the n – hexane completely. The seed flours were then milled/sieved to pass through 500mm mesh size. The differently processed full -fat seed flours were labeled F_1, F_2, F_3, F_4, and F_5 while their respectively defatted flours were labeled F_6, F_7,F_8, F_9 and F_{10} for raw dried, boiled, fermented, germinated and roasted seed flours.

CHEMICAL ANALYSIS

Proximate composition

The proximate composition of the seed flours was determined as follows. The moisture content, crude fat, ash, fibre were determined as described by AOAC (1990). The crude protein content was determined by using micro Kjeldahl method reported by Kirk and Sawyer (1991), (Nx6.25), while the carbohydrate was estimated by difference.

Ash Analysis

The ash analysis was carried out to determine the water-soluble ash, acid insoluble ash, soluble ash, alkalinity, potassium carbonate, sodium carbonate and potassium oxide alkalinity as described by Kirk and Sawyer (1991).

Energy Value

The energy value of the seed flours was determined using bomb calorimeter (Gallenkamp CBB – 300 – 010L, England). The seed flours were introduced into the bomb calorimeter and burnt in excess oxygen at pressure of 25atm. Rise in temperature due to burning was shown in the increasing deflection of galvanometer reading. Energy values were also estimated using the Atwater factor (Smith and Ojofeitimi, 1995)

Mineral Elements.

The mineral composition of the seed flours was determined using Atomic Absorption Spectrophotometer (AAS) (ALPHA 4, Conecticut U.S.A.), after wet oxidation of the seed flours as

described by IITA (1980). The total Phosphorus in each sample was determined using the Phosphovanado molybdate method (AOAC, 1990).

Amino acid analysis and protein evaluation acid stable amino acids were hydrolyzed with 6m HCl in the absence of air Blackburn (1978) while Sulphur amino acids were first oxidized with performic acid before it was treated with 6m HCl. The derivatised amino acids from above were separated by HPLC on a 25mm X 4.6mm spherisorb ODS 2 column using two waters 510 delivery systems. The solvents used were

a.) 0.14M Sodium acetate 850ml triethylamine pH 5.6
b.) 60% acetonitrate with a gradient of 0% for 2min 0 – 42% / 15 min (Convex curve) 100% / 4min.

Tryptophan content of the seed flours was determined as reported by Concon (1975) and modified by Ogunsua (1988). The amino acids obtained were used to evaluate the protein quality of the seed flours. Predicted Biological value (BV) was calculated using the regression equation of Morup Olesen (1976) as reported by Chavan *et al.*, (2001)

$$BV = 10^{2.15} \times q^{0.41}_{Lys} \times q^{0.60}_{Phe + Tyr} \times q^{0.77}_{met + Lys} \times q^{2.41}_{Thr} \times q^{0.21}_{Trp}$$

$$q = \frac{\text{ai sample}}{\text{ai reference}} \quad \text{for ai sample £ ai reference}$$

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$$q = \frac{\text{ai reference}}{\text{ai sample}} \quad \text{for ai sample }^{3}\text{ ai reference}$$

ai = mg of the amino acid per g of total essential amino acids

The predicted Protein Efficiency Ratio (PER) was calculated using one of the equations developed by Alsmeyer *et al.*, (1974) as stated below.

PER = - 0.464 + 0.454 [LEU] – 0.105 [TYR]

Fatty Acid Analysis

The oils extracted from the seed flours were methylated and their fatty acid composition determined as described by Metcaife *et al.*, (1966) using Gas – Liquid Chromatography (GLC). Heptadecanoic acid was used as the internal standard.

Phosphorus and Phytic acid were determined by extracting and precipitating the samples as described by Wheeler and Ferrel (1971).

Determinations were carried out in triplicates and significant differences were calculated using SPSS 10.0 computer Programme.

