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NUTRITIONAL EVALUATION OF FERMENTED, GERMINATED AND ROASTED PUMPKIN (*CUCURBITA MAXIMA*) SEED FLOUR

– Research paper –

Adeyanmola Oluwaseyi AKINTADE*, Olugbenga Olufemi AWOLU**, Beatrice Olawumi IFESAN**[1]

**Food Science and Technology Department, Ondo State University of Science and Technology, Okitipupa, Nigeria; **Food Science and Technology Department, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria*

Abstract: Freshly harvested pumpkin seed was processed into flour namely; raw (RPSF), fermented (FPSF), germinated (GPSF) and roasted (ROPSF). Various pumpkin seed flour obtained were investigated for nutritional properties using standard methods. Protein was higher in the fermented flour (35.85%) while the roasted flour (46.31%) possessed the highest fat content. Results obtained from processed pumpkin seed flour further revealed that glutamic acid (10.45- 13.02 g/100g) and linoleic acid (134.72- 419.70 µg/ml) were the most abundant amino acid and fatty acid, respectively. Processed pumpkin seed flour contained appreciable amount of minerals and demonstrated very good functional properties. It can be concluded that subjecting pumpkin seed to various processing methods enhanced its nutritional properties and may find application in food industry.

Keywords Pumpkin seed, fermentation, germination, roasting, nutritional evaluation.

INTRODUCTION

Cucurbita maxima (pumpkin) is a fruit vegetable and may be classified as one of the underutilized crops. Some other species include *Curcubita moschata* and *Curcubita pepo* (Yadegariet al., 2012). Pumpkin seeds can be consumed by different age groups as boiled, fried snack or added to stew to eat yam (Agbagwa and Ndukwu, 2004). They can serve as a good source of lipids, proteins, carbohydrates and other nutrients that are important for maintaining good health (Alfawaz, 2004). Pumpkin seeds have exhibited pharmacological properties such as anti-diabetic (Callet al., 2006), antifungal, antibacterial, anti-inflammation and antioxidant (Wang and Ng, 2003).

Fermentation as a processing method has been shown to enhance the biological enrichment of food substrates with elimination of anti-nutrients (McGovern et al., 2004). Fermented products have further benefits of enhancing bio-nutrients, minerals, flavour and aroma. Germination of seed improves its nutritional quality and causes an important change in biochemical and sensory characteristics (Shemi, 2013). It was reported that germination resulted in an appreciable reduction in the factors responsible for flatulence, thus increasing the intake and utilization of available protein and carbohydrate (Urbano et al., 2006). Roasting is often practiced in small-scale processing of oil seeds while the heat treatment is used to induce the development of desired colour, taste and flavour. There has been little work reported on effects of processing methods on pumpkin seed flour. Therefore, this work was done to investigate the effects of fermentation, germination and roasting on the nutritional properties of pumpkin seed flour.

MATERIALS AND METHODS

Materials

Pumpkin (*Curcubita maxima*) used for this study was planted at a farm in Iyin–Ekiti, Ekiti State, Nigeria. Mature fruits were harvested and brought to the laboratory for further processing. All the reagents used were of analytical grade.

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Preparation of pumpkin seed flours

Raw pumpkin seed flour

Mature pumpkin fruits were harvested and opened using a sharp knife. The seeds were manually

[1] Corresponding author. E-Mail addresses: bodewunmi2001@yahoo.co.uk ; obifesan@futa.edu.ng

removed, rinsed with water, allowed to drain in a strainer and oven dried at 50 ºC for 12 h using hot air rapid drying oven (Soyokaze type ASF-113S). The dried seeds were then milled and sieved through a 60 mesh (British standard screen). The fine pumpkin seed flour was packaged and stored in an air tight jar for further analyses (El-Adawy and Taha , 2001).

Fermented pumpkin seed flour

The fresh seeds were collected, boiled for 20 mins, allowed to drain and wrapped with blanched banana leaves. This was subjected to spontaneous fermentation for five days at room temperature. The seeds were then exposed to dry, milled, sieved to obtained fine flour and stored in an air tight jar (Ifesan et al., 2017)

Germinated Pumpkin Seed Flour

Fresh pumpkin seed was soaked in water for 12 h after which the water was drained, the steeped pumpkin seeds were spread on moistened jute bag, covered and left for 3 days with regular wetting and turning of seeds. After sprouting, the seeds were dried in the oven at 60 ºC for 4 h. The sprouts were removed from the dried seeds, milled into flour, sieved, kept in air tight container and labeled (Badau et al*.,* 2005).

