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NUTRIENT POTENTIAL AND EFFECT OF FERMENTATION PERIOD ON CHEMICAL COMPOSITION OF FLUTED PUMPKIN (*Telfera occidentallis*) SEED

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Objective: To determine the effect of fermentation periods on the chemical composition of boiled immature fluted pumpkin seeds. Methodology: Boiled immature pumpkin seed paste was fermented using local technology for 7, 8, 9 and 14 days respectively. Chemical compositions were evaluated using standard methods. Moisture (39-42.22%), protein (21.51-24.63%), and ash (2.08-2.32%) increased with fermenting period while fat (26.77-24.19%), crude fiber (1.93-1.70%), and CHO (8.18-4.95%) decreased. Most mineral increased with fermenting period with sample fermented for 14 days having the highest Ca, Mg, K, Na, Fe, I and Zn (68.36 mg, 75.87 mg, 240.36 mg, 76.39 mg, 5.61 mg, 7.62 mcg and 1.95 mg respectively). β-carotene, vitamins C, B_1 and B_6 decreased with fermenting period while sample fermented for 7 days had the vitamin B_2 and sample fermented for 9 days Vitamin B_3. Phytochemicals obtained in unfermented samples (<1 mg) reduced with fermenting period. Conclusion: key nutrients like protein, Ca, Fe, I and Zn in boiled fluted pumpkin increased with fermentation beyond 7 days. This implies intake of some nutrients could be enhanced by prolong fermentation.

Keywords: Fluted pumpkin seed, Fermentation, Protein, Minerals, Phytochymical

INTRODUCTION

Fluted pumpkin is a dioeciously perennial plant that belongs to the genus *Teliferia* and specie *occidentiles* (Agamator, 2016). The plant is cultivated in all the geopolitical zones of Nigeria and its consumption cut across all the tribes in the country. In South-eastern Nigeria the plant is called "ugu", 'ugwu in Hausa, and "efuru" among the Yorubas. The plant consists of majorly two varieties; the male and female varieties. The male variety has thinner stem and leave compare to the female variety and produces flower that does not pod. The female variety on the other hand consists of broader leaves, thicker stem and produces pods. The young or tender shoot and leaves are used as main ingredient in soup or sauce preparation. Pumpkin leave is a poor source of macronutrient but rich source micronutrient particularly vitamin C (USDA, 2006). The leaves have been implicated in the treatment of anaemia, chronic fatigue and diabetes (Alade, 2000; and Dina *et al*., 2006). The leave is also said to have hypolipdemic and hypocholestolemic effects (Eseyin *et al*., 2005; and Adaramoya *et al*., 2007). At maturity the female variety produces pod winch often weighs between ≤1 - ≥10 kg at maturity (Agamator, 2005). Matured seeds are majorly used for propagation and oil extraction (Agamator, 2005), while immature ones are cooked and eaten whole or ground into flour and use as soup thickener (Fegbemi *et al*., 2005); also boiled immature seed are fermented and use as a condiment known as "ogiri ugu" used as condiment in soup and sauce preparation in South-eastern Nigeria.

Fermentation is a traditional method often employed for preservation and increase of some nutrients and palatability of foods in developing countries. Fermentation

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is also known to increase digestibility of protein through the process of hydrolysis (Achinewhu and Isichei, 1990). Traditionally boiled immature fluted pumpkin seeds are usually fermented between 3 to 7 days to produce "ogiri ugu". Fermenting these seeds beyond 7 days could increase its nutrients values. This work was therefore designed to determine the effect of prolong fermentation period on the chemical composition of boiled pumpkin seed paste.

MATERIAL AND METHODS

Collection of Raw Material

Tender pumpkin pods were purchased from Akara Market, Isukwuato Local Government Area of Abia State, Nigeria

Selection of Pods with Immature Seeds

Traditional method of selecting immature pod was used for the selection. To select pods with immature seeds, two opposite edges of the same pod were pressed manually using the thump and the fore finger until the two edges meets. Edges that met without breaking were selected for the test while those that break were rejected as their seeds were assumed to be matured.

Extraction and Cleaning of Seed

The pods were cut open longitudinally manually using kitchen knife. The seeds were extracted and washed under tap water to remove the pulp surrounding them. The seeds were boiled on cooking gas under moderate heat for 1 h. The seed were then de-hulled manually using hand pressure. The cotyledons were mashed into smooth paste using local mortar and pestle and divided into five (5) parts. Each part weighed about 250 g.

