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Phenolic Profile and Antioxidant Activity of Brown and Yellow Varieties of Tigernut (*Cyperus esculentus L.*)

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ABSTRACT

Tigernut is a sedge with tuber planted and consumed in Nigeria but with no documented phenolics profile. This work determined the phenolic profile and the antioxidant activity of tigernut tubers grown and consumed in Nigeria. Tigernuts (yellow, YTG and brown, BTG)) were sorted, washed, dried, milled into powder, phenolics extracted with sodium hydroxide and analyzed with Gas chromatography (GC). The tigernuts contain significant concentration of hydroxybenzoic acids, hydrocinnamic acids and flavonoids. The major phenolic acids in YTG were ferulic acid (58.38 mg/100 g), p-hydroxybenzoic acid (29.12 mg/100 g), p-hydroxybenzaldehyde (16.47 mg/100 g) and vanillic acid (5.88 mg/100 g) while the major phenolic acids in BTG were vanillic (15.20 mg/100 g), p-coumaric (17.25 mg/100 g), caffeic (15.25 mg/100 g), ferulic (33.79 mg/100 g) and sinapinic acids (20.97 mg/100 g). Ferulic acid concentration was highest in the two samples. The concentration of the flavonoid compounds in YTG and BTG followed the same trend: flavones (52, 81%) > flavonols (36, 19%) > flavanones (7, 0%) > flavanols (5, 0%) > isoflavones (0, 0%). The 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) values were 86.20% and 37.5% (YTG) and 38.30% and 42.8% (BTG).

Keywords: Tigernut, phenolic profile, flavonoids, antioxidant activity.

1.0 Introduction

In recent years, research has focussed on the bioactive compounds present in many edible plants, due to their potential benefits for human health. Many of them have been reported to have antioxidant, anti-inflammatory, antibacterial, cytotoxic, antiviral and fungi static activities (Parker *et al.*, 2000; Adesegun *et al.*, 2008; Vlase *et al.*, 2014). The bioactive compounds are produced as a response to environmental stresses like microbial attack, insect/animal predation and ultraviolet radiations. The role of these metabolites is to increase plants resistance to these stresses by scavenging free radicals and other toxic compounds (Irina and Mohamed, 2012; Sharma *et al.*, 2012) which can result into diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome in human body. The bioactive compounds in plants with antioxidant activity include phenolic compounds, carotenoids, glucosinolates and different vitamins (Afify *et al.*, 2012).

Phenolic compounds classified as phenolic acids and derivatives, tannins, flavonoids, stilbenes and ligans are some of the most numerous and widely distributed groups of bioactive compounds in the plant kingdom (Dai and Mumper, 2010). Phenolic compounds are known to have antioxidant activities

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which prevent formation of free radicals, which are the major cause of various diseases such as cardiovascular and inflammatory diseases and cancer. They also have the ability to scavenge the free radicals thereby prevent the occurrence of the associated diseases (Vladimir-Knezevic, 2012). In addition, phenolic compounds contribute to the organoleptic properties of plant foods in term of astringency, bitter taste, colour and haze formation (Kobue-Lekalake *et al.*, 2007; Irina and Mohamed, 2012).

Tigernut (*Cyperus esculentus L.*), an underutilized sedge of the family Cyperaceae is a common tuber planted and consumed in Northern Nigeria and some parts of West Africa (Oladele and Aina, 2007). It is consumed mostly by many as snacks but sometimes for its medicinal value, mostly consumed by children and adults alike (Umerie *et al.*, 1997). Many health benefits have been attributed to consumption of tigernut. It has been hypothesized that tigernut consumption can reduce the incidence of diabetes because of its low sugar content (Shaker *et al.*, 2009) and high amino acid arginine which liberates the hormone insulin (Gambo and Da'u, 2014); sickle cell anaemia (Monago and Nwakwe, 2009); colon cancer and some heart related diseases because of its relatively high concentration of phenolic compounds (Oladele and Akinwande, 2013; Oloyede *et al.*, 2014). Monago and Nwakwe (2009) tested the anti-sickling activity of tigernut extracts on sickle cell haemoglobin. The authors observed up to 52% reduction in the sickle cell gelation. They attributed the anti-sickling activity to the bioactive compounds in tigernut. Meanwhile, substantive research findings have reported the presence of phenolic compounds in tigernut (Parker *et al.*, 2000; Oladele and Akinwande, 2013; Oloyede *et al.*, 2014). Tigernut was reported to have a relatively high total phenolic content which might be responsible for its antioxidant activity but there is no data on the phenolic profile. This study determined the phenolic profile and the antioxidant activity of tigernut grown in Nigeria with the aim of elucidating the health promoting potentials of the phenolic compounds.

