

Effects of Processing on the Nutrient and Anti-Nutrient Contents of Tiger Nut (*Cyperus Esculentus Lativum*)

Okoye JI* and Ene GI

Department of Food Science and Technology, Enugu State University of Science and Technology, P.M.B, Enugu, Nigeria

*Corresponding author: Okoye JI, Department of Food Science and Technology, Enugu State University of Science and Technology, P.M.B 01660, Enugu, Nigeria, E-mail: okoyejoseph6@gmail.com

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Abstract

The effects of germination and fermentation on the nutrient and anti-nutrient contents of tiger nut seed flours were investigated. The tiger nut seeds were sorted, cleaned and processed into raw, germinated and fermented flours. The flours produced were evaluated for proximate, mineral and anti-nutrient contents. The protein content of the flours increased significantly ($p < 0.05$) from $8.69 \pm 0.10\%$ in the raw sample to $8.91 \pm 0.02\%$ in the fermented flour compared to the sample processed by germination. The ash content of the samples also showed similar increase from 1.81 ± 0.01 to $2.24 \pm 0.01\%$, while the carbohydrate decreased from 58.97 ± 0.35 to $57.84 \pm 0.48\%$ with the raw and fermented samples having the highest and least values, respectively. The fat, crude fibre and energy contents of the processed flours also decreased significantly ($p < 0.05$) by germination and fermentation treatments compared to the raw sample with the least values recorded by the fermented flour. The mineral composition of the flours showed that the calcium, potassium, phosphorus, zinc and magnesium contents of the samples varied between 100.15 ± 0.07 - 104.00 ± 0.57 mg/100g, 487.10 ± 1.56 - 494.30 ± 2.69 mg/100g, 128.55 ± 0.07 - 132.61 ± 0.58 mg/100g, 4.00 ± 0.03 - 4.80 ± 0.03 mg/100g and 94.80 ± 0.57 - 100.02 ± 1.84 mg/100g, respectively. The anti-nutrient content of the samples also revealed that the tannin, oxalate, phytate, saponin, hydrogen cyanide and hemagglutinin levels of the processed samples were reduced significantly ($p < 0.05$) by fermentation than the germination treatment. The proximate, mineral and anti-nutrient contents of the flours evaluated showed that the processed flours have the potentials to be used as nutritional supplements in the preparation of low-cost bakery and confectionary products than the raw sample.

Keywords: Tiger Nut; Processing; Germination; Fermentation; Nutrient Content; Anti-Nutrient Content

Introduction

Legumes and pulses are important sources of protein in developing countries. They are consumed worldwide, especially in developing and under developed countries where the consumption of animal protein may be limited as a result of economic, social, cultural or religious factors [1]. However, the use of legumes as protein source is limited by the presence of anti-nutrients or toxic substances which interfere with digestive processes and prevent efficient utilization of their proteins. Anti-nutrients are those natural or synthetic substances which by themselves or through their metabolic products arising in living systems interfere with food utilization, causes certain physiological responses and affect the health and productivity of man and animal [2]. The naturally occurring anti-nutrients that are commonly found in leguminous seeds are protease inhibitors, saponins, oxalates, tannins, phytates, hydrogen cyanide and hemagglutinins. Protease inhibitors are globular proteins that bind irreversibly to protease enzymes, thereby inhibiting their proteolytic activities in the digestive tract of both man and animal. The presence of protease inhibitors in foods results in decrease in protein digestibility, pancreatic hypertrophy, growth inhibition and increase in demand for sulphur containing amino acids [3]. Saponins are a group of biologically active glucosides that are characterized by the formation of stable forms in aqueous solutions and hemolysis of red blood cells (erythrocytes) in the body [4]. Oxalates are those substances that have the ability to form complexes with certain minerals, particularly calcium in the diet, thus, making them nutritionally bioavailable for metabolism in human body [5]. Tannins are polyphenolic substances of higher molecular weights which have the ability to form complexes with protein and other macromolecules. The presence of tannins in foods causes remarkable reduction in protein bioavailability and digestibility in human subjects [6]. Phytates are those compounds that are capable of forming insoluble complexes with certain minerals such as calcium, iron, potassium, magnesium, zinc and manganese

