



## PHYTOCHEMICAL COMPOSITION AND FUNCTIONAL PROPERTIES OF FLOUR PRODUCED FROM TWO VARIETIES OF TIGERNUT (*Cyperus esculentus*)



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**Abstract:** Two varieties of tigernut (black and brown) were purchased from a local market in Wukari, Taraba State, Nigeria. The nuts were cleaned, washed, drained, dried (50°C) and milled into flour. The samples were analysed for phytochemical composition and functional properties using standard methods. The brown variety had higher values of moisture (4.10%), fat (28.0%) and energy (575.6 kcal/100g) while the black variety is relatively richer in ash (3.0%), protein (4.58%) and carbohydrate (70.32%). The black variety had a relatively higher phytate (0.22 mg/g), tannin (0.91 mg/g), saponin (0.17 mg/g), oxalate (0.001 mg/g) while the brown variety had higher values of polyphenols (1.25 mg/g), flavonoid (1.04 mg/g) and alkaloid (4.75%). The black variety had higher bulk density (0.462 g/ml), loose density (0.71 g/ml), water absorption capacity (0.82 ml/g) and, swelling capacity (2.86 g/g). Therefore, the two varieties of tigernut considered in this study have great potential for utilization in food processing and formulation.

**Keywords:** Tigernut, flour, variety, functional, phytochemical.

### Introduction

The search for lesser known and underutilized crops, many of which are potentially valuable as human and animal foods has been intensified to maintain a balance between population growth and agricultural productivity, particularly in the tropical and sub-tropical areas of the world (Enujuigba and Akanbi, 2005). The worsening food crisis and the consequent wide spread prevalence of malnutrition in developing and underdeveloped countries have resulted in high mortality and morbidity rates, especially among infants and children in low income groups (Enujuigba and Akanbi, 2005).

Tigernut (*Cyperus esculentus*) is an underutilized sedge of the family *Cyperaceae* which produces rhizomes from the base and tubers that are somewhat spherical (Cortes *et al.*, 2005). The plant is not really a nut but a tuber first discovered some 4000 years ago (Lowe and Whitewell, 2000). It has other names like yellow nutsedge, chufa, flatsedge, rush nut, water grass, earth almond, northern nut grass and nut grass (Lowe and Whitewell, 2000). Tigernut (*Cyperus esculentus*) is known in Nigeria as *aya* in Hausa, *ofio* in Yoruba and *akihausa* in Igbo. Tigernut (*Cyperus esculentus*) was reported as healthy and helps in preventing heart diseases, thrombosis and activates blood circulation. It helps in preventing cancer, due to high content of soluble glucose. It was also found to assist in reducing the risk of colon cancer (Adejuyitan *et al.*, 2009). The nut is rich in energy content (starch, fat, sugars and protein), mineral (phosphorus, potassium) and vitamins E and C (Belew & Belew, 2007). Tigernut (*Cyperus esculentus*) is suitable for diabetic persons and also helps in losing weight (Borges *et al.*, 2008). Tigernut flour has a unique sweet taste, which is ideal for different uses. It is a good alternative to many other flours like wheat flour, as it is gluten free and good for people who cannot take gluten in their diets. It is also used in the confectionery industry (Belew & Abodunrin, 2006). It is considered good flour or additive for the bakery industry, as its natural sugar content is fairly high, avoiding the necessity of adding too much extra sugar (Anderson *et al.*, 1994).

Tigernut (*Cyperus esculentus*) grows mainly in the middle belt and northern regions of Nigeria (Okafor *et al.*, 2003), where three varieties (black, brown and yellow) are cultivated (Umerie *et al.*, 1997). Among these, only two

varieties, yellow and brown are readily available in the market. The yellow variety is preferred to all other varieties because of its inherent properties such as bigger size, attractive colour and fleshier body (Belew and Abodunrin, 2006).

