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Nutritional Composition and Antioxidative Potentials of Fermented Fluted Pumpkin Seed (Ogiri) Extract on H_2O_2-Induced Oxidative Stress in Rats

Peter Ikechukwu Akwukwaegbu[1], Onyekachi Onyekwere[2,*], Agatha Kelechi Ugwu[3], David Christopher Bando[2], Kingsley Thompson James[1], Hannah Nuhu Tubasen[2], Jesse Polly Shingu[2], Abuchi Elebo[4]

[1]Department of Biochemistry, University of Port Harcourt, Choba, Rivers, Nigeria
[2]National Biotechnology Development Agency, Abuja, Nigeria
[3]Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria
[4]State Universal Basic Education Board, Kaduna, Nigeria

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Abstract Nutritional composition and antioxidative potentials of fermented fluted pumpkin seed (Ogiri) extract on hydrogen peroxide induced oxidative stress in rats were investigated using standard analytical methods. Traditional methods were employed for processing fluted pumpkin seed (Ogiri). A total of 24 albino rats weighing 75-150 g were divided into 4 groups of 6 rats each and kept to acclimatize for 1 week with normal rats feed and water. Oxidative stress was induced with H_2O_2 intraperitoneally at 200 µM/KgBW of rat on the 27th day for 3 days. Group A received normal rats feed and water only, group B received H_2O_2, while groups C and D received H_2O_2 + 100 mg/KgBW and H_2O_2 + 200 mg/KgBW of seed extract respectively. The proximate analysis revealed substantial percentage of crude fibre (7.06 %), crude lipid (28.84 %), crude protein (10.49 %) and carbohydrate (39.04 %). The energy value was (457.68 kcal/100g). Vitamins B_2, B_1 and C were predominant while the concentrations of phosphorus, magnesium and calcium occurred most. Flavonoids, alkaloids, total phenol and total carotene antioxidants were detected in the sample. There was a significant decrease ($P<0.05$) in the enzymatic antioxidant activity (CAT, SOD and GST) and a significant increase ($P<0.05$) in MDA concentration of H_2O_2 treated rats when compared to the control. Administration of fermented fluted pumpkin seed extract reversed this effect at the respective doses. In conclusion, fermented fluted pumpkin seed extract has good nutritional qualities and positively modulates antioxidant enzymes against lipid peroxidation and oxidative stress damage.

Keywords Nutritional Composition, Fluted Pumpkin Seed, Hydrogen Peroxide, Oxidative Stress, Antioxidant

1. Introduction

Oxidative stress when expressed in the gene or cells is responsible for a variety of health challenges such as cancer, atherosclerosis, cardiovascular disease and neurological disorders [1]. During oxidative stress, reactive oxygen species (ROS) are generated which led to damage to DNA base strand and protein linkage [2]. Over the years,

ROS such as hydrogen peroxide (H_2O_2) and oxygen (O_2) have proven to be second messengers that control cellular activities. H_2O_2 causes cell lysis through production of dangerous hydroxyl radicals that attack the DNA [3] resulting to lipid peroxidation (destruction of membrane lipids). Lipid peroxidation releases toxic products such as malondialdehyde (MDA) and 4-hydroxynonenal which bind with DNA molecules to form adducts that initiated apoptosis and mutagenesis [4]. Inefficient repair of DNA molecules caused cancer cells to grow [5]. It is imperative to discover ways to minimize the dangers of ROS-mediated damage to the DNA.

Fluted pumpkin (*Telfairia occidentalis*) belongs to the family of Cucurbitaceae that is widely eaten either as seed or leafy vegetable in Nigeria [6]. It is widely grown in the South Eastern part of Nigeria, South Africa and tropical regions of Africa with annual rainfall. Cucurbitaceous family includes crops like cucumbers, water melons, squashes and luffas melons. The term fluted is used to describe the shape of the female flowers which matures to fruits that are berry-like [7] in structure with many seeds.