Fermentation Process

Traditional fermentation method with some modification was adopted. The first part of paste was not fermented (control), while the other four (4) parts were each wrapped in blanched banana leave and tied in a polyethylene bag to provide warm humid environment for proper fermentation. The fermented samples were collected and chemically analysed at seventh, eighth, ninth, and fourteenth day of fermentation.

Chemical Analysis

The proximate compositions of the sample were determined using standard AOAC (2006) methods. Moisture was determined gravimetrically. The crude protein content was determined by micro-Kjeldahl method, using 6.25 as the nitrogen conversion factor. Crude fat was determined by Soxhlet extraction method using petroleum ether. Ash was determined by incinerating the samples at 600 ^{o}C in a muffle furnace. Carbohydrate was obtained by difference, while energy was calculated using the Atwater Conversion factors in KJ and Kcal (17 KJ/4 Kcal, 17 KJ/4 Kcal, and 37 KJ/9 Kcal, for protein, carbohydrate and lipid respectively.

Mineral elements were determined using wet-acid digestion method for multiple nutrients determination as described by the method of AOAC (2006). About 0.2 g of the processed sample material was weighed into a 150 ml Pyrex conical flask. Five (5.0) ml of the extracting mixture (H_2SO_4–Sodium Salicylic acid) was added to the sample. The mixture was allowed to stand for 16 hours. The mixture was then placed on a hot plate set at 30 ^{o}C and allowed to heat for about 2 hours. Five (5.0) ml of concentrated perchloric acid was introduced to the sample and heated vigorously until the sample was digested to a clear solution. Twenty (20) milliliters of distilled H_2O was added and heated to mix thoroughly for about a minute. The digest was allowed to cool and was transferred into a 50 ml volumetric flask and made up to the mark with distilled water. The digest was used for the determinations of calcium (Ca) and magnesium (Mg) by the ethylamine ditetra acetic acid (EDTA) Versanate complexiometric titration method. Potassium (K) and sodium (Na) were evaluated by flame photometry method and phosphorus (P) by the vanadomolybdate method using the spectrophotometer. The trace metals (zinc, iron, copper, and iodine) were determined using the atomic absorption spectrophotometer 969 instrument. The appropriate cathode lamp was fixed for each element. The sample was introduced to the atomizer and the value concentration of the element printed out as mgX/liter.

The β-carotene, riboflavin, niacin and thiamin of the products were determined spectrophotometrically as described by AOAC (2006). While ascorbic acid was determined using titration method as described by AOAC (2006). Gravimetric method (Harborne, 1973) was used to determine alkaloids. Alkaloid was determined using alkaline precipitation gravimetric method as described by Harborne (1973). Saponin and flavonoids were determined by gravimetric oven drying method as described by the method of AOAC (2006). Tannin content of the sample was determined spectrophometrically as described by Kirk and Sawyer (1991). Oxalalate and hydrogencyanide were determined as described by AOAC (2006).

Statistical Analysis

All determinations were done in duplicates. The data generated were entered into the computer and analyzed using Statistical Package for Social Sciences (SPSS version 18.0) Means and standard deviation obtained from the chemical analysis were calculated. Level of significance was accepted at $p<0.05$. Analysis of variance (ANOVA) was used to compare the values obtained.

RESULTS

Effect of Fermentation Period on Energy and Proximate Composition of Fluted Pumpkin Seed Paste

Energy and proximate composition of fluted pumpkin seed paste is shown on Table 1. Moisture increased with increase in fermenting period. Moisture of fermented samples (40.28-42.22%) was significantly higher than moisture obtained in unfermented sample (39.54%). Protein and ash also increased with fermenting period (23.19-24.63% vs 2.13-2.32% respectively) but values of protein and ash obtained for sample fermented for 14days (24.63% vs 2.32%) were not significantly ($p<0.05$) higher than those of sample fermented for 9 days (24.38% vs 2.27%. Fat and crude fiber reduced with fermentation period; fat and crude fiber (26.77%; 1.93%) contents of unfermented sample were significantly ($p<0.05$) higher than those of the fermented samples (25.47-24.19%; 1.85-1.70% respectively). Carbohydrate and energy calculated for unfermented sample (8.18% vs 1495 kcal) were significantly higher than those of the fermented samples (7.09-4.95% vs 1457-1397 kcal respectively).