Food contains various compositions of nutrients and phytochemical and could have important or deleterious effects in the body when consumed. The composition of the nutrients and phytochemical, usually leads to side effects found in most plants which may lead to excessive weight gain, constipation, allergies, diarrhoea, (Anonymous, 2009). For a food to be considered safe for human and animal health, its effect on these parameters need to be investigated to understand the nutritional potentials and safety of such foods with a view to determining their acceptability. This work therefore aimed at determining the chemical composition, phytochemical and functional properties of two varieties of tigernut flour in order to be able to explore its potentials in food formulation.

### Materials and Methods

#### Sample preparation

The two varieties of tiger nut used (black and brown) were obtained from a local market in Wukari, Taraba State, Nigeria. The nuts were cleaned, washed, drained, dried in an oven and ground into flour. The flour samples were sieved (45 µm mesh size). The black and brown tigernut flour were packaged in medium density polyethylene (ZipLock) bags, labeled black (BRF) and brown (BKF) and stored at 14°C until required for use.

#### Sample analyses

##### Proximate composition and energy

AOAC (2005) methods were used to determine moisture, protein, fat, crude fibre and ash contents while carbohydrate was calculated by difference. Total energy value (in cal/g) of each sample was calculated by multiplying the carbohydrate, fat and protein contents with their physiological fuel values (4, 9, 4 kcal, respectively).

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### ***Functional properties***

#### ***Water and oil absorption capacities***

Water and oil absorption capacities of the flour samples were determined by Beuchat (1977) methods. One gram of the flour was mixed with 10 ml of water or oil in a centrifuge tube and allowed to stand at room temperature ( $37 \pm 2^\circ\text{C}$ ) for 1 h. It was then centrifuged at  $200 \times g$  for 30 min. The volume of water or oil on the sediment water was measured. Water and oil absorption capacities were calculated as ml of water or oil absorbed per gram of flour.

#### ***Foam capacity and foam stability***

The method described by Narayana and Narasinga (1982) was used for the determination of foaming capacity (FC) and foam stability (FS). Two grams of flour sample was added to 50 ml distilled water at  $30 \pm 2^\circ\text{C}$  in a 100 ml measuring cylinder. The suspension was mixed and properly shaken to foam and the volume of the foam after 30 s was recorded. The forming capacity was expressed as a percentage increase in volume. The foam volume was recorded 1 h after whipping to determine the foam stability as a percentage of the initial foam volume.

#### ***Bulk density***

A 50 g flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density ( $\text{g cm}^{-3}$ ) was calculated as weight of flour (g) divided by flour volume ( $\text{cm}^3$ ) (Okaka and Potter, 1979).

#### ***Swelling capacity***

This was determined with the method described by Narayana and Narasinga (1982) with modification for small samples. One gram of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated to  $80^\circ\text{C}$  and held for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at  $1000 \times g$  for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as: swelling power = weight of the paste/weight of dry flour.

#### ***Determination of phytochemical composition***

##### ***Determination of alkaloid***

Determination of alkaloid was made by the method described by Harborne (1973). The alkaloid content was determined gravimetrically. Five grams of the sample was weighed and dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 h at  $28^\circ\text{C}$ . It was later filtered using Whatman No. 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous  $\text{NH}_4\text{OH}$  until the alkaloid was precipitated. The alkaloid precipitated in a weighed filter paper was washed with 1% ammonia solution, and dried in the oven at  $80^\circ\text{C}$ . Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

##### ***Determination of saponin***

The spectrophotometric method was used for saponin analysis as described by Brunner (1984). One gram of the flour sample was weighed into a 250-mL beaker and 100 mL iso-butyl alcohol was added. The mixture was shaken on a shaker (UDY Corporation, Fort Collins, CO) for 5 h

to ensure uniform mixing. The mixture was filtered through a Whatman No. 1 filter paper into a 100-mL beaker and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained was further filtered through a Whatman No. 1 filter paper to obtain a clear colourless solution. One milliliter of the colorless solution was homogenized into a 50-mL volumetric flask and 2 mL of 5%  $\text{FeCl}_3$  solution was added and made up to mark with distilled water and allowed to stand for 30 min for blood red color to develop. Standard saponin solutions (0–10 ppm) were prepared from saponin stock solution and treated with 2 mL of 5%  $\text{FeCl}_3$  solution as done for experimental samples. The absorbance of the sample as well as standard saponin solutions were read after color development on a Spectronic 21D spectrophotometer (Milton Roy, Houston, TX) at a wavelength of 380 nm. The percentage saponin was also calculated.