Seeds are fundamental part of human diet and are widely referred to as vital food [8]. The nutritional relevance of seeds cannot be over emphasized. Seeds are anticipated to be good sources of unsaturated fats, highly nutritive and supplies large amount of energy. Studies highlighted that the amount of energy provided by 1 gram of fat from digested seed is more than twice the amount from proteins and carbohydrates [9]. Fermentation of fluted pumpkin seeds produces "Ogiri", a local product peculiar to the South Easterners. Fluted pumpkin seeds can equally be utilized in diet formulation and production of marmalade [10].

Food sources and medicinal plants are presumed to be potent against ROS-induced damage [1]. Studies link intake of adequate diet and regular consumption of fruits and vegetables to lower risk of prostate [11] and oral [12] cancers. Antioxidants are powerful molecules against ROS that helps to subdue oxidative stress [13]. They act either directly by scavenging free radicals or indirectly by promoting body defense [1]. The need for the discovery of natural antioxidants from plants as against synthetic sources in the management of chronic illness has continued to attract research interest. Against this back drop, this study was undertaken to analyze the nutritional composition of fermented fluted pumpkin seeds and evaluate the protective potentials of this seed extract against hydrogen peroxide-induced oxidative damage.

2. Method

2.1. Sample Collection

Fluted pumpkin seed was obtained from a farmland in Orji in Owerri North L.G.A of Imo State, Nigeria.

2.2. Fermentation of Fluted Pumpkin Seeds (Ogiri)

The seeds were sorted to remove grit, dirt and winnowed. The sorted seeds were washed and boiled for 20 min in five times volume of water to soften the seeds. The softened seeds were dehulled and wrapped in leaves, kept in sacks and incubated in the earthen pot for 3 days. The seeds were grinded to paste by local mechanical means and stored for further analyses at -4^{o}C [14].

2.3. Preparation of Fermented Fluted Pumpkin Seed Extract

Exactly 1 Kg of the grinded seed paste was oven-dried at 50^{o}C and dissolved in hexane solvent to remove fats. The defatted extract was air-dried and re-extracted with butanol (1:10 w/v) to eliminate potential anti-nutrients. The defatted extract was allowed to dry again and suspended at pH 10.0 using deionized water. The resultant suspension was filtered to take away unwanted particles while the filtrate obtained was adjusted to acidic pH (pH 5.0). The filtrate was centrifuged for 15 min at 5000 rpm and 4^{o}C to separate the pellet from the supernatant. The pellet was lyophilized and stored at room temperature for further usage [15].

2.4. Chemical Analysis of Samples

Proximate composition of the sample was analyzed by [16] method, vitamin content was determined [17] with modifications, minerals analysis was done using acid digestion [18] while antioxidant composition was carried out using the method described by Pearson [19].

2.5. Experimental Design

A total of 24 male albino rats weighing 75-150 g used for this study were purchased from the animal house of Department of Physiology, University of Port Harcourt, Choba, Rivers State, Nigeria. The rats were divided into 4 groups of 6 rats each and kept to acclimatize for one week using normal rats feed and water. After acclimatization;

Group A received normal rats feed and water only;

Group B were fed on normal rats feed and water prior to H_2O_2 administration on the 27th day [20].

Groups C and D received normal rats feed supplemented with 100 mg/KgBW and 200 mg/KgBW respectively of seed extract for 27 days prior to treatment with H_2O_2. The study was terminated after 30 days [21].

H_2O_2 was administered via intraperitoneal at 200 µM per KgBW of rat. This dose was chosen based on its effectiveness at inducing cellular oxidative damage [1]. The rats were fasted overnight, decapitated through cervical dislocation and blood collected by direct heart puncture into EDTA bottles. The blood was centrifuged for 10 min at 300 revolutions per minute to obtain the plasma

which was used for oxidative stress assay [22].

2.6. Analysis of Oxidative Stress Parameters

Lipid peroxidation was carried out by thiobarbituric acid reaction [23] expressed by malondialdehyde (MDA) concentration, catalase (CAT) activity was done using [24], superoxide dismutase (SOD) activity was assayed according to [25] while glutathione S-transferase (GST) activity was determined using [26].

2.7. Statistical Analysis

Data obtained were subjected to descriptive and one-way analysis of variance (ANOVA) using statistical package for biological and social sciences (SPSS) version 21 Incidence and expressed as mean±standard deviation. Duncan's Multiple Range test was used for multiple comparisons. $P<0.05$ was taken to be significant.

