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Effect of fermentation methods on the mineral, amino and fatty acids composition of *Cyperus esculentus*

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Tiger nut (*Cyperus esculentus*) was subjected to different fermentation methods such as traditional, back slope and control. The raw and fermented samples were analyzed for mineral, amino and fatty acids. The results of mineral analysis revealed potassium and sodium as the most abundant mineral element with their value ranging from 546 to 91.6 mg/100 g and 64.00 to 3383.33 mg/100 g, respectively while copper was found in trace amount with value ranging from 0.03 mg/100 g to 0.05 mg/100 g. All the fermented samples shows significant increase in calcium ranging from 8.50 to 9.83 mg/100 g compared to raw samples (7.66 mg/100 g). Amino acid result showed arginine (23.02 g/100 g) as the most abundant amino acid present in back slope fermented tiger nut while tyrosine was the least amino acid (0.05 g/100 g). The oil in tiger nut showed a greater percentage of oleic acid (73.08%) which was recorded in back slope fermented milled sample. The overall result of the investigation revealed that back slope fermentation was the best method that may enhance mineral, amino and fatty acids content of tiger nut.

Key words: Tiger nut, mineral, amino acid, fatty acid, fermentation.

INTRODUCTION

Tiger nut (*Cyperus esculentus* var. sativa) is an under-utilized crop which belongs to the division magnoliophyta and was found to be a cosmopolitan perennial crop of the same genus as the papyrus plant (Odoemelan, 2003; Belewu and Belewu, 2007). Despite its name, tiger nut is a tuber. However, its chemical composition shares characteristics with tubers and nuts (Umerie et al., 1997). The tubers are spherical in shape and edible.

There are varieties of tiger nuts readily available in the market, which are brown and yellow varieties. The yellow variety is preferred to all other variety because of its inherent properties such as larger size, attractive colour and fleshy body. The yellow variety is also reported to yield more soluble extracts, contains lower fat more protein and possesses less anti-nutritional factors (Okafor et al., 2003). Its tubers can be eaten raw, roasted with sugar, soaked in water or processed into starch and flour (Oladele and Aina, 2007; Cortes et al., 2005). It can be processed into a milky beverage called “Horchata de Chufa” in Spain or “Atadwe” milk in Ghana (Rita, 2009).

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In Nigeria, tiger nut is well grown and available in semi-dried form in Nigerian markets where it is sold locally and consumed uncooked (Omode et al., 1995).

Tiger nut have long been recognized to contain almost twice the quantity of starch as potato or sweet potato tubers. This tuber is a good source of energy (carbohydrate, fibre and protein), minerals (mainly phosphorus and potassium), and vitamins E and C (Arafat et al., 2009).

Processing techniques such as boiling, roasting, fermentation and germination are means of improving the nutritional value of foods (Nergiz and Gokgoz, 2007). Although little study have been carried out on the effect of fermentation on the nutritional composition of tiger nut. It is therefore important to investigate the effect of different fermentation methods on the mineral, amino and fatty acids content of tiger nut. Therefore, this research was conducted to determine the effect of fermentation methods on mineral, amino and fatty acid contents of *Cyperus esculentus*.

MATERIALS AND METHODS

Source of tiger nut

Raw tiger nut were purchased from Adedeji market in Akure, Ondo State, Nigeria. The nuts were stored in the laboratory till the second day when they were sorted, weighed and washed.

Processing of tiger nut

The sorted and washed nut were divided into six portions designated A to F. Each of the portion contained 500 g of cleaned tiger nut. Part A was analyzed raw and this serves as control. Part B was fermented whole that is, submerged in 1500 ml of portable water in a cleaned container that was covered for four days at 25°C and allowed to ferment with indigenous micro flora (spontaneous). C was milled and subjected to spontaneous fermentation. Part D and E were fermented by addition of the steep water from the previously fermented culture used as starter culture (back slope) but part E was milled before fermentation while F was allowed to undergo control fermentation, in which pure culture of *Lactobaccilus plantarum* isolated in part B was used to inoculate the sixth part F. The fermented nuts were dried in oven at 50°C for 24 h and dry milled to powder using attrition mill. The milled samples were packaged in polythene prior to analysis.