when foods containing them are consumed by both man and animal. The presence of phytates in foods causes reduction in bioavailability and absorption of such minerals in the body. Hydrogen cyanide is a chemical compound that is liberated when cyanogenic glucosides are hydrolysed in the presence of water and enzyme. It is also known as prussic acid and the consumption of foods that contain greater amount of hydrogen cyanide can lead to cyanide toxicity in humans. The toxic effects of hydrogen cyanide toxicity range from diarrhoea to severe gastroenteritis which often leads to death [7]. Hemagglutinins are glycoproteins that are characterized by their ability to agglutinate the red blood cells. The presence of hemagglutinins in foods causes impairment of growth and reduction in food utilization in humans [2].

To improve the nutritional quality and organoleptic acceptability of leguminous seeds, these anti-nutrients can be drastically reduced or inactivated by the use of traditional processing techniques such as wet cooking, germination, fermentation, soaking in water, roasting, steam blanching and dehulling [8,9].

Tiger nut (*Cyperus esculentus lativum*) is an underutilized root vegetable and perennial crop that produces rhizomes from the base of the tuber that is somewhat spherical with a dimension of 8 to 16mm. Tiger nut can be used in different forms in Nigeria and other parts of the world. It can be eaten raw as snack or roasted, grated and baked. It can be also used for the preparation of ice-cream and beverage because of its high nutrient density and potential health benefits [10].

The fibrous seeds of tiger nut can be processed into flour which can be also used to complement wheat flour in the production of baked and fried food products such as breads, biscuits, pie crust, sausages, chin-chin, doughnuts and cakes. The seeds can be equally extracted for oil that can be used in the production of biodiesel. The oil extracted from tiger nut is of high nutritional quality due the presence of high amounts of phytosterols and essential fatty acids in the oil [11]. Tiger nut contains moderate amount of protein, dietary fibre and carbohydrate but is a rich source of minerals and vitamins, particularly calcium, phosphorus, potassium, magnesium, B – group vitamins and tocopherol (vitamin E). The tubers of tiger nut are used traditionally in the treatment of flatulence, diarrhoea, dysentery and indigestion in man due to the fact that it contains several amounts of phytochemicals which exhibit diverse pharmacological and biochemical actions when ingested and digested by man [12]. The regular consumption of tiger nut prevents stomach pain, promotes normal menstruation and prevents allergies in babies in pregnant women. Little information is available on the effects of processing on the nutrient and anti-nutrient contents of tiger nut. This study was, therefore, undertaken to investigate the effects of germination and fermentation on the nutrient and anti-nutrient composition of tiger nut seed flours.

Materials and Methods

The brown cultivar of tiger nut (*Cyperus esculentus lativum*) seeds used for the study was purchased from Mayor Market, Enugu, and Enugu State, Nigeria. The seeds were sorted, cleaned and divided into three equal portions of one kilogram (1kg) each. Two portions were subjected to different processing treatments (germination and fermentation), while the third batch was processed raw.

Preparation of Raw Tiger Nut Seed Flour

The raw tiger nut flour was prepared according to the method of Belewu and Alodunrin with slight modifications [13]. During preparation, one kilogram (1kg) of tiger nut seeds was sorted to remove the stones, dirt and other contaminants. The sorted seeds were cleaned thoroughly and soaked in 3 litres of potable water in a plastic bowl at room temperature (30 ± 20 °C) for 8h. The hydrated seeds were drained, rinsed, spread on the trays and dried in a cabinet dryer (Model HR 6200, UK) at 60 °C for 12 h with occasional stirring of the seeds at intervals of 30 min to ensure uniform drying. The dried seeds were dehulled by cracking them in the attrition mill followed by winnowing to remove the hulls. The dehulled seeds were milled in the attrition mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an airtight plastic container, labeled and stored in a freezer until needed for further use.