3. Results

Table 1. Proximate analysis of fermented fluted pumpkin seed (Ogiri)

Proximate content	Fermented fluted pumpkin seed (%)
Moisture	11.45±0.33
Ash	3.12±0.68
Crude fibre	7.06±0.77
Crude lipid	28.84±0.49
Crude Protein	10.49±0.79
Carbohydrate	39.04±0.82
Energy value	**457.68 kcal/100g**

Values are mean ± standard deviations of triplicate determinations.

The proximate analysis showed that carbohydrate content (39.04±0.82 %) was the highest while ash content (3.12±0.68 %) was the least in fermented fluted pumpkin seeds.

Table 2. Vitamin composition of fermented fluted pumpkin seed (Ogiri)

Vitamins	Fermented fluted pumpkin seed (mg/100g)
Vitamin A	7.96±0.23
Vitamin C	55.35±0.86
Vitamin B_1	92.42±1.27*
Vitamin B_2	96.08±1.38*
Vitamin B_3	1.36±0.09
Vitamin B_6	0.85±0.02

Results are mean± standard deviation of triplicate determinations

* values meet recommended dietary allowance of vitamin in adult male.

Table 2 shows that the highest and lowest concentrations of vitamin in fermented fluted pumpkin seeds are vitamin B_2 (96.08±1.38 mg/100g) and Vitamin B_6 (0.85±0.02 mg/100g) respectively.

Table 3. Mineral composition of fermented fluted pumpkin seed (Ogiri)

Minerals	Fluted pumpkin (mg/L)
Sodium	15.00±1.04*
Calcium	280.44±6.50
Iron	108.69±4.32*
Magnesium	650.25±9.66*
Potassium	77.00±1.60
Manganese	1.80±0.03
Phosphorus	2400.00±32.15*
Copper	1.20±0.02
Selenium	1.23±0.03

Results are mean± standard deviation of triplicate determinations

* values meet recommended dietary allowance of mineral in adult male.

Table 3 showed that the concentration of phosphorus (2400.00±32.15 mg/L) was highest while copper concentration (1.20±0.02 mg/L) was least in fermented fluted pumpkin seeds.

Table 4. Antioxidant composition of fermented fluted pumpkin seed (Ogiri)

Antioxidants	Fermented fluted pumpkin seed (%)
Flavonoids	15.00±1.04
Alkaloids	280.44±6.50
Total phenol	178.69±5.32
Total carotene	650.25±9.66

Results are mean± standard deviation of triplicate determinations

Table 4 revealed that the percentage of total carotene (650.25±9.66 %) was the highest in fermented fluted pumpkin seeds while flavonoids percentage (15.00±1.04 %) was the least.

Table 5 revealed that the activities of CAT, SOD and GST were significantly decreased ($P<0.05$) in group B when compared to group A however, treatment with fermented fluted pumpkin seed extract at 100 mg/kgbw and 200 mg/kgbw significantly increased ($P<0.05$) the concentrations of these enzymes. Furthermore, the MDA concentration was significantly increased ($P<0.05$) in group B when compared to group A while administration of the fermented fluted pumpkin seed extract at both concentrations significantly decreased ($P<0.05$) it.

Table 5. Effect of fermented fluted pumpkin seeds on plasma enzymatic antioxidant and MDA content in hydrogen peroxide induced rats

Groups	Enzymatic antioxidants activity (units/g protein)			MDA conc (nmol/mg protein)
	CAT	SOD	GST	
A (Normal rats)	6.22±0.87[a]	8.95±1.23[a]	234.10±5.50[a]	1.08±0.02[a]
B Control rats (H_2O_2 treated)	2.12±0.06[b]	2.16±0.07[b]	202.46±2.19[b]	3.80±0.24[b]
C (100 mg/KgBW seed extract)	4.72±0.54[c]	6.23±0.91[c]	228.22±4.13[a]	1.72±0.05[a]
D (200 mg/KgBW seed extract)	5.77±0.61[a]	7.04±0.96[c]	224.75±3.92[c]	1.64±0.03[a]

Data are mean±S.D of six determination (n=6). Values bearing different superscript letters "a, b,c" down the column show significant difference ($P<0.05$) when compared to groups A and B.