RESULTS AND DISCUSSION

Processing has significant ($p<0.05$) effects on the proximate composition of the Full fat and defatted fluted pumpkin seed flours Tables 1 and 2 respectively. The crude protein content of the full fat and defatted seed flours ranged between 29.2±0.7 – 35.06±0.8% and 65.50±1.5 – 70.53±1.6% respectively. The values observed are within the ranges reported for the Full fat seed by Asiegbu (1987), 30.1%; Ojolu (1978) 28.0%; Maduewesi (1975) 30%; and Longe *et al.* (1983), 26.6%. The protein content of fluted pumpkin seed is higher than some of the commonly consumed vegetable proteins in Nigeria, namely, melon seed (*Colocynthis citrullus*) 28.44%, Akobundu *et al.* (1982); peanut flour 24.3%; rapeseed 25%; sunflower flour 28.7% and it compared effectively with that of Soybeans 36% (Solsulski, 1983). The result obtained for the defatted seed flours agreed with the values reported by Oshodi and Fagbemi (1992), 67.5%. Thus, fluted pumpkin seed is a good source of protein. Fermentation and germination increased the crude protein content of full fat fluted pumpkin seed flours by 15.25% and 10.22% respectively while boiling reduced it by 1.64%. del. Rosario and Flores (1981), Kylen and McCready (1975) reported similar increases on mung bean and soybean respectively. Protein increase in fermented and germinated seeds was attributed to protein synthesis during germination and fermentation while the reduction noticed in the boiled seeds may be due to leaching (Kylen fluted pumpkin seed flours (Table 5) ranged between 26.55 ± 0.7 – 30.06 ± 0.8 KJ/g, while the value estimated using Atwater factor is 24.93 ± 0.2 – 28.01 ± 0.5 KJ/g. The energy value is close to the value reported by Longe *et al*. (1983) and Achinewhu and Isichei (1990), 32 – 33.33KJ/g and 27.0 – 27.2 KJ/g respectively. The little differences may be due to experimental conditions. The energy value of the fermented sample was the highest while the boiled sample was the least. This may be due to the increase in fat content of the fermented samples and leaching (especially fat) during boiling. Longe *et al*. (1983), Padmashree *et al*. (1987) and Agbede (2001) made similar observations on fluted pumpkin, cowpea and some under utilized legume seeds respectively. The values obtained using bomb calorimeter was higher than the estimated values from Atwater factor. This is contrary to the observation of Adeyeye (1995) on African yam bean. The bomb calorimeter determines the actual heat of combustion from the nutrients as well as the indigestible fibre in the seed (non-starch carbohydrate). This may make the determined energy high (not with standing the energy lost to the environment during experiment). However, both energy values followed the same trend. The daily energy requirement of an adult is 10,500 – 12,600 KJ, (FAO/ WHO / UNO 1985) and Bingham (1987), this can be obtained by consuming 400 – 475g of the seed flour daily. This amount is high, hence, the seed may only supplement daily energy requirement of man. The proportion of the total gross energy due to protein P^e**%** ranged between 19.57 – 22.90% while the utilizable energy due to protein NDPE % (assuming 60% utilization) ranged between 11.74 – 13.74%. This amount is above the safe level of 8%, hence, the seed may be enough to prevent Protein malnutrition (Araya, 1980) especially the fermented and germinated samples.

Minerals in the Seed Flours

Tables 6 and 7 show processing effects on the nutritionally important mineral composition and computed mineral, Phytates and Millimolar ratio of the seed flours respectively. The most abundant mineral element is K and it ranged between 4,207.47 ± 1.8 – 11,713.34 ± 1.6mg/kg. The least abundant minerals are Hg and Pb and ranged between 0.00 – 0.17 ± 0.01 and 0.12 ± 0.02 – 0.60 ± 0.02mg/kg respectively. Processing significantly ($P<0.05$) affected all the minerals in the seed flours. The high amount of K observed in the seed flour agreed with the observation of Olaofe and Sanni (1988), who reported that K is high in Plant foods from Nigerian soil. The minerals are close to the values reported by Longe *et al*. (1983) and values reported for some oil seeds (Robinson, 1975; Oshodi *et al.*, 1999). The K/Na ratio ranged between 15.27 – 72.45, it is greater than the recommended 1.0, and hence, consumption of the seed may be more beneficial to the body system by salting with NaCl. Apart from improving the taste, it will also enhance the salt balance of body fluid (Ranhotra *et al.*, 1998)

The calcium content of fluted pumpkin seed flour ranged between 63.50±1.6 – 131.79 ± 2.1mg/kg. The Ca content is low when compared with sunflower seeds (800 – 1,000 mg/kg) Robinson (1975) and 6,010mg/kg reported for benniseed (Oshodi *et al.*, 1999). Considering the macronutrients, K, Ca, Mg and P, the seed is a fair source of the minerals except Ca. High amount of Ca, K and Mg have been reported to reduce blood pressure (Ranhotra *et al.*, 1998). Fluted pumpkin consumption may serve this purpose. The micro minerals of Zn and Fe content of fluted pumpkin ranged between 43.28 ± 0.6 – 67.65 ± 1.1mg/kg and 39.34 ± 1.4 – 67.87 ± 1.1 mg/kg respectively. The Zn and Fe content is lower than the value reported by Longe *et al.*, (1983), Zn, 100mg / kg and Fe, 378 mg/kg. It is however, within the range reported for some oil seeds; sunflower, Robinson (1975); Zn (71 - 76 mg/kg), Fe (56 – 67mg/kg) and Oshodi *et al*. (1999); Zn (51 mg/kg). Fluted pumpkin seed can effectively supply Recommended Daily Allowance (RDA) of Zn and Fe for human of any age or Physiological condition (Bogert, 1973). This corroborates the report of Akoroda (1990) that *Telfairia occidentalis* leaf extract is administered as a blood tonic for convalescent persons. Zn is required to prevent growth and mental retardation in humans (NAS, 1977). The toxic elements, Hg, Pb, Cd and As ranged between 0.0 – 0.17 ± 0.01 mg/kg; 0.12 ± 0.02 – 0.63 ± 0.02mg/kg; 0.01 ± 0.006 – 0.05 ± 0.006 mg/kg and 0.0 – 0.31 ± 0.02 mg/kg respectively. The seed is generally low in toxic heavy metals and high values were only observed in the raw dried and germinated samples while processing especially fermentation and boiling reduced them significantly. The mass ratio of Cd: Zn has been reported to be very important in determining biochemical outcome of Cd toxicity. Zn had been reported to ameliorate the potential toxicity of cadmium by simple mass action effect (Pier and Bang, 1980). The ratio of Cd: Zn in the seed ranged between 1: 130 to 1: 3625 which is above the recommended 1: 100 (NAS, 1977) Hence, Cd though low in the seed flour, the predominant presence of zinc would further reduce it's toxicity.

