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Research Article
Nutritional Biomarkers of Tiger Nut (*Cyperus esculentus*) Intake in Albino Rats

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Abstract

Background and Objective: The use of Food Frequency Questionnaire (FFQ) and 24 hrs dietary recalls remain a challenging issue in dietary assessment as a result of errors in reporting methods and dietary information available is normally biased. This study is aimed at assessing the biomarkers of tiger nut (*Cyperus esculentus*) intake in Albino rats. **Materials and Methods:** Tiger nut samples were processed using the standard technique. Twenty-five male Wistar rats weighing between 100-120 g were grouped into five with five rats per group and each group was fed with a different concentration of tiger nut juice for 28 days. The functional groups and the nutritional biomarkers in the tiger nut samples as well as serum samples of rats fed with tiger nut, were assessed using FT-IR and LC-MS, respectively. **Results:** FT-IR spectroscopy confirms the appearance of functional groups OH, C = O and C-O at 3291-3268, 1744-1746 and 2926-3000 cm^{-1} bands, respectively. LC-MS analysis of serum sample of rats fed with 3 g kg^{-1} b.wt., of whole tiger nut showed the presence of pyridoxine (m/z 170.165), pristanic acid (m/z 299.33), hydroxynicotinic acid (m/z 140.126). At 1.5 g kg^{-1} b.wt., LC-MS analysis of the serum sample reveals the presence of proline (m/z 116.49), valine (m/z 118.033) and oxocholic acid (m/z 407.289). **Conclusion:** Serum proline, valine and oxocholic acids could be some of the potential biomarkers of tiger nut intake. However, more studies are needed to validate this finding.

Key words: Tiger nut, biomarkers, food intake, metabolomics, oxocholic acids, valine, cyperaceae, gastrointestinal disorders

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diet plays a key role in reshaping the risk of chronic diseases such as cardiovascular disease, diabetes, cancers and obesity[1]. Assessment of food intake in epidemiological studies was traditionally been conducted using a Food Frequency Questionnaire (FFQ), 24 hrs dietary recall or weighted food diaries. The validity and reliability of the tools were questionable due to the high rate of random and systematic measurement errors[2]. Metabolomics serves as an important tool for depicting a clear picture of the changes that occur in the metabolic profiles within a short- time or long-time diet[3]. Metabolomics aided in a thorough understanding of the characterization of metabolites in the biological samples[4]. Due to the errors that normally occur while using the traditional method in which the participant would say that he has taken something while he didn't take, metabolomics serves as an alternative for assessing "true" food taken by the participant[5].

The major concern of nutritional metabolomics was to discover peculiar metabolites to food intake and their effect on chronic diseases[5]. Fruits and vegetables are the fundamentals of a healthy diet and as such recommended for the cure and prevention of various diseases[6].

Tiger nut (*Cyperus esculentus* L.) is a root tuber that is a member of the family Cyperaceae. It is also known as yellow nutsedge, earth or ground almonds, "*Souchet*" in French, "*Ermandeln*" in German and "*Chufa*" in Spanish[7,8]. Tiger nut is found wild and cultivated in Africa, South America, Europe and Asia. Tiger nuts grow in the wild, along rivers and are cultivated on a small scale by rural farmers mostly in the northern states of Nigeria. It is locally called "*Aya*" in Hausa, "*Aki Awusa*" in Igbo, "*Ofio*" in Yoruba and "*Isipaccara*" in Effik[9]. Tiger nut is nutritionally rich[10]. The major fatty acid in the tuber is oleic (61% of total fatty acids), while other fatty acids include linoleic, palmitic and stearic[11]. Dietary fibre which has been reported to be effective in the treatment and prevention of many diseases has been indicated to be high in the tuber. These diseases include obesity, gastrointestinal disorders, coronary diseases, diabetics and colon cancer[12,13]. Phosphorus and calcium, which are important in the development and maintenance of bones and teeth are also present in appreciable levels[14].

Improvement in the value of tools for food intake assessment could be achieved with the aid of the use of qualitative biomarkers. Development of biomarkers of foods intake could be a long term goal in the application of metabolomics in the assessment of dietary and nutrient intake[15]. This study is aimed at assessing the biomarkers of tiger nut (*Cyperus esculentus*) intake in Albino rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biochemistry and Centre for Dryland Agriculture Laboratories, Bayero University Kano, Nigeria from February, 2019-March, 2020.