Preparation of Germinated Tiger Nut Seed Flour

The germinated tiger nut flour was prepared according to the method of Oladele, *et al.* [14]. During preparation, one kilogram (1kg) of tiger nut seeds was sorted to remove the stones, dirt and other extraneous materials. The sorted seeds were thoroughly cleaned and steeped in 3 litres of potable water in a plastic bowl at room temperature (30 ± 2 °C) for 24 h with a change of water at every 6 h to prevent fermentation. The hydrated seeds were drained, rinsed and immersed in 2% sodium hypochlorite solution for 10 min to disinfect the seeds. The seeds were rinsed for five consecutive times with excess water and cast on a damped jute bag, covered with a polyethylene bag and left for 24 h to fasten sprouting. The seeds were carefully spread on the jute bag and allowed to germinate at ambient temperature (30 ± 2 °C) and relative humidity of 95% in the germinating chamber for 96 h. During this period, the seeds were sprinkled with water at intervals of 8 h to facilitate germination. Non-germinated seeds were discarded and the germinated seeds were spread on the trays and dried in a cabinet dryer (Model HR 6200, UK) at 60 °C for 18 h with occasional stirring of the seeds at intervals of 30 min to ensure uniform drying. The dried malted seeds were cleaned and rubbed in-between palms to remove the sprouts and roots along with the hulls. The dehulled seeds were milled in the attrition mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an airtight plastic container, labeled and stored in a freezer until needed for further use.

Preparation of Fermented Tiger Nut Seed Flour

The fermented tiger nut flour was prepared according to the method of Adejuyitan, *et al.* [10]. During preparation, one kilogram (1kg) of tiger nut seeds was sorted to remove the stones, dirt and other extraneous materials. The sorted seeds were thoroughly cleaned and soaked in 3 litres of potable water in a plastic bowl at room temperature (30 ± 2 °C) for 8 h. The soaked seeds were drained, rinsed and wet milled in the attrition mill with 2 litres of potable water into fine slurry. The slurry obtained was stirred manually with a wooden stirrer for 3 min and sieved with a muslin cloth into a clean plastic bowl. The sieved slurry was transferred in a clean bag and immersed in a plastic bowl containing 2.5 litres of potable water. The slurry was allowed to ferment at room temperature (30 ± 2 °C) with the aid of naturally occurring microbial flora for 18 h. Thereafter, excess water was decanted and the fermented slurry was manually dewatered. The cake produced was spread on the trays and dried in a cabinet dryer (Model HR 6200, UK) at 60 °C for 24 h with occasional stirring of the cake at intervals of 30 min to ensure uniform drying. The dried fermented product was milled in the attrition mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an airtight plastic container, labeled and stored in a freezer until needed for further use.

Proximate Analysis

Proximate analysis was carried out on the samples in triplicate according to the standard methods of AOAC (2005). Moisture content was calculated after incineration at 105 °C to constant weight in a hot air oven (Thermo Scientific – UT 6200, Germany). The crude protein was determined by the Microkjeldahl method using 6.25 as a conversion factor. Fat was estimated by Soxhlet extraction method. Ash was determined gravimetrically after incineration in a muffle furnace (Carholite AAF-11/18, UK) at 550 °C for 24h. Crude fibre was calculated by difference after the ashing of the ash-free filter paper containing the insoluble materials from the hydrolysis and washing of moisture free defatted sample (0.5g). Carbohydrate was determined by difference. $100\% - (\% \text{ Moisture} + \% \text{ Crude protein} + \% \text{ Ash} + \% \text{ Fat})$. The energy content was calculated using the Atwater factor of 4.0 for protein and carbohydrate and 9.0 for fat.

Mineral Analysis

The calcium, potassium, phosphorus, zinc and magnesium contents of the samples were determined in triplicate using atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, Germany) according to the standard methods of AOAC (2005) [15].

Anti-Nutrient Analysis

The tannin, oxalate, saponin and hemagglutinin contents of the samples were evaluated in triplicate using atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, Germany) according to the standard methods of AOAC (2005) [15]. Phytate and hydrogen cyanide contents were estimated by solvent extraction gravimetric method of [16].