The Ca / P and Ca / Mg weight ratio (Table 7), ranged between 0.1 – 0.02 and 0.04 – 0.10 respectively. The values are low when compared with the recommended ratio of 1.0 and 2.2 respectively (NRC, 1989)**.**

This may be due to the low Ca content of the seed flour. Ca, P, and Mg are important in the formation of bones and teeth as well as in controlling the level of Ca in the blood of animals (NRC, 1989). Hence, Ca supplementation in diet based on the seed flour may be necessary to prevent Ca deficiency diseases like rickets.

The computed values of [K / (Ca+ Mg)] meq
2.2, [Phytates] / [Zn]; [Ca] / [Phytate], [Ca] [Phytates] / [Zn] and percentage [Phytate] / [P] molar ratio (Table 7), ranged between 3.49±0.03 – 5.76±0.09; 4.46±0.02 – 31.68±0.03; 0.09±0.003 – 0.71±0.02; 13.52±0.05 – 58.29±0.2 and 1.15±0.08 – 6.48±0.3% respectively. Phytic acid can form stable complexes with mineral ions rendering them unavailable for intestinal uptake (Lopez *et al.*, 2002). The computed values are considered to be low enough not to impair dietary Zinc bio availability and enhance phosphorus bioavailability (Udosen and Akpanabiatu, 1993; wise, 1983) The values are high in raw seed flour while processing especially fermentation reduced them. The values are lower than the values reported by Aremu and Abara (1992) as well as the values considered being critical to reduced dietary zinc bioavailability (0.5mol/kg) (Turnlund *et al.*, 1984).

Tables 8 and 9 show that the predominant fatty acid groups in fluted pumpkin seed oil are the unsaturated fatty acids. It ranged between 57.39±0.05 – 68.99±0.05% and consisted mainly oleic acid, 34.52±0.03 – 46.39±0.6% and linoleic acid, 11.0±0.6 – 30.94±0.2%. The saturated fatty acid in the seed ranged between 25.45±0.03 – 38.47±0.04% and consisted mainly Palmitic acid, 13.61±0.1 – 19.56±0.05% and stearic acid, 11.84±0.06 – 18.91±0.2%. The result obtained in this work is close to the values reported by Asiegbu (1987) i.e. oleic acid, 33.01%, linoleic acid 30.21%, Palmitic acid 13.35% and stearic acid 18.5%, though the author also reported some other fatty acids in very low concentrations. The oleic acid content of fluted pumpkin seed oil is higher than the values reported for some edible seed oils. Soybean oil (14.35%), Paul and South gate (1985); *Adenopus breviflores* brenth oil

(8.32%), (Oshodi, 1996); quinoa chenopodium wild seed oil, (24.5%) (Ruales and Nair 1993). The linoleic acid content of fluted pumpkin seed oil (11.0±0.6 – 30.94±0.2%), is lower than the values reported for soybean oil 52.0%, *Adenopus breviflores* 58.77% and quinoa seed oil 52.3%. It is comparable with the value reported for peanut oil 31.4% (Worthington, 1977). Thus fluted pumpkin seed oil contained less essential fatty acid than the named oils.

The oleic acid / Linoleic acid ratio (O/L) observed in fluted pumpkin seed oil ranged between 1.12 – 4.22, the highest was from the germinated sample while the least was from the fermented sample. The O/L of the seed oil is close to the values reported for peanut oil, 1.48 (Branch *et al.*, 1990) but less than cashew nut oil 3.69 – 6.40. High oil stability has been associated with high (O/L) value in hazelnut oil (Branch *et al.*, 1990) hence, fluted pumpkin oil may not be very stable.

The PS ratio (the relationship between all polyunsaturated fatty acid and saturated fatty acid) of fluted pumpkin oil ranged between 0.29±0.03 –1.22±0.02. (Table, 9) The PS value is much less than the values reported for some edible oils; soybean oil, 3.92; corn oil, 4.65; quinoa oil, 4.90; but it is close to olive oil, 0.65 and bambara groundnut oil, 1.41.

The low PS value of fluted pumpkin seed oil may be due to the low di and tri unsaturated fatty acids in the oil. The percentage of energy delivered by the linoleic acid in the seed oil (Table, 9) ranged between 6.22±0.1 – 24.56±0.07%. This is higher than the least value of 2.7% recommended for infant food by American Academy of Pediatricians (NRC, 1989). The seed oil can be used in infant weaning food formulation. Generally, processing has effects on the fatty acid composition of the seed oil.

The most abundant amino acids in fluted pumpkin seed flours (Table 10) are glutamic acid (132.04±0.02 – 152.30±0.2mg/g cp.), aspartic acid (124.61±0.03 – 130.78±0.02mg/g cp) and arginine (73.76±0.06 – 117.54±0.04mg/g cp.) while methionine (6.02±0.2 – 8.11±0.05mg/g cp.) is the least. Arginine is an essential amino acid for child growth (Robinson, 1987)