Roasted pumpkin seed flour

Fresh pumpkin seed was boiled with salt for 10 min, air dried and roasted in an earthen pot with coarse sand (which was properly washed and sun-dried before use). The roasted pumpkin seed was allowed to cool, milled into flour, sieved and then stored in a tight jar container for further analyses (Abdulrahamanet al., 2014).

Proximate analysis of processed pumpkin seed flour

Proximate content of processed pumpkin seed flour was determined according to Association of Official Analytical Chemists (AOAC, 2005).

Determination of amino acid profile of processed pumpkin seed flour

Amino acid content of raw, germinated, fermented and roasted pumpkin seed flour was determined following the method of AOAC (2005) and Benitez (1989). About 200 mg of dried, defatted various pumpkin seed flour was hydrolyzed using **7** ml of 6 N HCl at 105 °C for 22 h and the hydrolysate was filtered. The residue was rinsed with distilled water while the filtrate was evaporated on water bath at 40 °C. Amino acid of the samples was determined using PTH amino acid analyzer (model 120A).

Fatty acid profile determination of processed pumpkin seed flour

The fatty acid methyl esters (FAME) were analyzed using gas chromatography (Varian 3600 GC, Mississauga, ON). The data were processed using Class-VP data processor (Shimadzu Corporation, Columbia, MD). The FAMEs were separated on a fused silica capillary column (50 m x 0.32 mm, BPx-70, SGE Column, Pty. Ltd, Victoria, Australia) with the film thickness of 0.25 mm and temperature of 230 °C (Bozan and Temelli, 2002).

Mineral content of processed pumpkin seed flour

The mineral content of the samples were determined using energy dispersive X-ray fluorescence spectroscopy according to Jerkins (2000).

Determination of functional properties of raw, germinated, fermented and roasted pumpkin seed flour

The bulk density (BD), foaming capacity (FS), foaming stability (FS), least gelation (LG), oil absorption capacity (OAC) and water absorption capacity (WAC) of processed pumpkin seed flour were determined according to Fagbemi et al. (2005) while swelling index (SI) was carried out using the method described by Ukpabi and Ndimele (1990).

Statistical analysis

All the results were obtained in triplicate, subjected to analysis of variance ANOVA and the means were separated by New Duncan Multiple Range Test (NDMRT). Means and standard deviation of all the samples were calculated and compared. SPSS for Windows program version 21.0 was used to analyze the results obtained.

RESULTS AND DISCUSSION

Proximate composition of processed pumpkin seed

Table 1 revealed the proximate composition of raw, fermented, germinated and roasted pumpkin seed flour. Fermented pumpkin seed flour had the highest moisture content (7.34%), while that of germinated was the lowest (5.55%). The protein content (31.44 - 35.85%) of processed pumpkin seed flour was found to increase after various processing. Protein content of fermented pumpkin seed flour in this study is very close to 31.1%, 30.0 % and 33.0% reported for melon, gourd and different species of pumpkin seeds, respectively (Badifu and Ogunsua, 1991; Olaofe et al., 1994). Result obtained from this research is in agreement with previous reports which suggested that processing techniques; fermentation,

germination and roasting improved the nutritional quality of food products, especially the protein content (Enujiugha et al., 2003). The fat content (41.01 - 46.31%) of raw and processed pumpkin seed flour was observed to reduce during various processing. It may be suggested that shelf life of pumpkin seed flour will be prolonged as rate of rancidity will be slow and also contribute to the low energy of the samples (Fasasi, 2009). Germinated pumkin seed flour was found to possess highest fibre content (8.18%), followed by the roasted flour (7.54%), raw (6.67%) and fermented (6.64%). Ash content of raw and processed pumpkin seed flour ranged from 3.79% to 4.41% and processing significantly reduced the ash content of pumpkin seeds which is parallel to observations of Ohtsubo et al. (2005).