##### ***Determination of total phenolic compounds***

The samples (100 g) were extracted, by stirring with methanol 250 mL for 3 h. The extracted samples were then filtered through Whatman No. 1 filter paper, the residue was washed with 100 mL methanol, and the extracts were cooled. The extracts were evaporated to dryness under vacuum, using a rotary evaporator. The residues were dissolved with 10 mL of methanol and used for determination of total phenolic compounds. This determination was performed as gallic acid equivalents (mg/100g), by using Folin-Ciocalteu phenol reagent. The diluted methanol extracts (0.2 mL) were added, with 0.8 mL of Folin-Ciocalteu phenol reagent and 2.0 mL of sodium carbonate (7.5%), in the given order. The mixtures were vigorously vortex-mixed and diluted to 7 mL of deionized water. The reaction was allowed to complete for 2 h in the dark, at room temperature, prior to being centrifuged for 5 min at  $125 \times g$ . The supernatant was measured at 756 nm on a spectrophotometer. Methanol was applied as a control, by replacing the sample. Gallic acid was used as a standard and the results were calculated as gallic acid equivalents (mg/100 g) of the sample. The reaction was conducted in triplicate and the results were averaged (Brunner, 1984).

##### ***Determination of total flavonoid***

This was also determined according to the method outlined by Harborne (1973). Five grams of the sample was boiled in 50 mL of 2 mol/L HCl solution for 30 min under reflux. The contents were allowed to cool and then filtered through a Whatman No. 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

##### ***Determination of tannin content***

Tannin content of the flour samples was determined using the methods described by Harborne (1973). The sample (0.2 g) was measured in a 50-mL beaker; 20 mL of 50% methanol was added, covered with homogenizer, placed in a water bath at  $77\text{--}80^\circ\text{C}$  for 1 h, and the contents stirred with a glass rod to prevent lumping. The mixture was filtered using a double-layered Whatman No. 1 filter paper into a 100-mL volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. One milliliter of the sample extract was homogenized into a 50-mL volumetric flask, and 20 mL

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distilled water, 2.5 mL Folin-Denis reagent, and 10 mL of 17%  $\text{Na}_2\text{CO}_3$  were added and mixed. The mixture was made up to mark with distilled water, thoroughly mixed, and allowed to stand for 20 min when a bluish-green coloration developed. Standard tannic acid solutions in the range of 0–10 ppm were treated similarly as the 1 mL sample above. The absorbances of the tannic acid standard solutions as well as samples were read after color development on a Spectronic 21D spectrophotometer at a wave length of 760 nm. Percentage tannin was calculated.

### Determination of phytic acid

An indirect colorimetric method of Harborne (1973) was used for phytate determination. This method depends on an iron to phosphorus ratio of 4:6. A quantity of 5 g of the test sample was extracted with 3% trichloro acetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2 N HNO and the color read immediately at 480 nm. The standard solution was prepared from  $\text{Fe}(\text{NO}_3)_3$  and the iron content was extrapolated from a  $\text{Fe}(\text{NO}_3)_3$  standard curve. The phytate concentration was calculated from the iron results assuming a 4:6 iron:phosphorus molecular ratio.