Germination and fermentation improve the amino acids of the seed flour while boiling and roasting reduce the essential amino acids. The result agreed with the observation of Young and Varner (1959), Chropreeda and Fields (1984) and Asiedu *et al.*, (1993), on lettuce seeds, blends of soybean, corn meal and maize and sorghum respectively. Hence, fermentation or germination may be necessary (as the case may be) where the seed flours are to be used as ingredient for fabricated foods or in weaning food formulation. The amino acid pattern obtained in this study is close to the values reported for the raw and cooked fluted pumpkin seed by Longe (1983). The Lysine content of fluted pumpkin seed flour ranged between 37.5±0.2 – 66.64±0.03mg/g cp. that is comparable or higher than the lysine content of the reference egg protein (63mg/g cp.). Hence, fluted pumpkin seed may be mixed with cereals like maize, which are low in lysine for weaning food formulation (Chavan *et al.*, 2001). The total essential amino acid of fluted pumpkin ranged between 492.56±0.03 – 527.23±0.4mg/g cp. It is close to the reference egg protein, 566mg/gcp. (Paul *et al.*, 1980). It is also comparable with some oil seeds; Soybean flours (440 – 503mg/g cp.) Solsulski (1983); Kuri *et al.*, (1991) and peanut meal, (453mg/g cp.), Lusas (1979) and hull free defatted ‘egusi’ Flour *Colocynthis citrullus* 190mg/g cp. (Akobundu *et al.*, 1982). The seed may be a good substitute to “egusi” in local diet. The individual essential amino acid pattern of fluted pumpkin is comparable or slightly higher than the range of values required for all age groups, especially the fermented and germinated samples except tryptophan and the sulphur amino acids. This result agreed with the report of Bates *et al.*, (1977) and Asiedu *et al.,* (1993) that sprouting and fermentation improve the sulphur containing amino acid of soybean and sorghum / maize especially. The predicted biological value (BV) of fluted pumpkin protein ranged between 7.76±0.06 – 39.55±0.1. Processing technique affected the BV, fermentation (BV = 39.55±0.1); germination (BV = 36.85±0.03) improved the predicted biological value when compared with the raw dried seed (BV = 27.45±0.02). Boiling (BV = 17.66±0.03) and roasting (BV = 7.76±0.06) reduced the biological value of the seed flour. The Predicted BV of fluted pumpkin is higher than that of beach pea protein isolates (36.5 – 40.13) (Chavan *et al;* 2001). Since fermentation and germination improved the BV of fluted pumpkin, it may be a necessary processing step in the use of the seed flour for the formulation of infant food and livestock feed.

The predicted protein efficiency ratio (PER) and relative protein efficiency ratio (R – PER) of fluted pumpkin seed flours ranged between 0.66±0.02 – 1.24±0.1 and 26.40±0.2 – 49.60±2.6% respectively. The PER of fluted pumpkin is comparable with the value reported for some oil seeds; cotton seed, have PER 0.63 – 2.21 and R – PER of 60 – 80%; Peanut meal have R – PER of 30 – 45% and soy meal, 47 – 100%. It is however, less than the value reported for rapeseed, R – PER of 84 – 88%. Fermentation and boiling enhanced the PER and R – PER of fluted pumpkin when compared with the raw dried seed while they were reduced by germination and roasting. When the predicted PER of fluted pumpkin seed obtained in this work was compared with the INVIVO PER values reported by Achinewhu and Isichei (1990), the in vivo values (1.26, 1.49) for raw and fermented reported not to be significantly different from those fed on casein though slightly higher and the rats gained more weight. Hence, fermentation may be an effective means of improving the protein quality of fluted pumpkin seed. The amino acid scores of fluted pumpkin seed ranged between 0.44 – 25.34 (Table 11). Methionine, tryptophan and leucine are the limiting amino acids of the seed flours. Fermentation improved the chemical score of fluted pumpkin seed flour protein followed by germination. Roasted fluted pumpkin has the least tryptophan content resulting into very low chemical scores; this may be due to heat labile nature of tryptophan (Concon, 1975).

CONCLUSION

Processing affects the level of nutrients in fluted pumpkin seed flours. Germination and fermentation enhance protein quality of the seed flour, reduce deleterious elements and improve zinc bioavailability. The seed may be a potential source of nutrient in human diet if adequately processed. *In vivo* testing of the seed flours to confirm bioavailability of the nutrients may be recommended.

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Table 1: Proximate composition of processed full fat fluted pumpkin seed Flours (g/100g dry weight)

Component	Full fat fluted pumpkin				
	Raw Dried	**Boiled**	**Fermented**	**Germinated**	**Toasted**
Moisture	5.50±0.1	3.52±0.08	8.18±0.2	2.25±0.05	6.38±0.2
Crude protein	$30.42{\pm}0.7_{b}$	$29.92{\pm}0.7_{b}$	$35.06{\pm}0.8_{a}$	$33.53{\pm}0.8_{a}$	$30.59{\pm}0.7_{b}$
Crude fat	$50.49{\pm}1.2_{b}$	$42.74{\pm}1.0_{d}$	$56.91{\pm}1.3_{a}$	$42.23{\pm}1.0_{d}$	$46.04{\pm}1.1_{c}$
Total ash	$5.08{\pm}0.1_{a}$	$2.55{\pm}0.06_{b}$	$2.06{\pm}0.05_{c}$	$2.50{\pm}0.09_{b}$	$5.09{\pm}0.1_{a}$
Crude fibre	$2.60{\pm}0.06_{b}$	$2.49{\pm}0.06_{bc}$	$2.35{\pm}0.05_{c}$	$3.98{\pm}0.09_{a}$	$2.64{\pm}0.06_{b}$
Carbohydrate (by difference)	$11.41{\pm}0.3_{d}$	$22.30{\pm}0.5_{a}$	$3.62{\pm}0.08_{e}$	$17.76{\pm}0.5_{b}$	$15.64{\pm}0.4_{c}$

Values with different subscripts on the same row are significant ($p < 0.05$)

Errors are standard errors from the mean

Table 2: Proximate composition of processed defatted fluted pumpkin seed Flour (g/100g dry weight)