Sample collection: Two varieties of *Cyperus esculentus* (brown and yellow) were purchased from Rimi market, Kano metropolis, Kano State, Nigeria. The tubers were identified in the Department of Biological Sciences, Bayero University, Kano by Dr. Yusuf Nuhu with accession number BUKHAN 367. The sorted tiger nut samples were divided into two groups.

Sample preparation: A portion of the sorted tiger nut samples was washed, soaked and mashed using a mortar and pestle to obtain the whole tiger nut sample and then packaged in a transparent airtight container. The whole tiger nut sample was used for animal feeding.

The second portion of the sorted tiger nut samples was washed thoroughly in tap water and 500 g was then blended with 2500 mL of distilled water into slurry using a clean personal blender. Thereafter, the slurry was pressed exhaustively using a muslin cloth to extract the juice. The juice was freshly prepared before feeding. The tiger nut juice was used for animal feeding, FT-IR spectroscopy and LC-MS analysis.

Characterization of serum samples and tiger nut juice using liquid chromatography-mass spectroscopy and fourier-transform infrared spectroscopy: Protocol for LC-MS analysis (Generic method) using LC Waters e2695 separation module with W2998 PDA coupled to ACQ-QDA MS. The tiger nut juice as well as sera samples of rats fed with tiger nut, were analyzed using liquid chromatography (LC) tandem mass spectrophotometer (MS) as described by Piovesana *et al.*[16] with some modifications. The extracted samples were reconstituted in methanol and filtered through a polytetrafluoroethylene (PTFE) membrane filter with a 0.45 µm size. After filtration, the filtrate (10.0 µL) was injected into the LC system and allowed to separate on sunfire C_{18} 5.0 µm 4.6×150 mm column. The run was carried out at a flowrate of 1.0 mL min^{-1}. sample and column temperature at 25°C. The mobile phase consists of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with a gradient. From a ratio of A/B 95:5 this ratio was maintained

for a further 1 min, then A/B 5:95 for 13-15 min. Then A/B 95:5 to 17 and 19 min and finally 20 min. The PDA detector was set at 210-400 nm with a resolution of 1.2 nm and a sampling rate of 10 points/sec. The mass spectra were acquired with a scan range from m/z 100-1250 after ensuring the following settings: ESI in positive and negative ion modes, capillary voltage 0.8 kv (positive) and 0.8 kv (negative), probe temperature 600°C, flow rate 10 mL min^{-1}, nebulizer gas, 45 psi. MS set in automatic mode applying a fragmentation voltage of 125 V. The data was processed with Empower 3. The compounds were identified based on the following information: Elution order, retention time (tR), fragmentation pattern and Base m/z.

FT-IR protocol was done by mixing the tiger nut sample (20 mg) with 200 µL of 20% methanol. The mixture was incubated at 50°C for 20 min and then centrifuged at 13,000 rpm for 15 min. The supernatant was collected and stored at -20°C until analysis.

FT-IR spectra were measured using a Tensor 27 FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) equipped with a deuterated triglycine sulfate detector and a high-efficiency HTS-XT automation device. A 5-µL sample of the extracted supernatant was dispensed into a 384-well zinc selenide (ZnSe) plate and dried at 37°C on a hot plate for 20 min before loading into a 384-well dried ZnSe plate. Each spectrum was investigated at a range of 4000-400 at 4 cm^{-1} intervals of spectral resolution by averaging 128 scans. Each sample was analyzed in triplicate prior. OPUS Lab (ver. 6.5, Bruker Optics Inc.) was used for the evaluation of the spectra. For the baseline correction, the absorbances of the FT-IR spectral analysis at both 800 and 1800 cm^{-1} endpoints were adjusted to 0 and each spectrum was normalized to the same area to minimize experimental error[17,18].

Experimental animals: Twenty-five mice obtained from the Department of Physiology, Bayero University, Kano were used for this study and kept in well-ventilated cages under standard conditions The animals were acclimatized for 2 weeks and maintained under standard conditions of temperature (23±2°C), controlled humidity and a 12 hrs light/dark cycle. The mice were fed standard feed and water was given to them *ad libitum*. Ethical clearance was sought by the Research Ethics Committee of Bayero University Kano.

The animals were divided into five groups, each consisting of five mice and treated as shown below for 28 days:

- **Group I:** Control
- **Group II:** Received tiger nut juice (1.5 g kg^{-1} b.wt.)
- **Groups III:** Received tiger nut juice (3 g kg^{-1} b.wt.)
- **Group IV:** Received whole tiger nut (1.5 g kg^{-1} b.wt.)
- **Group V:** Received whole tiger nut (3 g kg^{-1} b.wt.)