Effect of Fermentation Period on Mineral Composition of Fluted Pumpkin Seed Paste

Mineral compositions of the samples are presented on Table 2. Values of most of the minerals increased with fermentation period. Ca, Mg, K, Na, Fe, I, and Zn (68.36 mg, 45.87 mg, 240.36 mg, 76.39 mg, 5.61 mg, 7.62 µg and 1.95 mg respectively) were significantly higher in sample fermented for 14 days while P and Cu (53.54 mg vs 0.50 mg) were significantly higher in sample fermented for 9days.

Effect of fermentation period on vitamin composition of fluted pumpkin seed paste

Effect of fermentation period on vitamin composition fluted pumpkin seed paste is shown on Table 3. Some of the vitamins reduced significantly with fermenting period. β-carotene, vitamin C, vitamin B_6 (1.75 µg, 2.67 mg, 0.53 mg) were significantly higher in the unfermented paste compared to values of β-carotene, vitamin C, vitamin B_6 (1.68-1.14 mg, 2.39-1.71 mg, and 0.47-0.38 mg respectively) found in fermented paste. Vitamin B_1 and B_3 were significantly higher in sample fermented for 8 days while vitamin B_2 was highest in sample fermented for 7 days.

Effect of Fermentation Period on Phytochemical Composition of Fluted Pumpkin Seed Paste

Phytochemical compositions of pumpkin seed paste as affected by fermentation is shown on Table 4. All phytochemical anlysed were less than 1mg. values of

Table 1: Effect of Fermentation Period on Energy and Proximate Composition of Fluted Pumpkin Seed Paste

Nutrients	T1	T2	T3	T4	T5
Moisture (%)	39.54^{e}±0.08	40.28^{d}±0.03	40.83^{c}±0.04	41.67^{b}±0.05	42.22^{a}±0.06
Protein (%)	21.51^{d}±0.13	23.19^{c}±0.01	23.77^{b}±0.05	24.38^{a}±0.31	24.63^{a}±0.18
Fat (%)	26.77^{a}±0.04	25.47^{b}±0.18	25.35^{b}±0.08	24.68^{c}±0.11	24.19^{d}±0.01
Crude fiber (%)	1.93^{a}±0.01	1.85^{b}±0.02	1.83^{b}±0.01	1.78^{c}±0.00	1.70^{d}±0.01
Ash (%)	2.08^{c}±0.02	2.13^{bc}±0.01	2.18^{b}±0.02	2.27^{a}±0.04	2.32^{a}±0.03
Carbohydrate (%)	8.18^{a}±0.18	7.09^{b}±0.11	6.06^{c}±0.06	5.23^{d}±0.21	4.95^{d}±0.08
Energy (kcal)	1495^{a}±0.47	1457^{b}±4.45	1447^{a}±2.28	1416^{d}±3.83	1397^{e}±1.85

Note: Values are mean±standard deviation of duplicate samples; means with similar superscript are not significantly ($p<0.05$) different from each other. T1 = unfermented fluted pumpkin seed paste; T2 = fermented fluted pumpkin seed paste for 7 days; T3 = fermented fluted pumpkin seed paste for 8 days; T4 = fermented fluted pumpkin seed paste for 9 days; T5 = fermented fluted pumpkin seed paste for 14 days.

Table 2: Effect of Fermentation Period on Mineral Composition of Fluted Pumpkin

Nutrient	T1	T2	T3	T4	T5
Ca (mg/100 g)	58.67^{e}±0.10	60.36^{d}±0.06	61.50^{c}±0.03	64.78^{b}±0.03	68.36^{a}±0.08
Mg (mg/100 g)	68.52^{d}±0.31	70.66^{c}±0.37	71.66^{c}±0.29	73.14^{b}±0.93	75.87^{a}±0.05
P (mg/100 g)	50.16^{c}±0.45	51.76^{bc}±0.27	52.07^{b}±0.33	53.54^{a}±1.07	50.71^{bc}±0.92
K (mg/100 g)	234.79^{e}±0.01	236.87^{c}±0.04	235.51^{d}±0.13	238^{b}±0.13	240.36^{a}±0.03
Na (mg/100 g)	74.61^{c}±0.01	75.34^{b}±0.37	76.27^{a}±0.04	75.52^{b}±0.16	76.39^{a}±0.01
Fe (mg/100 g)	4.34^{e}±0.02	4.73^{d}±0.04	5.04^{c}±0.03	5.17^{b}±0.01	5.61^{a}±0.01
Cu (mg/100 g)	0.04^{c}±0.00	0.05^{c}±0.00	0.04^{c}±0.00	0.50^{a}±0.01	0.41^{b}±0.02
I (mcg/100 g)	4.74^{e}±0.02	5.27^{d}±0.00	5.82^{c}±0.03	6.61^{b}±0.13	7.62^{a}±0.00
Zn (mg/100 g)	1.85^{b}±0.01	1.92^{a}±0.03	1.91^{a}±0.02	1.96^{a}±0.00	1.95^{a}±0.01