Component	Defatted fluted pumpkin				
	Raw Dried	**Boiled**	**Fermented**	**Germinated**	**Toasted**
Moisture	5.75±0.1	4.59±0.1	1.40±0.03	1.89±0.05	4.67±0.1
Crude protein	66.12±1.5	65.50±1.5	70.53±1.6	66.87±1.5	66.16±11.5
Crude fat	$8.35\pm0.2_b$	$6.53\pm0.2_c$	$7.93\pm0.2_b$	$8.10\pm0.2_b$	$9.73\pm0.2_a$
Total ash	$7.56\pm0.2_c$	$6.01\pm0.1_d$	$5.95\pm0.11_d$	$8.93\pm0.2_a$	$8.33\pm0.2_b$
Crude fibre	$5.50\pm0.1_b$	$5.34\pm0.1_{bc}$	$4.94\pm0.1_c$	$6.92\pm0.2_a$	$5.50\pm0.1_b$
Carbohydrate (by difference)	$12.47\pm0.3_b$	$16.62\pm0.4_a$	$10.65\pm0.2_c$	$9.18\pm0.2_d$	$10.28\pm0.2_c$

Values with different subscripts on the same row are significant ($p < 0.05$)

Table 3: Ash analysis of processed full fat fluted pumpkin seed flours (%)

Parameter	Full fat fluted pumpkin				
	Raw Dried	**Boiled**	**Fermented**	**Germinated**	**Toasted**
Water soluble ash	98.85±2.3	98.90±2.3	98.95±2.1	99.05±2.3	99.50±2.3
Acid insoluble ash	$0.15±0.006_c$	$0.20±0.06_b$	$0.01±0.0_d$	$0.01±0.0_d$	$0.41±0.01_a$
Soluble ash alkalinity	$20.97±0.5_a$	$5.49±0.1_c$	$1.99±0.05_d$	$9.49±0.2_b$	$21.44±0.5_a$
K_2CO_3 alkalinity	$1.45±0.03_a$	$0.38±0.01_c$	$0.14±0.006_d$	$0.66±0.02_b$	$1.48±0.03_a$
K_2O alkalinity	$0.99±0.02_a$	$0.26±0.006_c$	$0.09±0.0_d$	$0.45±0.01_b$	$1.01±0.02_a$
Na_2CO_3 alkalinity	$1.11±0.02_a$	$0.29±0.006_c$	$0.11±0.0_d$	$0.50±0.01_b$	$1.14±0.03_a$
Sulphated ash	$3.99±0.09_b$	$2.89±0.07_c$	$2.00±0.05_d$	$2.89±0.07_c$	$4.57±0.1_a$

Values with different subscripts on the same row are significant ($p < 0.05$)

Table 4: Ash analysis of processed defatted fluted pumpkin seed flours (%)

Parameter	Defatted fluted pumpkin				
	Raw Dried	**Boiled**	**Fermented**	**Germinated**	**Toasted**
Water soluble ash	98.30±2.3	97.75±2.3	94.66±2.3	98.01±2.3	98.45±2.3
Acid insoluble ash	$0.30±0.006_b$	$0.20±0.006_c$	$0.30±0.006_b$	$0.30±0.006_b$	$0.50±0.01_a$
Soluble ash alkalinity	$38.40±0.9_c$	$11.00±0.3_d$	$1.50±0.03_e$	$81.67±1.9_a$	$52.34±1.2_b$
K_2CO_3 alkalinity	$2.65±0.06_c$	$0.76±0.02_d$	$0.10±0.0_e$	$5.64±0.1_a$	$3.62±0.08_b$
K_2O alkalinity	$1.81±0.04_c$	$0.51±0.01_d$	$0.07±0.0_e$	$3.85±0.09_a$	$2.47±0.06_b$
Na_2CO_3 alkalinity	$2.04±0.05_c$	$0.58±0.01_d$	$0.08±0.0_e$	$4.33±0.1_a$	$2.77±0.06_b$
Sulphated ash	$9.06±0.2_b$	$7.07±0.2_c$	$6.76±0.2_c$	$7.26±0.2_c$	$9.92±0.2_a$

Values with different subscripts on the same row are significant ($P < 0.05$)

Table 5: Energy values (KJ/g) of processed full fat fluted pumpkin seed flour

Sample	**Gross energy Determined****	**Gross energy Calculated***	**P^e%**	**F^e%**	**NDPE%**
Full fat Fluted Pumpkin					
Raw Dried	$29.54\pm0.8_a$	26.11±0.3	19.57_b	73.11_{ab}	11.74_b
Boiled	$26.55\pm0.7_b$	24.93±0.2	20.18_b	64.82_c	12.11_b
Fermented	$30.06\pm0.8_a$	28.01±0.5	21.03_b	76.79_a	12.62_b
Germinated	$28.11\pm0.7_{ab}$	24.58±0.1	22.90_a	64.93_c	13.74_a
Toasted	$29.72\pm0.8_a$	25.17±0.6	20.42_b	69.13_{bc}	12.25_b

Values with different subscript on the same column are significant (pd” 0.05)

P^e% = Proportion of total energy due to protein

F^e% = Proportion of total energy due to fat

NDPE% = Utilizable energy due to protein

** Calculated using Atwater factor*

*** Obtained by using bomb colorimeter.*

Table 6: Processing effects on the mineral composition of fluted pumpkin seed flours. (mg/kg dry weight).