RESULTS

Table 1 presents the important functional groups in the tiger nut samples based on their characteristics bands. The whole tiger nut samples had characteristic bands at

Table 1: Functional group analysis of tiger nut samples and serum samples of rats fed tiger nut samples

Absorption (cm^{-1})	Class of compounds	Intensity	Assignments
Serum sample fed with whole tiger nut (3 g kg^{-1} b.wt.)			
3272.6	Alkynes, R-C≡C-H	s, sharp	≡C-H stretch
1636.3	Amides, R-C(O)-NR'R"	m-s	C=O stretch
Serum sample fed with whole tiger nut (1.5 g kg^{-1} b.wt.)			
3272.6	Alkynes, R-C≡C-H	s, sharp	≡C-H stretch
1636.3	Amides, R-C(O)-NR'R"	m-s	C=O stretch
Serum sample fed with tiger nut juice (3 g kg^{-1} b.wt.)			
3272.6	Alkynes, R-C≡C-H	s, sharp	≡C-H stretch
1636.3	Amides, R-C(O)-NR'R"	m-s	C=O stretch
Serum sample fed with tiger nut juice (1.5 g kg^{-1} b.wt.)			
3291.2	Alkynes, R-C≡C-H	s, sharp	≡C-H stretch
1636.3	Amides, R-C(O)-NR'R"	m-s	C=O stretch
Tiger nut juice			
3291.2	Alkynes, R-C≡C-H	s, sharp	≡C-H stretch
2926.0	Alkanes	S	C-H stretch
1744.4	Ketones	S	C=O stretch
1636.3	Amides, R-C(O)-NR'R"	m-s	C=O stretch
1461.1	Alkyls	S	C-H bend
1148.0	Alcohols, RR'R" C-OH (3°)	m-s	C-O stretch
1077.2	Alcohols, R-CH_2-OH (1°)	m-s	C-O stretch
998.9	Alcohols, C=C-CH(R)-OH	m-s	C-O stretch
Control			
3258.9	Amides, R-C(O)-NH_2	s, sharp	≡C-H stretch
1636.3	Amides, R-C(O)-NR'R"	m-s	C=O stretch

Table 2: LC-MS analysis of serum sample of rats fed with whole tiger nut

Peak numbers	Molecular weight	Measured mass [M+H] (m/z)	Compounds
Group V			
1	169.180	170.165	Pyridoxine
2	298.504	299.330	Pristanic acid
3	139.109	140.126	Hydroxynicotinic acid
4	226.350	227.290	Citronellyl butyrate
5	131.170	132.286	Isoleucine
6	115.130	116.490	Proline
7	198.170	199.336	Syringic acid
Group IV			
1	115.130	116.490	Proline
2	117.151	118.033	Valine
3	406.272	407.289	Oxocholic acid

Table 3: LC-MS analysis of serum sample of rats fed with tiger nut juice

Peak numbers	Molecular weight	Measured mass [M+H] (m/z)	Compounds
Group III			
1	131.170	132.510	Isoleucine
2	117.151	118.136	L-valine
3	519.660	520.365	Lysophosphatidylcholine
4	226.398	227.268	Heptyl ketone
Group II			
1	131.170	132.119	Isoleucine
2	298.460	299.247	Ricinoleic acid
3	198.170	199.259	Syringic acid
4	215.370	216.366	Solamine

Table 4: LC-MS analysis of tiger nut juice and control samples

Peak numbers	Molecular weight	Measured mass [M+H] (m/z)	Compounds
Tiger nut juice			
1	131.170	132.119	Isoleucine
2	198.170	199.259	Syringic acid
3	406.270	407.210	Oxocholic acid
4	117.151	118.231	Valine
5	318.121	319.090	Myricetin
6	103.312	104.330	D-valinol
Group I			
1	213.270	214.175	2-Noenoylglycine
2	388.450	389.383	Quassin
3	292.330	293.158	Phenylbutyrylglutamine
4	312.303	313.279	Phytanic acid

3272.6 cm^{-1} (Alkynes, R-C≡C-H) and (Alkynes R-C=CH) and 1636.3 cm^{-1} (Amides, R-C(O)-NR'R"). The result also showed absorption peaks at 1077.2-1148.0 cm^{-1} (Alcohols), 1461.1 cm^{-1} (Alkyls), 1636.3 cm^{-1} (Amide) and 2926.0 cm^{-1} (Alkanes) in the tiger nut juice.