Amino acid content of raw, fermented, germinated and roasted pumpkin seed flour

The essential and non-essential amino acid profile of pumpkin seed flour is presented in Table 2.

Table 1. Proximate composition (%) of raw and processed pumpkin seed flour

Samples	RPSF	FPSF	GPSF	ROPSF
Moisture	6.83 ± 0.43^{ab}	7.34 ± 0.35^{a}	5.55 ± 0.19^{c}	6.27 ± 0.14^{b}
Protein	31.44 ± 0.68^{b}	35.85 ± 0.55^{a}	33.89 ± 1.46^{a}	31.82 ± 1.63^{b}
Fat	46.31 ± 1.14^{a}	41.01 ± 0.29^{c}	44.06 ± 0.85^{ab}	43.25 ± 2.78^{bc}
Ash	4.41 ± 0.52^{a}	4.38 ± 0.58^{a}	3.79 ± 0.93^{a}	4.11 ± 0.52^{a}
Fibre	6.67 ± 0.40^{c}	6.64 ± 0.01^{c}	8.18 ± 0.23^{a}	7.54 ± 0.11^{b}
Carbohydrate	4.34 ± 0.04^{d}	4.78 ± 0.03^{b}	4.53 ± 0.04^{c}	7.01 ± 0.01^{a}

Means of triplicate determinations ± S.D with different superscripts on the same row are significantly different
RPSF - Raw pumpkin seed flour, FPSF - Fermented pumpkin seed flour, GPSF - Germinated pumpkin seed flour, ROPSF - Roasted pumpkin seed flour.

Table 2. Amino acid content (g/100g) of raw and processed *Cucurbita maxima* seed flour

Samples	RPSF	FPSF	GPSF	ROPSF	FAO/WHO 1991
Lysine	5.76 ± 0.03^{b}	4.75 ± 0.03^{d}	5.97 ± 0.08^{a}	5.31 ± 0.06^{c}	-
Histidine	2.44 ± 0.01^{b}	2.05 ± 0.07^{d}	2.84 ± 0.03^{a}	2.21 ± 0.04^{c}	
Glutamic	13.02 ± 0.00^{a}	11.55 ± 0.27^{b}	11.66 ± 0.31^{b}	10.45 ± 0.16^{c}	
Arginine	10.24 ± 0.09^{b}	9.64 ± 0.18^{c}	11.02 ± 0.09^{a}	8.52 ± 0.09^{d}	2.0
Aspartic	8.10 ± 1.00^{c}	7.78 ± 0.03^{d}	9.46 ± 0.16^{a}	8.44 ± 0.09^{b}	
Threonine	3.11 ± 0.06^{a}	2.42 ± 0.03^{c}	2.83 ± 0.06^{b}	2.25 ± 0.03^{d}	2.6
Serine	3.52 ± 0.06^{b}	3.02 ± 0.02^{c}	3.97 ± 0.03^{a}	4.05 ± 0.16^{a}	
Proline	3.03 ± 0.02^{c}	2.34 ± 0.11^{d}	3.96 ± 0.10^{a}	3.25 ± 0.00^{b}	
Glycine	2.26 ± 0.03^{b}	2.16 ± 0.02^{c}	3.52 ± 1.00^{a}	2.29 ± 0.01^{b}	
Alanine	5.53 ± 0.35^{a}	3.41 ± 0.00^{d}	3.98 ± 0.04^{c}	4.51 ± 0.04^{b}	
Cysteine	0.79 ± 0.06^{a}	0.73 ± 0.00^{a}	0.73 ± 0.00^{a}	0.54 ± 0.06^{b}	
Valine	4.47 ± 0.03^{b}	3.33 ± 0.06^{d}	5.12 ± 0.09^{a}	3.54 ± 0.03^{c}	2.8
Methionine	2.35 ± 0.00^{b}	2.04 ± 0.04^{d}	3.07 ± 0.08^{a}	2.19 ± 0.00^{c}	4.2
Isoleucine	3.51 ± 0.04^{c}	3.44 ± 0.04^{d}	4.62 ± 1.00^{a}	3.77 ± 0.04^{b}	4.2
Leucine	6.25 ± 0.06^{b}	5.57 ± 0.03^{d}	7.00 ± 0.00^{a}	5.84 ± 0.00^{c}	4.8
Tyrosine	3.19 ± 0.09^{c}	2.33 ± 0.19^{d}	4.39 ± 0.09^{a}	3.51 ± 0.10^{b}	
Phenylalanine	4.70 ± 0.09^{c}	4.17 ± 0.09^{d}	5.85 ± 0.00^{a}	4.97 ± 0.00^{b}	-
Tryptophan	1.53 ± 0.06^{a}	1.34 ± 0.03^{d}	1.50 ± 0.03^{b}	1.42 ± 0.00^{c}	-
TEAA	34.12	29.11	38.80	31.50	
TNEAA	49.68	42.96	52.69	45.56	