Statistical Analysis

The results were expressed as mean \pm standard deviation and the test for statistical significance was carried out using one-way analysis of variance (ANOVA). The Statistical Package for Social Sciences (SPSS, Version 20) software was used to determine significant differences. Significant means were separated using Duncan's New Multiple Range Test (DNMRT) and differences were considered significant at $p < 0.05$ [17].

Results and Discussion

Proximate Composition of Raw and Processed Tiger Nut Flours

The proximate composition of raw and processed tiger nut flours is presented in Table 1. The moisture content of the raw sample was 8.69%, while the moisture content of the processed flours increased significantly ($p < 0.05$) from 8.85% in the germinated sample to 8.91% in the fermented flour. The increase could be due to the imbibition of greater amount of water by the seeds as a result of prolonged steeping during fermentation. Similar result has been reported in fermented African locust bean seed flour [18]. The crude protein content of the samples increased significantly ($p < 0.05$) by fermentation than the germination treatment. The increase in protein content observed in the fermented flour could be attributed to decrease in concentration of carbohydrate which serves as a potential source of energy for fermentative microorganisms during fermentation. The result is in agreement with the results of earlier workers [19-21]. The fat content of the flours which ranged from 21.95 to 24.45% was reduced significantly ($p < 0.05$) by fermentation compared to the sample processed by germination. The decrease may be due to increase in the activities of lipolytic enzymes which tend to hydrolyse fat into free fatty acids and glycerol during fermentation, thereby, inhibiting the formation of protein-lipid or carbohydrate-lipid linkages which facilitate the easy extraction of the oil by the extracting solvent [10,22]. The ash content of the raw sample was 1.81%, while the ash content of the processed flours increased significantly ($p < 0.05$) from 2.11% in the germinated flour to 2.24% in the sample processed by fermentation. The increase in ash content of the fermented sample may be due to increase in biomass produced by fermentative microorganisms during fermentation. Similar result has been reported in fermented chickpea flour [23]. The crude fibre content of the raw sample was the highest (24.04%), while the crude fibre content of the processed samples which ranged from 22.40 to 23.00% was significantly ($p < 0.05$) lower in fermented flour compared to the sample processed by germination. The reduction in crude fibre content may be due to the enzymatic breakdown of fiber

components by lactic acid bacteria to volatile fatty acids for their nutrition during fermentation. The observation is in agreement with the report of [24] for germinated and fermented African yam bean seed flours. The carbohydrate content of the flours also decreased significantly ($p < 0.05$) from 58.02% in the germinated flour to 57.84% in the fermented sample compared to the highest value of 58.97% recorded by the raw sample. The reduction in carbohydrate during fermentation may be due to the utilization of some sugars by fermentative microorganisms for growth and other metabolic activities [25]. The energy content of the samples ranged from 4.65.19 to 488.29 KJ/100g with the raw and fermented flours having the highest and least values, respectively. The variation could be attributed to differences in the protein, fat and carbohydrate contents of the samples. Similar result has been reported by for boiled and roasted un decorticated castor oil seed flours [26]. The increase recorded in the crude protein content of the processed samples compared to the raw flour may be due to the reduction in the carbohydrate content and this may be regarded as apparent increase in protein content to complement the decrease in carbohydrate. However, both germination and fermentation treatments had greater effect in increasing the nutrient content of tiger nut seed flours.

Parameter	Raw	Germinated	Fermented
Moisture	8.69 ^c ±0.10	8.85 ^b ±0.04	8.91 ^a ±0.06
Crude protein	8.09 ^c ±0.07	8.49 ^b ±0.02	9.07 ^a ±0.13
Fat	24.45 ^a ±0.21	22.53 ^b ±0.18	21.95 ^c ±0.20
Ash	1.82 ^c ±0.01	2.11 ^b ±0.01	2.24 ^a ±0.01
Crude Fibre	24.04 ^a ±0.16	23.00 ^b ±0.15	22.40 ^c ±0.14
Carbohydrate	58.92 ^a ±0.35	58.02 ^b ±0.31	57.84 ^c ±0.48
Energy KJ/100g	488.29 ^a ±3.37	468.04 ^b ±3.18	465.19 ^c ±3.23