4. Discussion

The proximate analysis of fermented fluted pumpkin seeds (Ogiri) are shown in Table 1. Fermentation enhances microbial activities which in turn support the digestibility and nutrient content of food sample [27]. The moisture content (11.45%) obtained in this study was low when compared to values recorded by [14] for dehulled melon Ogiri seed (44.75%), dehulled soybean Ogiri seed (60.53 %) and dehulled African yam bean Ogiri seed (66.00 %) but higher than values obtained for full fat (3.00 %) and defatted (1.37%) seed flour of *Telfairiaoccidentalis* [28]. Moisture expresses the tendency of food sample to microbial attack.

Ash content shows the composition of mineral elements present in food sample. The ash content (3.12%) obtained in this study was higher than values for dehulled melon Ogiri seed (2.46%) and dehulled soybean Ogiri seed (2.66 %) but lower than dehulled African yam bean Ogiri seed (3.21%) [14], full-fat *Telfaira occidentalis* seed flour (5.25%) [28], full-fat seed flours of *Malus sylvestris* (3.66%) and *Citrullus vulgaris* (3.75%) [29].

Fibres confer physiological function on lipid breakdown and promote absorption of cholesterol and reabsorption of bile acid in the intestine [30]. The crude fibre content (7.06%) obtained in this study was higher than .33% obtained for defatted *Moringa oleifera* seed flour [31], 2.06%, 1.22% and 1.79% recorded in dehulled melon, soybean and African yam bean Ogiri seed sample respectively [14].

A decrease in crude lipid (fats) content (28.84 %) was observed when compared to dehulled melon Ogiri seed (40.27%) but higher than soybean Ogiri seed (26.88%) and African yam bean Ogiri seed (12.00%) [14] and 5.5% recorded in defatted *Citrullus vulgaris* flour by [32]. Fats in diets assist in the absorption and enhancement of flavours and promote palatability.

The crude protein content (10.49 %) found in fermented fluted pumpkin seed was similar to 9.86 % and 10.32 % recorded in dehulled melon seed and African yam bean seed respectively [14]. Similarly, this value corresponds to the findings of [33], [34] and [35] but higher than 46.55% in defatted *Telfairia occidentalis* seed flour [28], 17.13% in defatted *Moringa oleifera* seed flour [31] and 33.79% in apple seed flours [29]. The decrease in crude protein content recorded in this study might be linked to increased activities of proteolytic enzymes during fermentation to protein to amino acids [36].

The percentage of carbohydrate (39.04 %) was higher than 35.78 % in defatted *Telfairia occidentalis* seed flour [28] and 8.67 % recorded by Olagbemide and Philip [31] in full fat seed flour of *Moringa oleifera*. The higher percentages of crude protein, crude lipid and carbohydrate contributed to the high energy value (457.68 kcal/100g) obtained in this study.

Table 2 expressed the vitamin content of fermented fluted pumpkin seed. The result revealed appreciable amounts of vitamins B_2, B_1 and C and minimal concentrations of vitamins B_6 and B_3. Vitamins B_2, B_1 met the daily requirement of adult man. The concentrations of vitamin C (55.35 mg/100g) and A (7.96 mg/100g) were lower than concentrations in full fat *Telfairia occidentalis* seed flour [28]. Studies have attributed the preventive and protective potentials of edible plants to their vitamin content [37]. Vitamin C is notable for its fast wound healing tendency [38], vitamin A helps against night blindness while the B-complexes act as co-enzymes in biological reactions.

Minerals function as co-factors in biological reactions. The concentration of minerals contained in fermented fluted pumpkin seeds are substantial and can supplement the daily requirement of human need. Sodium and potassium are extracellular cation that helps to maintain osmotic balance. Sodium, iron, magnesium and phosphorus meet the daily requirement for adult male. Calcium is essential for stronger bone and neurological functions. The concentration of calcium (280.44 mg/L) obtained in this study can supply about one-third of the daily requirement of man [39]. Iron is vital for haemoglobin formation. The concentration of magnesium (650.25 mg/L) can meet the daily requirement of 500 mg in adult. The concentration of phosphorus was highest which implies fermented fluted pumpkin seed can serve as ideal supplement for children with rickets while selenium is essential for antioxidant formation [40].