2.0 Materials and Methods

2.1 Materials

The two varieties of tigernut (yellow, YTG and brown, BTG) used for the research work were obtained from a local market in New Bussa, Niger State, Nigeria in a dry form. The samples were differentiated with their skin colour and size. The brown variety is small with brown skin while the yellow variety is bigger with yellow skin. The phenolic acid standards used were purchased from Aldrich (Aldrich chemical Co., Milwankee, WI).

2.2 Sample preparation

The method described by Oladele and Aina, (2007) was used for the preparation of tigernut flour. The tigernut (Cyperus esculentus) samples were handpicked to remove dirts, stones, broken seeds and other contaminants. They were then washed in clean water and dried in an oven (Model FSOE, Labcon, South Africa) at 70°C for 24h before milling into powder with a local attrition mill to hasten extraction process.

2.2.1 Extraction of polyphenols

The extraction of phenolic compounds was done in two stages:

Stage 1: Fifty milligrams of the sample was extracted with 5 ml of 1M NaOH for 16 h on a shaker at ambient temperature (Provan *et al.*, 1994). After the extraction, it was centrifuged (5000 x g), rinsed with water and centrifuged again. The supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2 h to release the conjugated phenolic compounds (Whitehead et al., 1983). The heated extract was cooled, titrated with 4M HCl of pH < 2.0, diluted to 10 ml with deionised water and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification. At stage 2, the residue was extracted with 5 ml of 4M NaOH, heated to 160°C in Teflon as described by Proven *et al.*, (1994). After cooling, the mixture was filtered. Supernatant was collected and the residue washed with deionised water. The

supernatants were combined and adjusted to pH < 2.0 with 4M HCl. The filtrates were combined for further purification.

2.2.2 Purification of extracted polyphenols

The polyphenol extract was purified using Proven *et al.*, (1994) method. An aliquot (5-15 ml) of the various supernatants was passed through a conditional varian (Varian Association, Harbor city, CA) bond Elut PPL (3 ml size with 200 mg packing) solid phase extraction tube at ~5 ml/ mm attached to a visiprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (-60Kpa) until the resin was thoroughly dried after which the PA's were eluted with 1 ml ethylacetate into gas chromatography autosampler vials. The PPL tubes were conditioned by first passing 2 ml of ethylacetate followed by 2 ml water (pH < 2.0).

2.2.3 Derivatization (Silylation)

Following the extraction steps, the concentrated extract of about 2 ml in the GC vial was derivatized by adding 20 µl of derivatizing agent, Bis (trimethylsilyl) trifluoroacetamide (BSTFA). The silicone septum corked vial was allowed into the water bath with hanger to stand upright in the water with a magnetic stirrer at 45°C for 10 min. A gas chromatography (GC) (model HP 6890) powered with HP Chemstain Rev.A.09.01 (1206) with split injector ratio (20:1) and nitrogen as the carrier gas was used. The GC has inlet temperature of 250°C with 30 m x 0.25 mm x 0.25 µm HP-1 capillary column. The oven of the GC operates at initial temperature of 60°C for 5 min followed by first ramping at 15°C/min for 15 min and second ramping at 10°C for 4 min.