Table 2 presents the result of the LC-MS analysis of serum samples of group IV and V (rats fed whole tiger nut at 1.5 and 3.0 g kg^{-1} b.wt., respectively). It showed the presence of pyridoxine (m/z 170.165), pristanic acid (m/z 299.33), hydroxynicotinic acid (m/z 140.126), citronellyl butyrate (m/z 227.29), isoleucine (m/z 132.286), proline (m/z 116.49) and syringic acid (m/z 199.336), valine (m/z 118.033) and oxocholic acid (m/z 407.289) in both samples.

Table 3 showed the compounds found in the serum sample of groups I and II (rats fed with tiger nut juice at 1.5 and 3.0 g kg^{-1} b.wt., respectively). Isoleucine (m/z 132.51), L-valine (m/z 118.139), lysophosphatidylcholine (m/z 520.365), heptyl ketone (m/z 227.268), ricinoleic acid (m/z 299.247), syringic acid (m/z 199.259) and solamine (m/z 216.366) biomarkers were detected in the LC-MS chromatogram.

Table 4 present the result of the LC-MS analysis of the control samples and tiger nut juice. It showed the presence of isoleucine (m/z 132.119), syringic acid (m/z 199.259), oxocholic acid (m/z 407.210), valine (m/z 118.231), myricetin (m/z 319.090) and D-valinol (m/z 104.330) in the tiger nut juice. LC-MS analysis of the control sample reveals the

presence of biomarkers including 2-noenoylglycine (m/z 214.175), quassin (m/z389.383), phenyl-butyryl glutamine (m/z 293.158) and phytanic acid (m/z 313.279).

DISCUSSION

The result of the FTIR analysis revealed the formation of certain important functional groups due to oxidation of the -OH groups in the samples. The following important functionalities were revealed: The band at 3291-3268 cm^{-1} was due to bonded -OH groups in the sample[19-21]. The band at 1744-1746 cm^{-1} in tiger nut was due to carbonyl (C=O) stretch, which confirmed the formation of oxidized compounds. Other important absorption bands which confirmed the formation of carboxyl groups attributable to further oxidation of the carbonyl groups were observed around 1647-1637 cm^{-1} [20]. The presence of stretch and bend (alkanes) was indicated by the appearance of the bands around 2926-3000 cm^{-1} and 1458-1420 cm^{-1}, respectively[22]. The peaks at 1144-850 cm^{-1} are characteristic absorptions of C-O stretch in C-O-C and C-O-H of the glycosidic links in the samples[20,23].

The LC-MS study of the tiger nut samples shows important chemicals present in the tiger. Some of the bioactive compounds are used for the discovery of drugs (as starting materials) and also in modern and traditional medicine[24]. Pyridoxine, pristanic acid, hydroxynicotinic acid and citronellyl butyrate are believed to possess hypotensive effects. These compounds are also known to possess antimicrobial activity[25]. It has been reported that it may be used for the treatment of prostate cancer due to its cell death inducer and cytotoxic effect[26].

The properties of these various phytochemical components show that *C. esculentus* nut (tiger nut) may possess anti-microbial, anti-inflammatory, antioxidant, lipid moderating and immune-boosting effects. Tiger nut has also been documented to aid in activating the circulation of blood, prevention of heart disease, treatment of bacterial infection and urinary tract infection[27].

The findings of this study imply that tiger nut consumption could be assessed quantitatively with the aid of objective biomarkers. Therefore, food and nutrient intake studies may find this useful in studies involving the assessment of tiger nut intake. However, the need for more studies to validate the findings of this research is necessary. This study is limited to animal studies but first on biomarkers of tiger nut intake, to the best of our knowledge. Future studies are expected to use human volunteers in a more robust approach.

CONCLUSION

FT-IR used for functional group identification confirms the appearance of functional groups OH, C=O, C-O and C-H at 3291-3268, 1744-1746, 2926-3000 and 1458-1420 cm^{-1} bands. LC-MS study of the tiger nut samples revealed the presence of bioactive compounds including pyridoxine, pristanic acid, hydroxynicotinic acid and citronellylbutyrate. Hence, these biomarkers could be used to ascertain dietary compliance in nutrition research.

SIGNIFICANCE STATEMENT

This study discovers the biomarkers of tiger nut intake that can be beneficial in dietary and nutrient intake assessment. This study will help the researchers to objectively assess compliance with dietary intake reported by volunteers in dietary assessment studies.

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