### Determination of oxalate content

Oxalate was determined by AOAC (2005) method. One gram of the sample was weighed in a 100-mL conical flask. Seventy-five milliliters of 3 mol/L  $\text{H}_2\text{SO}_4$  was added and the solution was stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman No. 1 filter paper. The sample filtrate (extract) (25 mL) was collected and titrated against hot (80–90°C) 0.1 N  $\text{KMnO}_4$  solution to the point when a faint pink color appeared that persisted for at least 30 s. The concentration of oxalate in each sample was obtained from the calculation: 1 mL 0.1 permanganate = 0.006303 g oxalate.

### Sensory analysis

Sensory evaluation was performed on the products by twenty-member untrained sensory panelists randomly selected. A 9-point hedonic scale was used in scoring the products (Ijarotimi and Keshinro, 2012) so as to help determine the degree of acceptability in terms of mouth feel/texture, appearance, flavour, over all acceptability.

## Results and Discussion

### Proximate composition

Percentage moisture, fat and protein values of black and brown varieties of tignernut are presented in Fig. 1. The proximate composition of food is a major index of nutritious potentials of food crops. The brown variety had higher values of moisture (4.10%), fat (28.0%) while the black variety is relatively richer in ash (3.0) and protein (4.58%). Moisture content, ash and protein obtained for both samples were close to values obtained for soybean and tignernut flour blends reported by Emmanuel-Ikpeme *et al.* (2012). The low moisture of both samples would have a positive effect on shelf stability. Result obtained for fat content of the brown variety corroborated earlier value of 24.3% reported by Ekeayanwu and Ononogbu (2010). Fat is important in diet because it promotes absorption of fat and soluble vitamins (Bogert *et al.*, 1994).

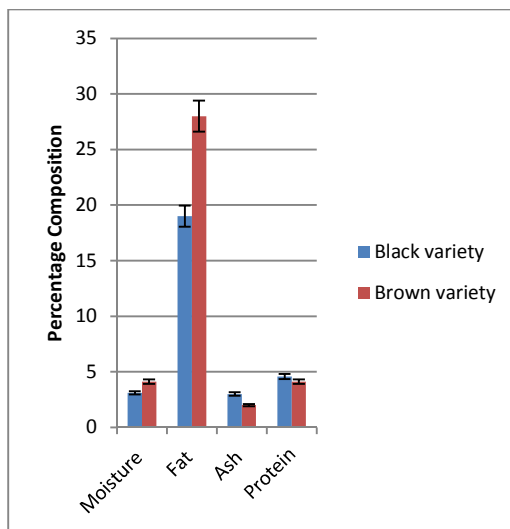


Fig. 1: Proximate composition of black and brown varieties of tignernut

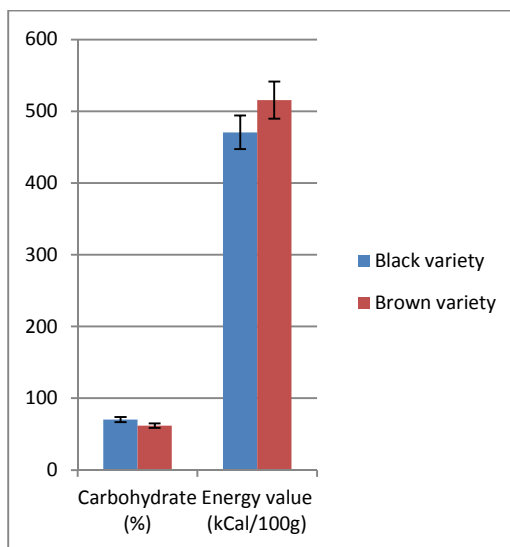


Fig. 2: Carbohydrate content and energy values of black and brown variety of tignernut

Fig 2 shows the percentage composition of carbohydrate (61.78–70.32%) and energy values (470.6–515.6 kcal) of black and brown varieties of tignernut. The black variety had a higher carbohydrate composition of 70.32% while the brown variety had a higher energy content of 515.6 kcal/100g. The calorific value of the two varieties—black and brown tignernut (470.6 and 515.6 kcal, respectively) showed that it could be a reliable source of energy and can thus provide a large portion of the daily requirement of 2,500–3,000 kilocalories (Ekeayanwu and Ononogbu 2010) for adults if large quantities are consumed as it usually the case in the northern Nigeria. Carbohydrate composition and energy values obtained for both samples differ from those reported for yellow variety of tignernut by Oladele and Aina (2007) which could be due to relatively low moisture level (3.10–4.10%) of the samples under study. The ash content of the two varieties (3.0 and 2.0%) were within the range of 1.5–2.5 recommended by Pomeranz and Clifton (1981) and