Means of triplicate determinations ± S.D. with different superscripts on the same column are significantly different. FPSF - Fermented pumpkin seed flour, GPSF - Germinated pumpkin seed flour, ROPSF - Roasted pumpkin seed flour, TEAA - Total essential amino acids, TNEEA - Total non essential amino acids

It was observed that germination and roasting improved some of the amino acids present in pumpkin seed flour while there were reductions in all the amino acid values of fermented sample.

The result from this study supports earlier studies by Ijarotimi (2012) and Shemi (2013) who observed increases and decreases in some amino acids after germination and fermentation of wheat grains and germination and roasting of *C. maxima* respectively. The values of total non-essential amino acid (42.96 - 52.69 g/100g) of the pumpkin seed flour samples were higher than the essential amino acid (29.11 - 38.80 g/100g). The essential amino acid content of the processed pumpkin seed flour was found to compare well with the Food and Agriculture Organization/World Health Organization (FAO/WHO, 1991) reference standard with the exception of methionine.

Fatty acid profile of raw, fermented, germinated and roasted pumpkin seed

The most abundant saturated fatty acids in processed pumpkin seed are palmitic acid (59.92 - 144.74 µg/ml) and stearic acid (32.75- 79.02 µg/ml) while linoleic acid (134.72-419.70 µg/ml) and arachidonic acid (11.27 - 15.20 µg/ml) are found to be the most abundant unsaturated fatty acid in the processed pumpkin seed flour (Table 3). The result is similar to the findings of El-Adawy and Taha (2001) on some oilseeds. The high percentage of polyunsaturated fatty acid (PUFA) of the pumpkin seed flour may enable it to exhibit some nutritional benefits. High consumption of PUFA was reported to decrease the risk of coronary heart diseases (El Sohaimy, 2012). The ratio of PUFA: total saturated fatty acid (TSFA) was highest in fermented flour (1.93) followed by the roasted sample (1.42), germinated (1.29) and raw pumpkin seed (1.08). Pumpkin seed oil may find application in cooking and other industrial food production processes where oil that is rich in fatty acids is desired.

Table 3. Fatty acid profile (µg/ml) of raw and processed *Cucurbita maxima* seed flour

Fatty acids	Carbon chain	RPSF	FPSF	GPSF	ROPSF
Butyric	C4	18.45	N.D	19.39	N.D
Caproic	C6	N.D	N.D	18.26	N.D
Capric	C10	N.D	N.D	21.32	N.D
Undecylic	C11	N.D	9.13	N.D	N.D
Lauric	C12	17.85	17.92	17.95	N.D
Myristic	C14	18.00	18.76	18.71	18.01
Myristoleic	C14:1	9.54	10.08	10.00	9.56
Pentadecylic	C15	9.14	9.24	9.21	N.D
Palmitic	C16:0	59.92	144.74	115.84	62.18
Palmitoleic	C!6:1	38.63	117.09	89.28	40.79
Margaric	C17	9.63	10.14	9.88	9.65
10Z Heptadecenoic	C17:1	N.D	10.33	10.11	N.D
Stearic	C18	32.75	79.02	61.56	35.65
Linoleic	C18:2	134.72	419.70	270.24	142.51
γ – linolenic	C18:3	83.60	245.45	161.44	88.54
Arachidic	C20	19.32	22.39	20.92	19.48
Gondoic	C20:1	9.83	10.05	10.17	9.82
Dihomo- γ – linolenic	C20:3	10.56	12.31	12.19	10.89
Arachidonic	C20:4	11.27	15.20	13.93	11.99
Eicosapentaenoic	C20:5	10.74	12.66	12.43	11.11
Heneicosylic	C21	10.04	10.09	10.20	10.16
Behenic	C22	19.58	20.52	19.98	19.63
Erucic	C22:1	N.D	11.49	11.17	10.99
Docosahexaenoic	C22:6	13.02	14.75	12.88	13.04
Tricosylic	C23	N.D	10.40	10.34	N.D
Lignoceric	C24	20.29	20.80	20.51	20.34
Nervonic	C24:1	N.D	10.95	10.54	N.D
TSFA		234.97	373.15	374.07	195.10
MUFA		58.00	169.99	141.27	71.16
PUFA		263.91	720.07	483.11	277.28
PUFA/TSFA		1.08	1.93	1.29	1.42