Note: Values are mean±standard deviation of duplicate samples; means with similar superscript are not significantly ($p<0.05$) different from each other. T1 = unfermented fluted pumpkin seed paste; T2 = fermented fluted pumpkin seed paste for 7 days; T3 = fermented fluted pumpkin seed paste for 8 days; T4 = fermented fluted pumpkin seed paste for 9 days; T5 = fermented fluted pumpkin seed paste for 14 days.

Table 3: Effect of Fermentation Period on Vitamin Composition of Fluted Pumpkin Seed Paste

Nutrient	T1	T2	T3	T4	T5
Beta carotene (mcg/100 g)	1.75^{a}±0.00	1.68^{b}±0.01	1.42^{c}±0.01	1.28^{d}±0.01	1.14^{e}±0.01
Vitamin B_1 (mg/100 g)	0.24^{a}±0.00	0.27^{b}±0.01	0.30^{bc}±0.01	0.25^{c}±0.00	0.19^{d}±0.01
Vitamin B_2 (mg/100 g)	0.16^{b}±0.01	0.19^{a}±0.01	0.15^{bc}±0.01	0.12^{cd}±0.01	0.11^{d}±0.01
Vitamin B_3 (mg/100 g)	0.73^{b}±0.01	0.74^{ab}±0.00	0.78^{a}±0.03	0.60^{c}±0.01	0.47^{d}±0.02
Vitamin B_6 (mg/100 g)	0.53^{a}±0.01	0.47^{b}±0.01	0.45^{b}±0.00	0.42^{c}±0.00	0.38^{d}±0.01
Vitamin C (mg/100 g)	2.67^{a}±0.04	2.39^{b}±0.01	2.32^{b}±0.11	1.91^{c}±0.01	1.71^{d}±0.01

Note: Values are mean±standard deviation of duplicate samples; means with similar superscript are not significantly ($p<0.05$) different from each other. T1 = unfermented fluted pumpkin seed paste; T2 = fermented fluted pumpkin seed paste for 7 days; T3 = fermented fluted pumpkin seed paste for 8 days; T4 = fermented fluted pumpkin seed paste for 9 days; T5 = fermented fluted pumpkin seed paste for 14 days.

tannin, saponin, flavonoid, oxalate, alkaloid and hydrogencyanide (0.38, 0.61, 0.44, 0.67, 0.41 and 0.84 mg respectively) found in unfermented fluted pumpkin seed paste were significantly ($p<0.05$) higher than amounts (0.26-0.13, 0.47-0.18, 0.33-0.09, 0.42-0.10, 0.38-0.13, and 0.71-0.19 mg respectively) found in pumpkin seed fermented at different periods.

DISCUSSION

Some of the proximate components (protein, ash and moisture) obtained for fermented fluted pumpkin seed paste were significantly higher than those obtained for the unfermented sample. Protein, fat, crude fiber and carbohydrate found in unfermented sample were however lower than values reported in other similar studies (Akwaowo *et al.*, 2000; Christian, 2007; and Udoh, 2017). Difference in proximate values could be a function of their moisture content. Moisture, protein and fat were the major proximate components of flute pumpkin seed. Protein and moisture increased with fermenting period while fat decreased. Percentage increase in protein ranged between of 7.5-14.5%. Though protein increased with fermentation period, it was however observed that the increment was not significant between the 9th and 14th day; this may imply that

Table 4: Effect of Fermentation Period on Phytochemical Composition of Fluted Pumpkin Seed Paste