Hydrogen peroxide is largely recognized as a bleaching substance and utilized as sterilizer in the pharmaceutical field [1]. Despite, its economic relevance, H_2O_2 has been implicated as one of the known reactive oxygen species (ROS) that produces free radical which led to oxidative

stress and cellular damage [41]. It enters cells and combines with Cu^{++} and Fe^{++} to form highly reactive hydroxyl radicals which fight the protein, lipid and DNA components of the cells leading to oxidative damage [1]. Free radical damage to DNA causes numerous negative effects such as cancer, mutagenesis, human pathological diseases and age-related illness [42]. Antioxidants such as flavonoids, alkaloids, phenols and ascorbates helped to scavenge free radicals to prevent oxidative stress related injury.

The result of the enzymatic antioxidants and MDA content in hydrogen peroxide administered rats (Table 5) obtained in this study showed that fermented fluted pumpkin seed significantly enhanced all oxidative stress parameters evaluated as compared to chosen concentrations in H_2O_2 treated rats. As regards this study, the antioxidant composition in fermented fluted seed extract (Table 4) might be reason behind their modulatory effects by directly scavenging the hydroxyl free radicals on H_2O_2 treated rats. Similar findings were obtained by Ademiluyi *et al.* [43] and Ogunyinka *et al.* [15] in their respective studies on the "modulatory effects of dietary inclusion of garlic (*Allium sativum*) on gentamycin-induced oxidative stress in rats" and "modulatory influence of *Parkiabiglobosa* protein isolate on biomarkers of oxidative stress in streptozotocin-induced diabetic rats". This implies fermented fluted pumpkin seed can be utilized as good source of natural antioxidant with pharmacological relevance.

The activities of CAT, SOD and GST were significantly decreased in group B rats (Table 5) and might be attributed to the uncontrolled administration of hydrogen peroxide [1]. CAT and SOD are the two essential free scavenging antioxidant enzymes. CAT neutralizes H_2O_2 to oxygen and water while SOD acts as catalyst that minimizes the severe effect of superoxide radical during its conversion to H_2O_2[44, 45]. On the contrary, glutathione is a complex enzyme that comprises peroxidases, S- transferases and reductases. It protects oxidative cell damage by decreasing disulfide bonds in cytoplasmic proteins to cysteines [46]. Results obtained here complement the findings of Aitken and Roman [47], Singh *et al.* [48] and Ogunyinka *et al.* [15]. Treatment with fermented fluted pumpkin seed extract significantly elevated the activities of these oxidative cellular enzymes in a dose dependent manner with 200 mg/KgBW offering better relief. Generally, enzymatic antioxidants offer first line defence in response to oxidative stress damage on hydroxyl radical species [49].

Furthermore, significant increase in levels of thiobarbituric acid reactive substances (TBARS) expressed by elevated concentrations of malondialdehyde (MDA) in H_2O_2 treated rats obtained in this study showed oxidative damage. Similar reports were recorded by [50], [52] and [15]. According to Florence *et al.* [52], cellular damage from ROS emanates through lipid peroxidation of unsaturated fatty acids. The decrease in the concentration of MDA expressed in groups C and D highlights the protective tendency of fermented fluted pumpkin seed extracts against H_2O_2 induced oxidative stress damage.

5. Conclusions

This study therefore recommends the use of fermented fluted pumpkin seed as an essential part of human diet and backs the extract as positive antioxidant modulant against lipid peroxidation and oxidative stress in hydrogen peroxide treated rats. It equally expressed that the modulatory potential of this seed extract occurred in a dose-dependent manner with 200 mg/KgBW offering better protection efficiency.

Further studies can be carried out to determine the bioactive constituents and mechanism of action of fermented fluted pumpkin seed extract on H_2O_2-induced oxidative stress in rats.

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