Chemical analysis

Mineral analysis

The mineral composition (potassium, sodium, calcium, magnesium, zinc, iron and copper) of each sample was determined by wet ashing method followed by reading of the level of mineral. Triplicate samples of 1 g each were weighed into porcelain crucibles and placed in a muffle furnace. The temperature was raised gradually to 450°C. The sample was ashed at 450°C for 5-6 h. After cooling to room temperature, the ash was dissolved in 1 ml of 0.5% HNO_3. The sample volume was brought to 100 ml, and the levels of mineral present were analyzed by Atomic absorption spectrophotometer Buck 201 VGP. The mineral content was calculated using the formula below.

$$\text{Mineral (mg/100 g)} = \frac{R \times V \times D}{Wt}$$

Where, R = Solution concentration obtained from graph, V = Volume of sample digest, D = Dilution factor and Wt = Weight of sample. Sodium (Na) and K were analyzed using flame photometer (Perkin-Elmer, 1982).

Amino acid determination

Amino acid composition was determined by the method of Spackman et al. (2006) 2.0 g of each sample was weighed into the extraction thimble and the fat extracted with chloroform methanol mixture using a Soxhlet extraction apparatus. The extraction lasted for 5-6 h. The defatted samples (30 to 35 mg) were weighed into glass ampoules. Seven milliliters of 6 MHCl were added and oxygen was expelled by passing nitrogen gas into the ampoule (to avoid possible oxidation of some amino acid during hydrolysis). Each glass ampoule was then sealed with a Bunsen flame and put into an oven at 105 ± 5°C for 22 h. The ampoule was allowed to cool before breaking open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 ml of acetate buffer and stored in a plastic specimen bottle and kept in the deep freezer. The amount loaded was between 5 to 10 µl. This was dispensed into the cartridge of the analyzer. The TSM (technicon sequential multisample amino acid analyzer) analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 min. The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height. The norcleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$\text{NE} = \frac{\text{Area of norcleucine peak}}{\text{Area of each amino acid}}$$

Fatty acid determination

Fifty milligram (50 mg) of fat extracted from raw and fermented tiger nut was esterified for 5 min at 95°C with 3.4 ml of the 0.5 M KOH in dry methanol. The mixture was neutralized using 0.7 M HCL. About 3 ml of boron triflouride (14%) in methanol was added. The mixture was heated for 5 min at the temperature of 90°C to achieve complete methylation process. The Fatty Acid Methyl Esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for gas chromatography analysis and 1 µL was injected into injection port of gas chromatography (Alejandro, 2013).

Statistical analysis

The experiment was carried out in triplicates. Data obtained were analyzed by one-way analysis of variance and mean were compared by Duncan's multiple range tests (SPSS 17.0 version). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Mineral composition (mg/100 g) of raw and fermented

Table 1. Mineral composition of raw and fermented Tiger nut (mg/100 g).

Mineral	Raw	TFM	TFW	BFM	BFW	CF
Ca	7.66^{c}±0.28	9.33^{ab}±0.28	9.50^{ab}±0.50	8.50^{bc}±1.00	9.00^{ab}±0.50	9.83^{a}±0.28
Cu	0.04^{a}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.05^{a}±0.06	0.03^{a}±0.00	0.04^{a}±0.00
Fe	0.09^{b}±0.00	0.23^{ab}±0.30	0.14^{b}±0.00	0.13^{b}±0.00	0.14^{b}±0.00	0.41^{a}±0.00
K	606.33^{b}±0.57	533.66^{f}±1.15	562.00^{d}±1.00	577.00^{c}±1.00	546.00^{e}±1.00	91.6^{a}±1.00
Na	3383.33^{a}±28.8	3250.00^{b}±0.00	3366.67^{a}±76.37	3166.66^{b}±76.37	2950.00^{b}±50.00	64.00^{c}±1.00
Pb	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00
Zn	0.07^{c}±0.00	0.64^{a}±0.10	0.00^{e}±0.00	0.51^{b}±0.00	0.02^{d}±0.00	0.51^{b}±0.38