2.3 Determination of the antioxidant activities

2.3.1 Measurement of radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH analysis was carried out as described by Molyneux (2004). Sample (2g) was extracted with 10 ml 80% methanol at room temperature with constant stirring for 20 minutes. After decantation, 0.5 ml of the extract was mixed with 0.5 ml of 0.125mM DPPH solution in methanol. The absorbance of the reaction mixture was read in a spectrophotometer at 515nm after an hour and compared with the standard antioxidant BHT. The control sample was 1 ml 80% methanol. The scavenging of the DPPH radical by the extract was calculated by

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}}$$

Where $A_{control}$ = Absorbance of control sample, A_{sample} = Absorbance of the sample at 515 nm

2.3.2 Measurement of radical scavenging activity using 2,2'-azino-bis-3-ethylbenz-thiazoline-6-sulphonic acid (ABTS)

The radical scavenging activity of the tigernut extract with ABTS was determined using the method described by Pellegrini *et al.*, (2006). The ABTS was dissolved in deionized water to a 7mM concentration. ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS solution with 2.45mM potassium persulfate and allowed the mixture to stand in the dark room at room temperature for 15 h. ABTS solution was diluted with deionized water to an absorbance of 0.7 at 734nm. Solvent was read as blank. After the addition of 100 ?l of aqueous extract solution of 3 ml of ABTS solution, the absorbance was read at room temperature for 20 min after agitation of the mixture. The % inhibition was calculated by

$$\frac{Ab - Ae}{Ab} \times 100$$

Where A_b = absorbance of the blank, A_e = absorbance of the extract.

2.4 Statistical analysis

The data obtained were subjected to one way analysis of variance (ANOVA) while the means were separated using Duncan multiple range test (DMRT).

3.0 Results and Discussion

3.1 Total phenolic content (TPC)

Phenolic compounds are plant phenols with antioxidant property. Various research findings have correlated phenolic content with antioxidant activity. The higher the concentration of total phenolic compounds, the higher the antioxidant activity (Jayaprakasha *et al.*, 2008; Lemos *et al.*, 2013). The total phenolics of tigernut consist majorly of phenolic acids and flavonoids. Although, other phenols are present, they were at minute concentrations. The total phenolic content of YTG and BTG were 351 mg/100g and 134 mg/100g respectively. The TPC of YTG was more than 2.5 folds of BTG. Variation in phenolic values among varieties of produce (e.g. cherry fruit, sorghum and tigernut) has been observed by other researchers. Xiao *et al.* (2015) and Afify *et al.* (2012) observed variation in the total phenolic contents of cherry wine and sorghum varieties respectively. Parker *et al.* (2000) reported TPC range between 5.63 and 64.9 mg/100 g for tigernut while Badejo *et al.* (2014) reported TPC of 21.67 mg/100 ml for tigernut aqueous extract drink. The difference in the values could be due to the methods of extraction and analysis. The highest value obtained by Parker *et al.* (2000) was from tigernut extracted with 0.1M NaOH while the least was from 1M NaOH. The 1M NaOH used for extraction in this work might add to the high value obtained. Also, the TPC determined by Folin-Ciocalteu method is usually higher compared to the TPC by summation of the individual phenolic compounds (Rodríguez-Roque *et al.*, 2013). The values obtained in this work (134 and 351 mg/100g) are higher compared to 78 – 125 mg/100g reported for eggplant (Chumyam *et al.*, 2013), 3.09 mg TAE/100mg for sorghum (Sikwese and Duodu, 2007) and 109 - 116 mg/100g for sorghum (Afify *et al.*, 2012) but lower to 2131 mg/100g reported for peanuts flour (Taha *et al.*, 2012). The composition of an agricultural produce can be affected by many factors which include variety, location of planting and agricultural practices among others. Tigernut can therefore be said to have a relatively high TC.