Mineral	Full fat fluted pumpkin				
	Raw Dried	**Boiled**	**Fermented**	**Germinated**	**Toasted**
K	$11,388\pm4.7_b$	$4,207\pm1.8_e$	$7,130\pm2.8_d$	$11713\pm1.6_a$	$7540\pm2.9_c$
Na	$303.04\pm1.8_b$	$275.48\pm2.8_c$	$311.48\pm1.7_a$	$161.67\pm2.5_e$	$247.69\pm1.0_d$
Ca	$77.99\pm1.6_c$	$93.40\pm1.2_b$	$131.79\pm2.1_a$	$88.38\pm0.9_b$	$63.50\pm1.6_d$
Mg	$1,898\pm0.9_b$	$1,113\pm1.7_e$	$1,299\pm2.6_d$	$2,032\pm1.3_a$	$1,346\pm2.2_c$
Cu	$40.60\pm0.5_a$	$40.97\pm1.2_a$	$23.43\pm1.8_b$	$25.58\pm1.1_b$	$38.51\pm1.1_a$
Mn	$21.96\pm1.2_a$	$22.28\pm1.2_a$	$8.44\pm0.5_c$	$15.86\pm0.6_b$	$11.22\pm0.7_c$
Zn	$45.95\pm1.2_b$	$43.28\pm0.6_b$	$67.65\pm1.1_a$	$65.36\pm0.7_a$	$45.43\pm1.1_b$
Sn	$10.79\pm0.6_a$	0.00_c	0.00_c	0.00c	7.05±0.2b
P	$10,577\pm1.9_b$	$5,811\pm3.6_e$	$12,479\pm3.5_a$	6,484±2.2d	$9,114\pm4.0_c$
Fe	$59.06\pm1.2_b$	$47.07\pm1.2_c$	$67.85\pm1.1_a$	$39.34\pm1.4_d$	$58.36\pm1.4_b$
Al	$23.06\pm3.5_a$	$20.33\pm0.03_b$	$18.98\pm0.4_b$	$11.14\pm0.08_d$	$16.29\pm1.3_c$
Ni	$0.02\pm0.006_a$	$0.01\pm0.006_a$	$0.01\pm0.003_a$	$0.02\pm0.002_a$	0.00_b
Mo	$15.09\pm1.2_b$	$6.13\pm1.2_d$	$10.88\pm1.2_c$	$16.05\pm1.2_a$	$12.19\pm0.7_b$
As	$0.31\pm0.01_c$	$0.32\pm0.01_c$	0.00_d	$0.56\pm0.02_b$	$0.92\pm0.02_a$
Hg	$0.17\pm0.01_a$	$0.07\pm0.01_b$	$0.09\pm0.006_b$	0.00_d	$0.04\pm0.006_c$
Pb	$0.31\pm0.03_c$	$0.12\pm0.02_d$	$0.63\pm0.02_a$	$0.51\pm0.02_b$	$0.60\pm0.06_{ab}$
Se	$0.04\pm0.006_a$	$0.04\pm0.006_a$	$0.03\pm0.006_{ab}$	$0.02\pm0.006_c$	$0.03\pm0.006_{ab}$
Cd	$0.05\pm0.006_a$	$0.01\pm0.006_b$	$0.01\pm0.002_b$	$0.05\pm0.006_a$	$0.04\pm0.006_a$

Values with different subscripts on the same row are significant ($p < 0.05$)

Table 7: Computed mineral and phytate millimolar ratios for fluted pumpkin seed flours

Mineral/phytate	Full fat fluted pumpkin				
	Raw dried	Boiled	Fermented	Germinated	Toasted
K/Na (wt)	$37.58\pm1.1_b$	$15.27\pm0.7_e$	$22.89\pm1.1_d$	$72.45\pm0.8_a$	$30.44\pm0.8_c$
Ca/P (wt)	0.01 ± 0.001	0.02 ± 0.006	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
Ca/Mg (wt)	$0.04\pm0.006_b$	$0.08\pm0.006_{ab}$	$0.10\pm0.03_a$	$0.04\pm0.006_b$	$0.05\pm0.006_b$
[K/(Ca + Mg)] meq. 2.2	$5.76\pm0.09_a$	$3.49\pm0.03_d$	$4.98\pm0.03_c$	$5.52\pm0.04_{ab}$	$5.35\pm0.2_b$
[Phytate]/[Zn] (molar ratio)	$31.68\pm0.3_a$	$10.19\pm0.01_c$	$4.46\pm0.02_e$	$9.90\pm0.2_d$	$13.98\pm0.06_b$
[Ca]/[Phytate] (molar ratio)	$0.09\pm0.003_d$	$0.35\pm0.03_b$	$0.71\pm0.02_a$	$0.22\pm0.02_c$	$0.16\pm0.02_{cd}$
[Ca][Phytate]/[Zn] (molar ratio)	$58.29\pm0.2_a$	$22.92\pm0.01_b$	$13.52\pm0.05_e$	$21.38\pm0.2_c$	$20.84\pm0.06_d$
[Phytate]/[P] (%molar ratio)	$6.48\pm0.3_a$	$3.60\pm0.1_c$	$1.15\pm0.08_d$	$4.74\pm0.03_b$	$3.30\pm0.2_c$

Values with different subscripts on the same row are significant ($p < 0.05$)

Table 8: Processing effect on the fatty acid composition of fluted pumpkin seed oil (% Total fatty acid composition)