Values are (mean±SD) of replicates. Values with the same alphabet are not significantly different at (p =0.05). RAW: Raw, TFM: traditional fermented milled, TFW: traditional fermented whole, BFM: back slope fermented milled, BFW: back slope fermented whole, CF: controlled fermented sample.

tiger nut is shown in Table 1. Sodium was the most abundant mineral with value 3383.3 mg/100 g which was recorded in raw sample while copper (0.03 mg/100 g) is the least mineral obtained which was found in back slope fermented whole sample. Micronutrients such as potassium, sodium and calcium were found to be appreciable in tiger nut samples analysed earlier reported by Bosch et al. (2005) and, Oladele and Aina (2007). Potassium and sodium are important in maintaining the normal water balance, conservation of osmosis and acid balance in the body. Potassium is necessary for the metabolism of carbohydrates and proteins. It also protects the internal arterial walls against any damages, prevents haemorrhages and brain/heart attack (Oladele et al., 2009). Hence, tiger nut is a good source of these elements.

The result of amino acid composition revealed that tiger nut is rich in essential amino acid such as lysine, threonine, leucine, phenylalanine and cystine. The most concentrated essential amino acid lysine (5.14 g/100 g) was recorded in back slope fermented whole sample. Tyrosine (0.50 g/100 g) was the least amino acid which was recorded in raw sample (Table 2). Some essential amino acid (threonine, leucine, phenylalanine and cystine) present in back slope fermented whole tiger nut were found to compare favourably with food and Agriculture Organization Standard (FAO, 1998). Back slope fermented whole sample showed significant increase ($p \leq 0.05$) in lysine (5.14 g/100 g), Threonine (3.15 g/100 g), leucine (4.39 g/100 g), phenylalanine (3.25 g/100 g) and cystine (2.56 g/100 g) content when compared to FAO standard (Table 3).

Oyetayo and Agbaje (2012) has earlier reported that amino acids of fermented Acha was higher than the raw sample. Also Oyetayo et al. (2007) reported that food rich in total essential amino acid will contribute to the supply of essential amino acid in diet. Amino acids distribution in controlled fermented sample is smaller to what was obtained in traditional and backslope fermented samples, this may be due to the effect of sterilization on the control sample, high temperature denature protein.

LAB fermentation has been shown to improve the nutritional value and digestibility of foods (Nout, 2009). The acidic nature of the fermentation products enhances the activity of microbial enzymes at a temperature range of 22-25°C (Mokoena et al., 2005). The enzymes, which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively. This is in line with this paper finding.

Results shown in Tables 2 and 3 show an increment in some nonessential and essential amino acid of fermented samples when compared with unfermented (raw) sample, these was in agreement with Steinkraus report (1997) that bacterial enzymatic hydrolysis may enhance the bioavailability of protein and fat and increase the production of free amino acids, short chain fatty acids and also reported that fermentation increase biological environment of food substrates with protein essential amino acids and vitamins.

The result of the fatty acid composition of oil extracted from raw and fermented tiger nut are as shown in Table 4. This study shows that all the sample contain appreciable amount of oleic acid with value recorded ranging from 64.91 to 73.08%, but low in erucic acid with values ranging from 0.02±0.00 to 0.05±0.01%. Key et al. (1986) reported that epidemiological studies also suggested that the presence of a high proportion of monounsaturated acid especially oleic acid in the diet is linked with a high reduction in the risk of coronary heart diseases. Oleic acid is also reported to be useful for building cellular membranes, attracting oxygen to tissues, to transform energy into nerve impulses, and as precursors to molecules of cellular communication such as prostaglandins (Odutuga et al., 1992).

Result also reveals that traditional fermentation had reduces the value of linolenic acid from 0.65 to 0.57%. High percentage of linolenic acid is not desirable in edible oils because of the off-flavours and potentially harmful oxidation products formed. As reported by Warner and Gupta (2003), a decrease from 2 to 0.8% linolenic acid content in oils improved flavor quality and oxidative

Table 2. Amino acids composition of raw and fermented tiger nut (g/100 g).