FPSF- Fermented pumpkin seed flour GPSF- Germinated pumpkin seed flour, ROPSF- Roasted pumpkin seed flour, TSFA- Total saturated fatty acid, MUFA- Monounsaturated fatty acid, PUFA- Polyunsaturated fatty acid, N.D- Not detected

Mineral composition raw, fermented, germinated and roasted pumpkin seed

The result of the mineral content of pumpkin seed flour on Table 4 revealed that it contained appreciable amount of minerals with potassium having the highest value (102.40 - 147.65 mg/100g) and manganese the least (0.00 - 0.07 mg/100g). El-Adawy and Taha (2001) reported that pumpkin seed contained moderate concentrations of minerals with only fair amounts of copper and zinc. It was observed that processing methods especially fermentation showed greatest retention while increases were noticed in potassium, sodium, and phosphorous, magnesium, iron, zinc and copper. Increase in mineral content during fermentation and germination couldbe as a result of activities of microorganism and bio-synthesis during processing (Ijarotimi, 2012). The ratio of Na/K (0.36 - 0.42 mg/100g) for raw and processed pumpkin seed flour is within the recommended limit of less than 1.0 (Food and Nutrition Board: Institute of Medicine, 2002). This may suggest that pumpkin seed flour is suitable for consumers that are placed on diets with low concentration of salt.

Functional properties of raw, fermented, germinated and roasted pumpkin seed

Processing methods employed showed significant remarkable effect on functional properties of pumpkin seed flour (Table 5). Water absorption capacity of the samples ranged from 137.17 to 195.70% with fermented sample having the highest. Water absorption capacity is an expression of the amount of water available for gelatinization and the ability of flour to absorb water.

Table 4. Mineral composition (mg/100g) of raw and processed *Curcubita maxima* seed flour

Samples	RPSF	FPSF	GFSF	ROPSF
Potassium	$102.40^{1} \pm 0.40^{2\,d}$	147.65 ± 0.05^{a}	143.10 ± 0.10^{b}	103.30 ± 0.10^{c}
Sodium	43.05 ± 0.05^{c}	56.10 ± 0.10^{a}	51.20 ± 0.20^{b}	42.85 ± 0.15^{d}
Calcium	4.59 ± 0.01^{a}	3.36 ± 0.02^{c}	3.37 ± 0.01^{b}	3.00 ± 0.01^{d}
Magnesium	1.57 ± 0.01^{b}	1.46 ± 0.01^{c}	2.50 ± 0.00^{a}	1.15 ± 0.01^{d}
Phosphorous	42.66 ± 0.02^{d}	58.73 ± 0.01^{b}	61.01 ± 0.01^{a}	51.34 ± 0.03^{c}
Zinc	0.27 ± 0.01^{c}	0.30 ± 0.00^{b}	0.25 ± 0.00^{d}	0.43 ± 0.00^{a}
Iron	2.63 ± 0.01^{c}	3.64 ± 0.12^{b}	3.65 ± 0.01^{a}	1.47 ± 0.02^{d}
Copper	0.53 ± 0.00^{b}	0.56 ± 0.00^{a}	0.45 ± 0.00^{c}	0.12 ± 0.01^{d}
Manganese	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.07 ± 0.06^{a}
Na/K	0.42 ± 0.00^{a}	0.38 ± 0.00^{c}	0.36 ± 0.00^{d}	0.41 ± 0.01^{b}

Means of triplicate determinations ± S.D. with different superscripts on the same row are significantly different at $p<0.05$. RPSF - Raw pumpkin seed flour, FPSF - Fermented pumpkin seed flour, GPSF - Germinated pumpkin seed flour, ROPSF - Roasted pumpkin seed flour.