Fatty acid	Fluted pumpkin seed oil				
	Raw Dried	**Boiled**	**Fermented**	**Germinated**	**Toasted**
Palmitic acid $C_{16:0}$	$16.09\pm0.05_b$	$16.23\pm0.1_b$	$13.61\pm0.1_c$	$19.56\pm0.04_a$	$16.00\pm0.02_b$
Stearic acid $C_{18:0}$	$14.23\pm0.1_c$	$16.88\pm0.1_b$	$11.84\pm0.06_e$	$18.91\pm0.1_a$	$13.20\pm0.2_d$
Oleic acid $C_{18:1}$	$40.40\pm0.2_b$	$40.04\pm0.04_b$	$34.52\pm0.03_c$	$46.39\pm0.06_a$	$40.25\pm0.1_b$
Linoleic acid $C_{18:2}$	$27.47\pm0.1_c$	$23.34\pm0.06_d$	$30.94\pm0.2_a$	$11.00\pm0.6_e$	$28.74\pm0.04_b$
Unidentified peaks	$1.81\pm0.02_d$	$3.51\pm0.03_c$	$9.09\pm0.05_a$	$4.14\pm0.05_b$	$1.81\pm0.04_d$
Total Unsaturated Fatty Acid (TUFA)	$67.87\pm0.1_b$	$63.38\pm0.08_d$	$65.46\pm0.03_c$	$57.39\pm0.05_e$	$68.99\pm0.05_a$
Total Saturated Fatty Acid (TSFA)	$30.32\pm0.2_c$	$32.11\pm0.06_b$	$25.45\pm0.03_e$	$38.47\pm0.04_a$	$29.20\pm0.1_d$
Essential Fatty Acid (EFA) ($C_{18:2}$ + $C_{18:3}$)	$27.47\pm0.05_c$	$23.34\pm0.05_d$	$30.94\pm0.06_a$	$11.00\pm0.1_e$	$28.74\pm0.08_b$

Values with different subscripts on the same row are significant ($p < 0.05$)

Table 9: Processing effect on the fatty acid distribution according to saturation on fluted pumpkin seed oil

Fatty acid	**Fluted pumpkin seed oil**				
	Raw Dried	**Boiled**	**Fermented**	**Germinated**	**Toasted**
Saturated Fatty Acid	$30.32 \pm 0.2_{c}$	$32.11 \pm 0.06_{b}$	$25.45 \pm 0.06_{e}$	$38.47 \pm 0.04_{a}$	$29.20 \pm 0.1_{d}$
Monounsaturated Fatty Acid	$40.40 \pm 0.2_{b}$	$40.04 \pm 0.03_{b}$	$34.52 \pm 0.03_{c}$	$46.39 \pm 0.04_{a}$	$40.25 \pm 0.1_{b}$
Diunsaturated Fatty Acid	$27.470.06_{c}$	$23.34 \pm 0.05_{d}$	$30.94 \pm 0.07_{a}$	$11.00 \pm 0.1_{e}$	$28.74 \pm 0.08_{b}$
Total Triunsaturated fatty acid	ND	ND	ND	ND	ND
P/S ratio	$0.91 \pm 0.03_{b}$	$0.73 \pm 0.01_{c}$	$1.22 \pm 0.05_{a}$	$0.29 \pm 0.03_{d}$	$0.98 \pm 0.05_{b}$
% Energy due to linoleic acid	$19.84 \pm 0.03_{b}$	$13.19 \pm 0.03_{c}$	$24.56 \pm 0.07_{a}$	$6.22 \pm 0.1_{d}$	$19.79 \pm 0.09_{b}$
Oleic / Linoleic ratio	$1.47 \pm 0.003_{c}$	$1.72 \pm 0.006_{b}$	$1.12 \pm 0.07_{d}$	$4.22 \pm 0.09_{a}$	$1.40 \pm 0.06_{c}$

Values with different subscripts on the same row are significant ($p < 0.05$)

P/S Ratio: ratio of polyunsaturated to saturated fatty acid

ND: Not detected

Table 10: Amino acid Composition of Raw and Processed Full fat Fluted Pumpkin Seed Flours (mg/g crude protein)