Amino acid	Raw	TFW	TFM	BFW	BFM	CF
Alanine	2.77^{e}±0.12	3.42^{bc}±0.02	3.27^{cd}±0.00	3.56^{ab}±0.04	3.10^{d}±0.08	3.76^{a}±0.24
Arginine	17.78^{c}±0.51	21.53^{b}±0.51	20.93^{b}±0.00	23.02^{a}±0.13	19.91^{c}±0.25	21.27^{b}±0.34
Aspartic	5.01^{d}±0.09	5.95^{b}±0.05	5.43^{c}±0.06	7.07^{a}±0.19	5.11^{d}±0.09	6.08^{b}±0.11
Cystine	1.97^{e}±0.10	2.38^{b}±0.03	2.28^{c}±0.00	2.56^{a}±0.00	2.14^{d}±0.00	2.42^{b}±0.07
Glutamic	5.79^{e}±0.18	7.64^{b}±0.04	6.39^{c}±0.07	8.63^{a}±0.15	6.11^{d}±0.07	7.64^{b}±0.11
Glycine	2.40^{e}±0.10	3.03^{c}±0.03	2.89^{c}±0.02	3.80^{a}±0.19	2.70^{d}±0.04	3.31^{b}±0.03
Histidine	2.02^{c}±0.11	2.41^{a}±0.02	2.23^{b}±0.01	2.50^{a}±0.03	2.08^{c}±0.02	2.42^{a}±0.01
Isoleucine	0.78^{d}±0.03	1.71^{c}±0.01	1.24^{d}±0.03	2.80^{a}±0.35	0.89^{d}±0.01	1.81^{b}±0.05
Leucine	2.74^{d}±0.07	3.91^{b}±0.10	3.36^{c}±0.10	4.39^{a}±0.13	2.51^{e}±0.04	4.12^{b}±0.19
Lysine	3.31^{e}±0.09	4.89^{b}±0.08	4.06^{c}±0.05	5.14^{a}±0.11	3.87^{d}±0.05	4.91^{b}±0.10
Methione	0.57^{d}±0.01	0.91^{b}±0.02	0.76^{c}±0.04	1.11^{a}±0.15	0.71^{c}±0.01	1.11^{a}±0.06
Phenylalanine	1.80^{cd}±0.04	2.42^{bc}±0.04	2.24^{bc}±0.04	3.25^{a}±0.85	1.38^{d}±0.80	2.59^{b}±0.04
Proline	1.50^{d}±0.00	2.03^{b}±0.06	1.80^{c}±0.06	2.44^{a}±0.00	1.68^{c}±0.06	2.14^{b}±0.17
Serine	2.14^{e}±0.08	2.62^{b}±0.01	2.48^{c}±0.01	2.91^{a}±0.09	2.32^{d}±0.03	2.57b^{c}±0.01
Threonine	2.24^{f}±0.02	2.78^{c}±0.03	2.55^{d}±0.02	3.15^{a}±0.05	2.42^{e}±0.02	2.89^{b}±0.05
Tyrosine	0.50^{d}±0.00	0.83^{b}±0.00	0.66^{c}±0.00	1.15^{a}±0.16	0.58c^{d}±0.08	1.07^{a}±0.08
Valine	1.65^{d}±0.07	2.31^{b}±0.06	2.10^{c}±0.03	3.21^{a}±0.11	1.71^{d}±0.07	2.41^{b}±0.04

Values are (mean±SD) of replicates. Values with the same alphabet are not significantly different at (p =0.05). RAW: Raw, TFM: traditional fermented milled, TFW: traditional fermented whole, BFM: back slope fermented milled, BFW: back slope fermented whole, CF: controlled fermented sample.

Table 3. Essential amino acid of raw and fermented tiger nut (g/100 g of protein) compared with FAO std`

Amino acid	Raw	BFW	FAO value
Lysine	3.31±0.09	5.14±0.11	4.20
Threonine	2.24±0.02	3.15± 0.05	2.80
Valine	1.65±0.07	3.21± 0.11	4.20
Methionine	0.57±0.01	1.11± 0.15	2.20
Isoleucine	0.78±0.03	2.07± 0.35	4.20
Leucine	2.74±0.07	4.39 ± 0.13	4.20
Tyrosine	0.05±0.00	1.15± 0.16	2.80
Phenylalanine	1.80 ±0.04	3.25± 0.85	2.80
Tryptophan	-	-	1.40
Cystine	1.97± 0.10	2.56 0.00	2.20

Values are (mean±SD) of replicates. RAW: raw, BFW: backslope fermented whole.