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Ekeanyanwu and Ononogbu (2010). The black and brown tiger nut gave a relatively high level of fiber (12.3 and 13.5%, respectively). The existence of a casual relationship between the absence of fiber in diet and the incidence of wide range of diseases in man; notably diabetes, mellitus, obesity and coronary. Heat disease has long been reported (Eastwood 1974; Mendelhoff 1978; Ekeanyanwu and Ononogbu, 2010).

### Phytochemical composition

Phytochemical composition of black and brown varieties of tigernut is presented in Fig. 3. Phytate, tannin, polyphenols, flavonoid, saponin, oxalate and alkaloid content of the black and brown variety of tiger nut are 0.22 and 0.16 mg/g, 0.91 and 0.53 mg/g, 1.07 and 1.25 mg/g, 0.88 and 1.04 mg/g, 0.17 and 0.006 mg/g, 0.003 and 0.0023 mg/g, 3.7 and 4.75%, respectively. The black variety is relatively richer in phytate (0.22 mg/g), tannin (0.91 mg/g), saponin (0.17 mg/g) and oxalate (0.003 mg/g), while the brown variety had higher values of phenol (1.25 mg/g), flavonoid (1.04 mg/g) and alkaloid (4.75%) contents. Values obtained for tannins, phytates and oxalate for both samples were lower than 2.37 mg/100g, 21.42 mg/100g and 13.12 mg/100g, respectively reported by Oladele *et al.* (2009). However, values obtained for polyphenols and alkaloids were higher than values reported by the same authors. Saponin contents of both samples were lower than the value reported by Ekeanyanwu and Ononogbu (2010). Alkaloids, saponins and tannins are known to have antimicrobial activity, as well as other physiological activities (Sofowara 1993, Evans 2005). Some have been used either as an analgesic, antispasmodic, bactericidal agents (Frantisek, 1991).

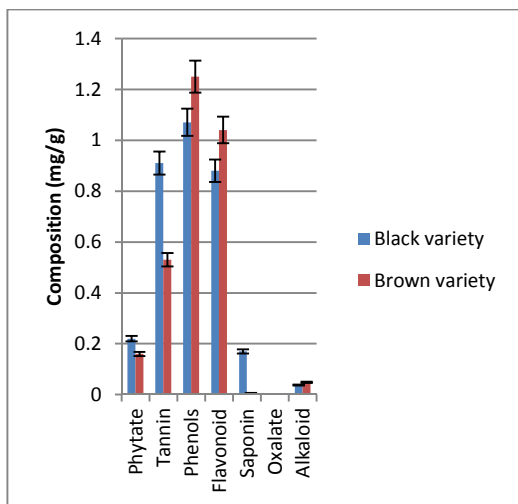


Fig. 3: Phytochemical composition of black and brown varieties of tigernut

Saponins have been reported to be useful in reducing inflammation of upper respiratory passage and also chiefly as foaming and emulsifying agents and detergent (Frantisek, 1991). Tanin compounds have antimicrobial activities and are responsible for preventing and treating urinary tracts infections and other bacterial infections (Ekeanyanwu *et al.*, 2010). The result of the determination of phytochemical test indicated that the tuber possess some

biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine (Ekeanyanwu *et al.*, 2010). Due to inherent high polyphenolic and alkaloids contents, both black and brown varieties of tigernut should be subjected to processing. Oladele *et al.* (2009) reported reduction in phytates, tannin, polyphenols, oxalate and alkaloid contents of processed tigernut.