Full fat fluted pumpkin Amino acid	Raw Dried	Boiled	Fermented	Germinated	Toasted
Asp	$130.78\pm0.07_a$	$127.05\pm0.03_c$	$128.21\pm0.1_b$	$126.13\pm0.03_d$	$124.610.03_e$
Glu	$152.30\pm0.7_a$	$133.51\pm0.3_d$	$132.04\pm0.02_e$	$143.01\pm0.06_c$	$147.900.4_b$
Ser	$45.80\pm0.06_c$	$38.110.06_d$	$32.53\pm0.04_e$	$47.24\pm0.02_b$	$48.80\pm0.06_a$
Gly	$64.54\pm0.01_a$	$61.00\pm0.09_c$	$52.020.05_e$	$61.81\pm0.07_b$	$59.70\pm0.1_d$
His*	$36.59\pm0.03_c$	$30.15\pm0.09_e$	$40.290.05_a$	$38.55\pm0.06_b$	$35.88\pm0.006_d$
Arg*	$113.51\pm0.02_b$	$117.540.04_a$	$73.76\pm0.06_d$	$105.91\pm0.02_c$	$114.51\pm0.05_b$
Thr*	$36.80\pm0.06_c$	$34.08\pm0.2_e$	$40.050.006_b$	$42.25\pm0.006_a$	$35.48\pm0.02_d$
Ala	$35.71\pm0.02_b$	$31.34\pm0.08_d$	$35.490.03_c$	$30.14\pm0.02_e$	$35.96\pm0.006_a$
Pro	$49.80\pm0.06_b$	$43.46\pm0.06_c$	$56.440.03_a$	$40.55\pm0.02_d$	$41.04\pm0.02_d$
Tyr*	$54.95\pm0.02_c$	$50.38\pm0.02_d$	$47.310.06_e$	$58.08\pm0.03_b$	$66.34\pm0.03_a$
Val*	$51.90\pm0.06_c$	$56.25\pm0.1_a$	$47.450.7_d$	$55.04\pm0.02_b$	$51.86\pm0.03_c$
Met*	$6.56\pm0.02_c$	$6.02\pm0.2_c$	$8.11\pm0.05_a$	$7.56\pm0.03_b$	$6.52\pm0.03_c$
Cys*	$24.71\pm0.03_b$	$17.96\pm0.02_c$	$25.790.03_a$	$16.66\pm0.01_d$	$18.35\pm0.03_c$
Ile*	$49.34\pm0.02_a$	$46.56\pm0.08_b$	$46.440.03_b$	$39.96\pm0.02_c$	$38.28\pm0.04_d$
Leu*	$49.34\pm0.01_a$	$46.56\pm0.06_b$	$46.440.02_b$	$39.96\pm0.006_c$	$38.28\pm0.04_d$
Phe*	$47.56\pm0.02_d$	$49.25\pm0.1_b$	$54.650.03_c$	$58.78\pm0.03_a$	$49.48\pm0.04_b$
Lys*	$42.25\pm0.1_c$	$40.20\pm0.1_d$	$66.64\pm0.03_a$	$54.82\pm0.06_b$	$37.50\pm0.2_e$
Trp*	$7.08\pm0.04_c$	$4.23\pm0.1_d$	$13.78\pm0.04_a$	$9.66\pm0.01_b$	$0.08\pm0.02_e$
TAA	$998.87\pm1.2_a$	$933.65\pm0.05_e$	$947.440.3_d$	$976.11\pm0.02_b$	$950.57\pm0.02_c$
TEAA	$519.94\pm0.6_b$	$499.180.08_d$	$510.71\pm0.06_c$	$527.23\pm0.4_a$	$492.56\pm0.03_e$
TEAA/TAA (%)	$52.05\pm0.9_c$	$53.46\pm0.06_b$	$53.90\pm0.06_b$	$54.01\pm0.06_a$	$51.82\pm0.02_d$
EAA/NEAA	$1.09\pm0.01_c$	$1.15\pm0.02_b$	$1.17\pm0.03_a$	$1.17\pm0.03_a$	$1.08\pm0.02_c$
TSAA (Met + Cys)	$30.62\pm0.02_b$	$23.98\pm0.06_e$	$33.90\pm0.02_a$	$24.22\pm0.01_d$	$24.87\pm0.01_c$
ArEAA (Phe +Tyr)	$102.51\pm0.1_c$	$99.63\pm0.2_d$	$101.96\pm0.02_c$	$116.86\pm0.006_a$	$115.82\pm0.02_b$
BV	$27.41\pm0.02_c$	$17.66\pm0.03_d$	$39.55\pm0.1_a$	$36.85\pm0.03_b$	$7.76\pm0.06_e$
PER	$1.24\pm0.1_a$	$1.13\pm0.01_a$	$1.14\pm0.02_a$	$0.73\pm0.02_b$	$0.66\pm0.02_b$
R- PER (%)	$49.60\pm2.6_a$	$43.20\pm0.9_b$	$45.60\pm0.1_b$	$29.20\pm0.3_c$	$26.40\pm0.2_d$

Values with different subscripts on the same row are significant Pd" 0.05; TAA = Total amino acids; TEAA = Total Essential amino acids; TSAA = Total sulphur amino acids (Met + Cys); EAA = Essential aromatic amino acids; BV = Biological Value; PER = Protein Efficiency Ratio

Table 11: Amino acid scores (%) of the essential amino acids of fluted pumpkin seed flours

Amino acid	Full fat fluted pumpkin seed flours					Egg ref**
	Raw Dried	Boiled	Fermented	Germinated	Toasted	(mg/g protein)
Asp						
Glu						
Ser						
Gly						
His*	152.46	125.63	167.88	160.63	149.50	24
Arg*	186.08	192.69	120.92	173.62	187.72	61
Thr*	72.16	66.82	78.53	82.84	69.57	51
Ala						
Pro						
Tyr*	125.95	137.38	118.28	145.20	165.85	40
Val*	68.29	74.01	62.43	72.42	68.24	76
Met*	20.50	20.38	25.34	23.63	20.38	32
Cys*	133.67	99.78	143.28	92.56	101.94	18
Ile*	88.11	83.14	82.93	66.00	68.36	56
Leu*	59.45	56.10	55.95	48.14	46.12	83
Phe*	93.25	96.57	107.16	115.25	97.02	51
Lys*	67.06	63.81	105.78	87.02	59.52	63
Trp*	39.33	23.50	76.56	53.67	0.44	18
Chemical score	20.50	20.38	25.34	23.63	0.44	
1st limiting amino acid	Met	Met	Met	Met	Trp	
2nd limiting amino acid	Trp	Trp	Leu	Leu	Met	

R-PER= Relative Protein Efficiency Ratio; * = Essential amino acids