3.2 Phenolic acids

The phenolic acids composition and concentration of YTG and BTG are shown in Table 1. YTG and BTG were different in phenolic acids concentration (114.96 and 169.21 mg/100g respectively) and composition. The phenolic acids in the tigernut samples which consist of 26 compounds can be classified into hydroxybenzoic acids, hydroxycinnamic acids and other phenols. Hydroxybenzoic acids and hydrocinnamic acids are significant in concentration compared to other phenols which occur as traces. Hydroxybenzoic acids have a general structure derived from benzoic acid but differ according to hydroxylation

Table 1: Phenolic acid concentration (mg GAE/100g) of yellow (YTG) and brown (BTG) tigernut

Phenolic acid	YTG	BTG
Hydroxybenzoic acids		
P-hydroxybenzoic acid	29.12	2.18
Protocatechuic acid	0.61	0.79
Vanillic acid	5.88	15.20
Syringic acid	4.58×10^{-4}	4.12
p-hydroxybenzaldehyde	16.47	ND
Total	52.08	22.29
Hydrocinnamic acids		
p-coumaric acid	0.84	17.25
O-coumaric acid	4.20 x 10-4	5.39
Caffeic acid	1.07	15.25
Ferulic acid	58.38	33.79
Sinapinic acid	8.53×10^{-1}	20.97
Gallic acid	1.74	3.95×10^{-3}
Total	62.88	92.65
Total phenolic acids	**114.96**	**114.94**

YTG - Yellow tigernut, BTG - Brown tigernut, ND - Not detected. Values are means of two determinations

and methoxylation of the aromatic ring while hydroxycinnamic acids are derivatives of cinnamic acid. The hydroxycinnamic compounds are esters found on the arabinose side-chains of arabinoxylans and lignin in the cell wall (Ishii, 1997). They are involved in co-pigmentation process with anthocyanins, which affect the colour of materials (Cabrita *et al.*, 2008). Cinamic acid derivatives are more potent in antioxidant activity than benzoic acid derivatives because of the presence of a conjugated double bond on the side chain (Fukumoto and Mazza, 2000). The hydroxybenzoic acids detected in tigernut include protocatechuic acid, hydroxybenzoic acid, hydroxybenzaldehyde, vanillic acid and syringic acid while the hydroxycinnamic acids include gallic, caffeic, coumaric, ferulic and sinapinic acids. The hydroxybenzoic acids constitute 45% and 19% while hydrocinnamics constitute 54% and 81% of the phenolic acids of YTG and BTG respectively. The YTG and BTG contained 25 and 23 phenolic compounds respectively. The concentration of the phenolic acids in the two varieties of tigernut varied. The concentration of the hydroxybenzoic acids in YTG and BTG were 52 and 22 mg/100g while the hydrocinnamics concentrations were 63 and 93 mg/100g respectively. The YTG contained higher hydroxybenzoic acids content than BTG while BTG contained higher hydroxycinnamic acids than YTG. The variation could be attributed to the difference in the variety and composition. The major phenolic acids in YTG,were ferulic acid, p-hydroxybenzoic acid and P-hydroxybenzaldehyde with 58.38, 29.12 and 16.47mg/100g respectively while BTG contains 33.79 mg ferulic acid, 20.97 mg sinapinic acid, 17.25 mg p-coumaric acid and 15.25mg caffeic acids respectively as the major phenolic acids. Ferulic acid concentration was the highest in the two samples but the ferulic acid in YTG is higher than in BTG. Parker et al. (2000) reported that the major phenolic acids in tigernut are coumaric acid, ferulic acid and its derivatives, p-hydroxybenzaldehyde and vanillic acid. The authors reported higher concentration of the phenolic acids in the skin than the peeled tuber of tigernut. Ferulic acid, a derivative of caffeic acid, has the ability to scavenge free radicals and inhibits the activity of cytotoxic enzymes (Mancuso and Santangelo, 2014). Ferulic acid also affected the pasting properties of starch (Beta and Corke, 2004) and it has been proposed as a potential treatment for many disorders such as cancer, diabetes mellitus and skin diseases (Mancuso and Santangelo, 2014). The presence of ferulic acid in a substantial concentration in tigernut makes tigernut a potential source of treatment for many free radicals induced diseases especially in the areas where it is grown. On the other hand, phenyl-6`-O-malonyl-beta-D-glucoside (4.18×10^{-6}) and chlorogenic acid (2.51×10^{-5}) were the lowest in YTG and BTG respectively. Other phenolic compounds such as capsaicin, curcumin, cinamic acid, eugenol, isoeugenol, rosmarinic acid, 2-phenylethyl-beta-D-glucoside and piperic acid were though detected in the two samples, they were present at a very low concentration. Only salicylic acid was not detected in YTG whereas p-hydroxybenzaldehyde, coumestrol and Phenyl-6'-O-malonyl-beta-D-glucoside were not detected in BTG. Phenolic acids such as capsaicin in pepper have strong antioxidant and anti-inflammatory properties which can modulate oxidative defence system in cells (Tsao, 2010).