Values are mean ± standard deviation of triplicate determinations. Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table 1: Proximate Composition (%) of Raw and Processed Tiger Nut Flours

Mineral Content of Raw and Processed Tiger Nut Flours

The mineral content of raw and processed tiger nut flours is given in Table 2. The calcium content of the raw sample was 100.15mg/100g, while the calcium content of the processed flours increased significantly ($p < 0.05$) from 102.80mg/100g in germinated sample to 104.00mg/100g in the fermented flour. The observed increase in calcium content of the fermented flour could be attributed to the synthesis of the mineral element by fermentative microorganisms during fermentation [27]. The process of fermentation and germination is associated with the enhancement of calcium bioavailability in legumes. The potassium content of the flours which ranged from 487.10 to 494.30mg/100g increased significantly ($p < 0.05$) by fermentation compared to the sample processed by germination treatment. The increase in potassium content could be due to the synthesis of the mineral element by fermentative microbial flora during fermentation. The observation is in agreement with the report of [28] for fermented chickpea seed flour. The phosphorus content of the samples also increased significantly ($p < 0.05$) from 129.71mg/100g in the germinated flour to 132.61mg/100g in the fermented sample. The increase could be due to the synthesis of the mineral element by microorganisms during fermentation. Similar result has been reported in fermented pigeon pea flour [29]. The zinc content of the samples was significantly ($p < 0.05$) lower in germinated flour compared to the sample processed by fermentation treatment. The reduction may be due to leaching of the mineral element into the steeping water during germination. The significant ($p < 0.05$) reduction in zinc content of the germinated sample observed in this study is in consonance with the reports of previous investigators [30,31]. The magnesium content of the raw sample was 94.80mg/100g, while the magnesium content of the processed flours ranged from 97.70 to 100.92mg/100g with the fermented sample having the highest value. Magnesium has been reported to increase with the process of fermentation in legumes due to microbial synthesis than germination [32]. Generally, fermentation had greater effect in enhancing the micro-nutrient content of tiger nut seed flour than the germination treatment.

Parameter	Raw	Germinated	Fermented
Calcium	100.17 ^c ±0.54	102.80 ^b ±0.57	104.00 ^a ±0.59
Potassium	487.10 ^c ±2.56	487.75 ^b ±2.64	494.30 ^a ±2.72
Phosphorus	128.55 ^c ±0.07	129.71 ^b ±0.08	132.61 ^a ±0.10
Zinc	4.00 ^b ±0.03	4.20 ^b ±0.03	4.80 ^a ±0.03
Magnesium	94.80 ^c ±0.45	97.70 ^b ±0.53	100.92 ^a ±0.57

Values are mean ± standard deviation of triplicate determinations. Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table 2: Mineral Composition (mg/100g) of Raw and Processed Tiger Nut Flours