Table 5. Functional properties of raw, fermented, germinated and roasted pumpkin *seed* flour

Samples	RPSF	FPSF	GPSF	ROPSF
WAC (%)	137.17 ± 0.14^{d}	195.70 ± 0.59^{a}	166.31 ± 0.62^{c}	176.77 ± 0.54^{b}
OAC (%)	97.69 ± 0.58^{c}	78.42 ± 0.02^{d}	109.20 ± 1.04^{b}	117.64 ± 0.04^{a}
SI (g/ml)	0.52 ± 0.02^{c}	1.00 ± 0.02^{a}	0.70 ± 0.01^{b}	0.29 ± 0.02^{d}
LBD (g/ml)	0.41 ± 0.01^{b}	0.43 ± 0.01^{a}	0.40 ± 0.02^{b}	0.36 ± 0.01^{c}
PBD (g/ml)	0.65 ± 0.01^{a}	0.58 ± 0.01^{c}	0.64 ± 0.01^{a}	0.63 ± 0.01^{b}
FS (%)	8.34 ± 0.02^{c}	11.65 ± 0.03^{a}	10.04 ± 0.04^{b}	8.32 ± 0.02^{c}
FC (%)	25.02 ± 0.02^{c}	25.43 ± 0.37^{b}	28.78 ± 0.01^{a}	22.97 ± 0.03^{d}
LG (%)	60.00 ± 17.32^{b}	46.67 ± 11.55^{c}	63.33 ± 5.77^{a}	46.67 ± 11.65^{c}

Means of triplicate determinations ± S.D with different superscripts on the same row are significantly different at $p<0.05$ RPSF - Raw pumpkin seed flour, FPSF - Fermented pumpkin seed flour, GPSF - Germinated pumpkin seed flour, ROPSF - Roasted pumpkin seed flour, WAC - Water absorption capacity, OAC - Oil absorption capacity, SI - Swelling index, LBD - Loose bulk density, PBD - packed bulk density, FS - Foam stability, FC- Foam capacity, LG - Least gelation.

This result may be an indication that processed pumpkin seed flour can be employed in baking industry. The oil absorption capacity for raw and processed pumpkin seed flour (78.42-117.64%) is greater than the values reported earlier for some nuts and seeds; pumpkin seed flour (87%), gourd seed (6%) and soy flour (84.4%) (Olaofe et al., 1994). It was observed that swelling index of the samples ranged from 0.29 - 1.00 g/ml and may be a function of size of flour particles, types or variety and processing methods. Loose bulk density (0.36 g/ml- 0.43 g/ml) and packed bulk density (0.58 - 0.65 g/ml) of the raw and processed pumpkin seed were found to be within the same rannge with bulk density (0.42 - 0.55 g/ml) of processed full fat fluted pumpkin flour (Fagbemi, 2006). In diets, loose bulk density is a property that promotes easy digestibility of food products, particularly among children (Osundahunsi and Aworh, 2002). High bulk density is preferred for the preparation of food materials with high nutrient content (Hassan et al., 2013). The foaming capacity (22.97 - 28.78%) of raw and processed pumpkin seed flour in this study are higher than foaming capacityof 13.2% recorded for *Telfairia occidentalis* (Oshodi and Fagbemi, 1992). Pumpkin seed flour may be considered in the production of ice-cream and beer where foaming is an attribute.

CONCLUSIONS

Findings from this work revealed that pumpkin seed flour may offer a balanced meal. Essential amino acid content of the processed pumpkin seed flour was observed to compete well with reference standard. Generally, the processing methods used produced flour with better nutritional values than the raw sample. The study also showed that the pumpkin seed oils contain both unsaturated and saturated fatty acid and could be utilized as edible cooking oil. Functional properties of processed pumpkin seed flour showed that it can find application in some food preparations which include; weaning formula, ice cream, sausage and comminuted foods.

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