3.3 Flavonoids

The flavonoids of YTG and BTG varied and consist of 21 compounds but only seven (7) with significant values are shown (Table 2). The flavonoid compounds of the tigernut samples can be classified into isoflavones, flavones, flavonols, flavanones, flavanols and other phenols. Flavonoids, apart from conferring colour, can inhibit enzymes, act as chelating agent and protect cells against free radicals. This is due mainly to their antioxidant activity (Hargrove *et al.*, 2011; Irina and Mohamed, 2012).

The flavonoids concentration of YTG and BTG are 236 and 19 mg/100g respectively. Oladele and Akinwande (2013) reported 202 and 195 mg/100g

of flavonoids in yellow and brown tigernut respectively. The variation in TPC values were attributed to the method of analysis used by the authors. The flavonoid concentration of YTG and BTG was higher than in most vegetables. Saikia and Mahanta (2013) reported 40.76, 35.14, 112.68 and 32.97mg/100g for carrot, cabbage, tomato and green pea respectively while Dajanta *et al.* (2013) reported 138-350 mg/100g for soybean. Vlase *et al.* (2014) also reported 130 mg/100g for Hyssopus officinalis. Compared to some carbohydrate rich food, the flavonoids concentration of YTG was higher than 46 – 55mg/100g reported for sorghum (Afify *et al.*, 2012).

Quercetin, a flavonol compound, had the highest concentration (60.63 mg/100g) in YTG while apigenin, a flavone was highest (7.91 mg/100g) in BTG. The kaempferol concentration (24.44 mg/100g) of YTG was almost seven folds compared to BTG (3.62 mg/100g). Platelets aggregation and 12-hydroxyeicosatetraenoate (12-HETE) synthesis in the system increase human risk to many diseases. Quercetin is an inhibitor of thrombin - induced and ADP-induced platelets aggregation and 12-HETE synthesis (Pace-Asciak *et al.*, 1995). Consumption of tigernut may therefore prevent coagulation of blood platelets and synthesis of 12-HETE thereby reduce the risk of diseases such as sickle cell anaemia, thrombosis and coronary heart diseases.

The flavone (apigenin and luteolin) of YTG (123 mg/100g) was eight folds of 15 mg/100g obtained for BTG. The flavone of YTG was the highest among the flavonoids. Also, the flavonols (kaempferol and quercetin) concentration of YTG (85 mg/100g) was 24 times higher than that of BTG (3.62 mg/100g). Flavonoids extract with apigenidin and luteolinidin inhibited α-amylase and aromatase activity (Hargrove *et al.*, 2011).

Aromatase enzyme breaks the production chain of estrogen-dependent breast cancer while inhibition of α-amylase prevents production of rapidly digestible starch (RDS) which can lead to postprandial hyperglycaemia. High glycaemic index foods increase the risk of obesity, diabetes and cancer (Warren *et al.*, 2003). The high concentration of apigenin and luteolin suggests tigernut as a potential nutraceutical food which can prevent diseases such as obesity, diabetes and cancer. The two tigernut samples also contain catechin and its derivatives though at varying concentrations. YTG is richer in catechin and epicatechin than BTG (Table 2). Catechin and epicatechin are monomers of tannins; high molecular weight phenolic compound with high antioxidant activity (Awika and Rooney, 2004). This might add to the ability of tigernut to reduce postprandial glycaemia. The presence of catechin might also contribute to the colour and antioxidant activity of tigernut. Takahama *et al.* (2013) and Miao *et al.* (2015) reported the inhibitory effect of cyanidin-catechin pigment and catechin derivatives (epicatechin, epicatechin gallate and epigallocatechin gallate) on α-amylase respectively. The pigment inhibited the hydrolysis of starch by α-amylase.