Anti-Nutrient Content of Raw and Processed Tiger Nut Flours

The anti-nutrient content of raw and processed tiger nut flours is given in Table 3. In all the anti-nutrients examined, germination and fermentation resulted in decreased levels of the anti-nutrients. The tannin content of the raw sample was 21.96mg/100g, while the tannin content of the processed flours decreased significantly ($p < 0.05$) from 1.24mg/100g in germinated flour to 1.05mg/100g in the fermented sample. The decrease in tannin during fermentation may be due to its solubility in water. Similar reduction in tannin content during fermentation of legumes has been reported by other workers [33-35]. Tannins at their safe level (below 100ppm) have some health benefits [2]. They play significant roles in the prevention of cavities, diarrhoea, tooth decay and heart diseases. The oxalate content of the flours which ranged from 1.19 to 22.33mg/100g was significantly ($p < 0.05$) lower in fermented flour compared to the sample processed by germination. The reduction in oxalate content could be attributed to leaching and the action of microorganisms during fermentation. The observation is in agreement with the reports of other researchers in fermented African locust bean and chick pea flours [17,22,36]. Oxalates affect calcium, magnesium and protein metabolism in man. They also react with calcium to form calcium oxalates which are responsible for the formation of kidney stone in human subjects [37]. The phytate content of the samples decreased significantly ($p < 0.05$) from 1.13mg/100g in the germinated sample to 1.06mg/100g in the fermented flour compared to the phytate content of the raw sample which was 21.51mg/100g. The decrease in phytate content of the fermented sample may be due to increased microbial activities during fermentation. A wide range of microflora has been known to possess phytase activity which may be partly responsible for the reduction in the phytate content of the fermented sample [21]. Phytate related compounds have been reported to have beneficial effect as an antioxidant in human subjects [38]. The saponin content of the raw sample was 20.43mg/100g, while the saponin content of the processed samples ranged from 1.09 to 1.87mg/100g with the fermented flour having the least value (1.09mg/100g). The reduction in saponin content during fermentation could be due to its solubility in water coupled with leaching process. Saponins when extracted and purified can be used for the preparation of certain hormonal and fertility drugs [39]. Also, saponins at the recommended safe level (below 22.4mg/g) reduce blood lipids and possess antioxidant effect in humans [40]. The hydrogen cyanide content of the samples decreased significantly ($p < 0.05$) from 1.87mg/100g in the germinated flour to 1.68mg/100g in the fermented sample compared to the raw sample which had the value of 63.40mg/100g. It has been reported that the hydrogen cyanide levels of less than 50mg/kg body weight is not poisonous, 50 to 100mg/kg body weight is moderately poisonous and over 100mg/kg body weight is highly poisonous [41]. It can be therefore seen that the levels of hydrogen cyanide observed in processed samples were far below the levels that can cause cyanide toxicity in human subjects. The hemagglutinin content of the samples which ranged from 2.31 to 33.15mg/100g also decreased significantly ($p < 0.05$) in fermented flour compared to the sample processed by germination. The reduction observed in hemagglutinin content of the fermented sample may be as a result of increased microbial enzyme activities during fermentation [42]. In effect, the result showed that fermentation had greater effect in reducing the levels of anti-nutrients naturally present in tiger nut seeds than the germination treatment.

Parameter	Raw	Germinated	Fermented
Tannin	21.96 ^a ±0.14	1.24 ^b ±0.03	1.05 ^c ±0.01
Oxalate	22.33 ^a ±0.17	1.30 ^b ±0.03	1.19 ^c ±0.04
Phytate	21.51 ^a ±0.13	1.13 ^b ±0.01	1.06 ^c ±0.01
Saponin	20.43 ^a ±0.15	1.87 ^b ±0.07	1.09 ^c ±0.01
Hydrogen Cyanide	63.40 ^a ±1.67	1.87 ^b ±0.07	1.68 ^c ±0.06
Haemagglutinin	33.15 ^a ±1.03	2.62 ^b ±0.11	2.31 ^c ±0.09

Values are mean ± standard deviation of triplicate determinations. Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table 3: Anti-Nutrient Content (%) of Raw and Processed

Conclusion

The germination and fermentation treatments affected the nutritional value of tiger nut seed flours. The higher crude protein, calcium, potassium, phosphorus, zinc and magnesium contents observed in the processed tiger nut flours showed significant improvement in their nutritional value compared to the raw sample. The higher nutrient density of the fermented sample confers higher nutritional status on the flour compared to the sample processed by germination treatment. In addition, the levels of tannin, oxalate, phytate, saponin, hydrogen cyanide and hemagglutinin were also reduced drastically by germination and fermentation treatments. Knowing the health implications of these anti-nutrients to man and animal, the reduction in the levels of the anti-nutrients during processing of tiger nut seeds is very vital for the safety of the product. Although germination significantly improved the nutritional value of the product, fermentation resulted in the production of flour of better nutritional quality that will enhance the utilization of the product as a nutritional supplement in the preparation of a wide range of low-cost baked and fried food products.

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