Phytochemical	T1	T2	T3	T4	T5
Tannin	0.38^{a}±0.01	0.26^{b}±0.01	0.22^{b}±0.02	0.18^{c}±0.02	0.13^{d}±0.00
Saponnin	0.61^{a}±0.01	0.47^{b}±0.01	0.31^{c}±0.01	0.23^{d}±0.01	0.18^{e}±0.02
Flavonoid	0.44^{a}±0.01	0.33^{b}±0.01	0.25^{c}±0.01	0.17^{d}±0.01	0.09^{e}±0.01
Oxalate	0.67^{a}±0.02	0.42^{b}±0.02	0.36^{c}±0.01	0.23^{d}±0.00	0.10^{e}±0.00
Alkaoid	0.41^{a}±0.01	0.38^{b}±0.02	0.28^{c}±0.00	0.20^{d}±0.01	0.13^{e}±0.00
Hydrogencyanide	0.84^{a}±0.01	0.71^{b}±0.01	0.45^{c}±0.00	0.34^{d}±0.02	0.19^{e}±0.00

Note: Values are mean±standard deviation of duplicate samples; means with similar superscript are not significantly ($p<0.05$) different from each other. T1 = unfermented fluted pumpkin seed paste; T2 = fermented fluted pumpkin seed paste for 7 days; T3 = fermented fluted pumpkin seed paste for 8 days; T4 = fermented fluted pumpkin seed paste for 9 days; T5 = fermented fluted pumpkin seed paste for 14 days.

optimum fermentation period for protein increment for pumpkin seed could be 9 days. When compared with other study protein value for unfermented sample was higher than 21.51% reported for unfermented fluted pumpkin seed while value obtained for sample fermented for 9 days was similar value (24.40%) reported for fermented sample in that study (Akwaowo *et al.*, 2000).

Crude fiber and fat values varied in all the samples; and each reduced significantly with fermentation period. Reduction in crude fiber could be due to microbial activity while reduction fat could be attributed to increase in lipolytic enzymes activities. When compared with other study crude fiber reported for fermented fluted pumpkin (3.98%) in a similar study (Udoh, 2017) was higher than values obtained in current study. Difference in fiber values could be attributed to difference in maturity levels of fluted pumpkin seed used in the two studies.

Carbohydrate and energy were also observed to decrease significantly with fermentation period. This observation was also made by Inyang and Zakari (2008). Decrease in carbohydrate could be due to increase activity of amylolytic emzyme which are known to hydrolyse starch and other complex carbohydrate to simpler sugars (Onweluzo and Nwabugwu, 2009).

Macro-minerals obtained were generally low, this was expected as most other seeds also poor sources of macro-minerals (Stadimayr, 2012). Most of the minerals were however observed to increase with fermentation period, with sample fermented for 14days having the highest Ca, Mg, K, Na, Fe, I, and Zn. Increase of these minerals could be due to increase in ash earlier observed. Amount of ash obtain in food item is usually relative to its mineral content. Also fermentation is said to increased minerals such as Ca and P through hydrolysis of phytate and oxalate (Enwere, 1998).

Most of the vitamins (with the exception of vitamins B_2 and B_3) analysed were significantly higher in unfermented fluted pumpkin seed paste. Vitamins B_2 and B_3 increased with fermenting period with day 7 having the highest B_2 value and day 8 the highest B_3 value. Increase of B-vitamins content and decreased trypsin inhibitor activity using substrate fermentation has been reported (Prinyawiwatkul *et al.*, 1997).

Phytochemicals obtained were all low; interestingly the values obtained decreased with fermentation period. Amounts obtained may not likely pose toxicity problems to man since they are below toxicity levels (Anigo, *et al.*, 2010; and Ndukwe and Ikpeama, 2013). Reduction of flavonoid was however detrimental as flavonoid is known to have antioxidant properties and also enhances healthy circulation (Ejike and Ajileye, 2007). Reduction in phytochemicals levels could be due to microflora activities and secreted polyphenol oxidase (Achinewhu and Isihei, 1990).

CONCLUSION

Moisture, protein and ash increased with fermenting period while fat, fiber, CHO and energy decreased. Sample fermented for 14 days had the highest Ca, Mg, K, Na, Fe, I and Zn. While sample fermented for 9days had the highest Cu and P. vitamins and phytochemicals reduced with fermentation period except for vitamins B_2 and B_3.

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