Table 2: Flavonoids concentration (mg GAE/100g) of yellow (YTG) and brown (BTG) tigernut

Flavonoids	YTG	BTG
Kaempferol	24.44	3.62
Quercetin	60.63	3.76×10^{-3}
Naringenin	16.16	2.38×10^{-3}
Apigenin	50.58	7.91
Luteolin	72.17	7.29
Epicatechin	6.58	1.09×10^{-3}
Catechin	5.61	8.83×10^{-4}
Total	236.17	18.82

YTG - Yellow tigernut, BTG - Brown tigernut. Values are means of two determinations

In YTG, only carvacrol was not detected whereas in BTG, five compounds (glycitein, eriocitrin, epigallocatechin-3-O-gallate,4-O-methyl-epigallocatechin and rutin) were not detected. The concentration of the flavonoid compounds in the two tigernut samples followed the same trend: flavones > flavonols > flavanones > flavanols >

isoflavones. In BTG, only flavones and flavonols are significant in concentration whereas only isoflavones are insignificant in YTG. The flavonoids of YTG are made of 52% flavones, 36% flavonols, 7% flavanones and 5% flavanols while BTG consist 81% flavones and 19% flavonols.

3.4 Antioxidant activity

The antioxidant activity of tigernut as measured by radical scavenging activity using ABTS and DPPH solutions is presented in Table 3. The % inhibition of the free ABTS radicals by the extracts of YTG and BTG were 86.20 and 37.50 % while the DPPH values were 38 and 42.80 % respectively. The ABTS value for YTG was 2.5 fold of BTG value whereas the DPPH value of YTG was lower than that of BTG. Radical scavenging activity of materials has been correlated with its polyphenol concentration. This shows that YTG portends more antioxidant power than BTG. Saikia and Mahanta (2013) and Carciochi *et al.* (2014) reported a similar trend for various vegetables and quinoa seeds respectively. The ABTS value for YTG was higher than the DPPH value while a reverse was observed in BTG. Variation in ABTS and DPPH values among plant varieties have been observed by many researchers. Afiffy *et al.* (2012) reported a higher ABTS values for three sorghum variety while Chumyam *et al.* (2013) reported a higher DPPH over ABTS values for four cultivars of eggplants. The observed differences in the ABTS and DPPH values among varieties can be attributed to the difference in suitability of the determination methods though the two methods are suitable for the analysis of radical scavenging activity. The phenolic compounds in YTG and BTG exhibited different scavenging mechanisms toward the ABTS and the DPPH radicals, and may be able to reduce lipid oxidation via chain-breaking reactions.

Table 3: Antioxidant properties of yellow (YTG) and brown (BTG) tigernut

Sample	ABTS (% inhibition)	DPPH (% inhibition)
YTG	86.20 ± 5.01	38.00 ± 3.21
BTG	37.50 ± 1.03	42.80 ± 3.07

Means and standard deviation of three determinations.

4.0 Conclusion

Tigernut contains high concentrations of polyphenols such as phenolic acids, flavonoids and other phenolic compounds. The two tigernut variety (YTG and BTG) studied differ in total polyphenol content, phenolic acids and flavonoids concentration. YTG was higher in hydroxybenzoic acids while BTG was higher in hydrocinnamic acids concentration. The major phenolic acids in YTG were ferulic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, sinapinic, p-coumaric acid, caffeic and vanillic acids while the major phenolic acids in BTG were vanillic, p-coumaric, caffeic, ferulic and sinapinic acids. Ferulic acid concentration was highest in the two tigernut samples. The concentration of the flavonoid compounds in the two tigernut samples followed the same trend: flavones > flavonols > flavanones > flavanols > isoflavones. The tigernut samples showed moderate antioxidant activity.

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