### Functional Properties

Bulk density (packed), loose density, oil absorption capacity, water absorption capacity, swelling capacity, swelling index and least concentration for gelation of black and brown varieties of tiger nut as presented in Fig. 4 are 0.463 and 0.439 g/ml; 0.71 and 0.614 g/ml; 0.46 and 0.62 ml/g; 0.82 and 0.73 ml/g; 2.86 and 2.14 g/g; 0.36 and 0.36; and 0.2 and 0.22. The black variety is relatively higher in bulk density, loose density, water absorption capacity and swelling power than the brown variety. Values obtained for loose and packed densities were lower than 0.62 and 0.55 g/ml respectively reported for yellow variety by Oladele and Aina (2007).

Water absorption capacities of the samples were also lower than 1.26 ml/g reported for yellow variety by Oladele and Aina (2007). Water absorption capacity describes flour-water association ability under limited water supply. This result suggests that tigernut flour may find application in baked products e.g. biscuits. The relatively low oil absorption capacity shows that tigernut may be a lower flavour retainer than African yam bean (1.42 ml/g) flour (Eke and Akobundu (1993). The low oil absorption capacity of tigernut might be due to low hydrophobic proteins which show superior binding of lipids (Kinsella, 1976).

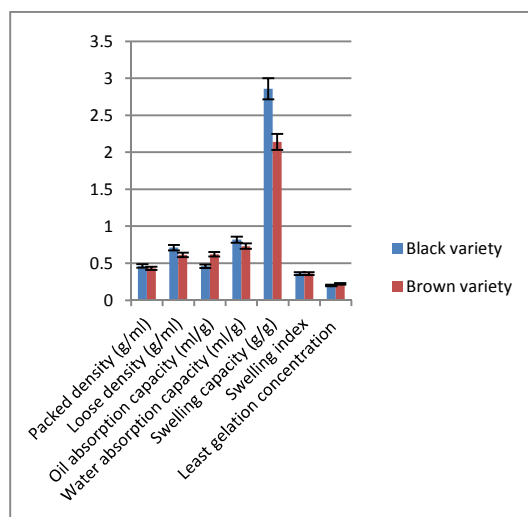
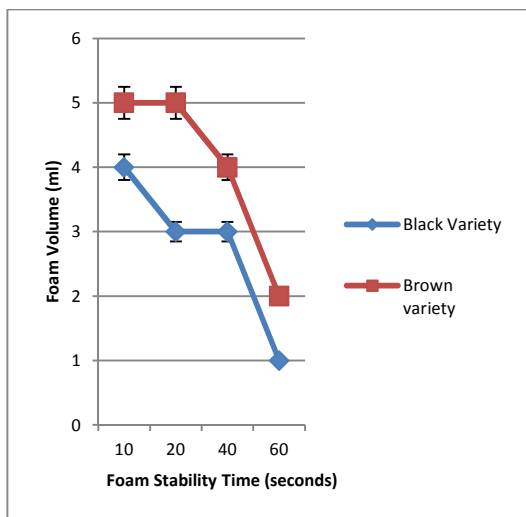


Fig. 4: Functional properties of black and brown varieties of tigernut

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**Fig. 5: Foam stability of black and brown varieties of tigernut**

Fig. 5 shows the foam stability of black and brown varieties of tigernut at different time intervals (10, 20, 40 and 60 s). For both varieties, foam stability decreased with time. The brown variety had better foam stability at various times considered. Food materials with good foaming capacity and stability are useful in the formulation of aerated foods. Foam formation and stability are dependent on protein type, pH, surface tension, viscosity and processing method (Eltayeb *et al.*, 2011).

**Conclusion**

It is evident that the two varieties (brown and black) of tigernut considered in this study have great potential for utilization in food systems. It is a rich source of fat with moderate amount of protein. The low bulk density also suggests its application in infant food formulation. The phytochemical composition of tiger nut could be a potential in the production of antibodies and improvement of biological body systems of the consumer.

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