Table 4. Fatty acids composition of raw and fermented tiger nut (%).

Fatty acid	Raw	TFM	TFW	BFM	BFW	CF
Arachidic	4.93^{a}±0.02	4.23^{c}±0.15	4.71^{b}±0.01	2.77^{d}±0.02	4.32^{c}±0.02	5.40^{a}±0.40
Arachidonic	0.06^{a}±0.00	0.13^{c}±0.01	0.10^{d}±0.00	0.04^{b}±0.00	0.05^{ab}±0.01	0.00^{a}±0.00
Behenic	0.05^{b}±0.01	0.09^{a}±0.01	0.08^{a}±0.01	0.03^{e}±0.0	0.04^{c}±0,00	0.00^{a}±0.00
Capric	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00
Caprylic	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00±^{a}0.00	0.00^{a}±0.00
Erucic	0.03^{bc}±0.00	0.05^{a}±0.01	0.04^{a}±0.00	0.02^{c}±0.00	0.02^{c}±0.00	0.00^{a}±0.00
Lauric	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00

Table 4. Contd.

Fatty acid	Raw	TFM	TFW	BFM	BFW	CF
Lignoceri	0.11^{b}±0.01	0.23^{a}±0.05	0.22^{a}±0.01	0.07±0.00	0.06^{c}±0.02	0.00^{a}±0.00
Linoleic	9.00^{b}±0.01	10.12^{a}±0.01	7.74^{c}±0.00	10.17^{a}±0.01	10.45^{a}±0.01	8.86^{c}±0.01
Linolenic	0.65^{c}±0.01	0.71^{c}±0.01	0.57^{d}±0.00	3.50^{a}±4.61	0.90^{b}±0.00	0.66^{c}±0.01
Margaric	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00
Myristic	1.73^{a}±0.02	1.13^{c}±0.01	1.15^{c}±0.04	0.73^{e}±0.02	1.02^{d}±0.02	1.28^{b}±0.01
Oleic	69.77^{b}±0.02	70.61^{b}±0.01	69.33^{b}±0.03	73.08^{a}±0.01	69.10^{b}±0.01	64.91^{c}±0.01
Palmitolic	0.04^{d}±0.00	0.08^{c}±0.00	0.28^{b}±0.01	0.03^{d}±0.00	0.03^{d}±0.01	0.32^{a}±0.01
Plamitic	10.23^{b}±0.01	9.51^{c}±0.01	10.75^{b}±0.18	9.16^{c}±0.01	10.55^{b}±0.02	12.43^{a}±0.03
Stearic	3.38^{c}±0.02	3.16^{d}±0.01	5.34^{b}±0.02	3.01^{d}±0.01	3.40^{c}±0.11	6.10^{a}±0.00

Values are (mean±SD) of replicates. Values with the same alphabet are not significantly different at (p =0.05). Raw: Raw, TFM: traditional fermented milled, TFW: traditional fermented whole, BFM: back slope fermented milled, BFW: back slope fermented whole, CF: controlled fermented sample.

stability of fried foods. This therefore shows that the lower the linolenic acid content in oil, the more suitable is the oil for frying. This indicates that tiger nut oil is a good source of edible oil for cooking and frying that may be useful for the fight against cardiovascular diseases (Muhammad et al., 2011).

Conclusion

This study established the effect of different fermentation methods on the mineral, amino and fatty acids content of tiger nut (*Cyperus esculentus)*. The result of mineral composition revealed that tiger nut was rich in potassium, sodium and calcium. Also tiger nut is a poor source of copper and zinc. Back slope fermented sample was found to be high in the following amino acid: Arginine, glutamic, lysine and aspartic acid. Oleic is the most abundant fatty acid present in tiger nut. In conclusion, back slope fermentation is the best processing method that enhances the mineral, amino and fatty acids content of tiger nut. This method is the best because there is an increase in essential amino acid such as lysine, threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and cystine recorded in back slope fermentation method

Conflict of interests

The authors did not declare any conflict of interest.

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