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RESEARCH ARTICLE

# CHARACTERIZATION AND FATTY ACID AMINATION OF TIGER NUT TUBER OIL

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## ABSTRACT

Oil was extracted from tiger nut (*Cyperus esculentus*) tubers at a percentage oil yield of 4.72% using Soxhlet extraction method. The extracted oil was characterized using Gas Chromatography Flame Ionization Detector (GC-FID) and a fatty acid percentage composition of 35.69%, 20.78%, 18.03%, 14.25%, 9.35%, and 1.90% of Oleic acid, Linolenic acid, Lauric acid, Myristic acid, Linoleic acid and Eicosapentaenoic acid respectively was obtained, giving a total percentage fatty acid composition of 100%. A Fourier Transform Infrared Radiation (FTIR) spectroscopy was carried out on the tiger nut tuber oil which measured the oil's absorbance of infrared light at various wavelengths that led to the determination of the oil's molecular composition and structure. Furthermore, the triglycerides in the tiger nut tuber oil were aminated with diethylamine for synthesis of fatty acid amides. An FTIR characterization of the resulted fatty amides revealed the presence of many functional groups. The fatty acid profile of the tiger nut tuber oil and the derivatives of their fatty acids (fatty acid amides) were indicators that tiger nut tuber oil has huge exploitable potentials for utilization in food, nutrition, cosmetics, pharmaceuticals, therapeutics, medicines, and plastics. Thus, inclusion of tiger nut tuber as a source of vegetable oil will reduce over-dependence on conventional oil seeds and scale up its economic value as well as strengthen the Nigeria's economy.

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# INTRODUCTION

Tiger nut is not really a nut but a small tuber of the family *Cyperaceae* which produces rhizomes from the base and tubers that are spherical with the size of a pea nut (Muhammed *et al*., 2011).The Cyperaceae are monocotyledonous plants with up to 4000 species worldwide (Ekeanyanwu and Ononogbu 2010) that are of very little economic value with the exception of *Cyperus papyrus* which is used in the manufacture of paper and *Cyperus esculentus* L (tiger nut) which is edible (Simpson and Inglis 2001). Though tiger nut is widely grown across the globe, it has been poorly researched leading to their limited applications (Adejuyitan 2011; Yu *et al*., 2022). Of course, tiger nut has high yield and potentials for comprehensive utilization (Bazine and Arslanoğlu 2020). Tiger nut (*Cyperus esculentus*) is a fast-growing grass-like plant taking an average of three months to mature and often it is mistaken for weeds. It is a perennial crop (from rhizomes ending in hard tubers) that is erect with yellowish-green leaves and triangular stem (20-60 cm tall) and grows best in well-drained sandy or loamy soils; and its production increases as the ambient temperature increases (Warra 2013). Tiger nut has become naturalized in Nigeria, Ghana and Sierra-Leone (Emurigho *et al*., 2020). It is locally sold in Nigerian markets in fresh, semi-dried and dried forms for consumption. The tiger nut has a nutrient composition of 22.14–44.92% lipids, 3.28–8.45% proteins, 23.21–48.12% starch, 8.26–15.47% fibers, 1.60–2.60% ashes, and bioactive compounds (Adel *et al*., 2015; Yul *et al*., 2022). It is also rich source of iron, phosphorus, magnesium, potassium, calcium, and vitamins E, C and B (Alobo and Ogbodo 2007; Maduka and Ire 2018). Tiger nut (*Cyperus esculentus*) tubers as food, can be eaten raw, roasted, dried and baked (Adejuyitan *et al*., 2009). According to Bazine and Arslanoğlu (2020), tiger nuts tubers can be utilized in production of flour, beverage, confectioneries, sweeteners, milk, yoghurt, feed source, soap and vegetable oil. Of course, it is daunting to observe that such crop repleted with innumerable industrial potentials capable of reviving the economies of countries like Nigeria are ignorantly relegated. The relegation of tiger nut tubers is due to lack of detailed information about its utilizable characteristics and that of its derivatives. For example, tiger nut tuber oil is a vegetable oil (composed of unsaturated fatty acids) with both edible and non-edible applicable potentials (Warra 2013; Oyedele, Oladipo and Adebayo 2015; Mohdaly, 2019; Yul *et al*., 2022). Its fatty acid profile is similar to olive oil. The oil in tiger nut tuber is extracted using organic solvents such as petroleum ether, hexane, etc. or through laboratory press (Ali Rehab and El Anany 2012) and is now produced on a commercial scale for the European market (Yeboah *et al*., 2012). The oil is golden brown in color. It has a rich nutty taste and is a fantastic component of beauty products. This oil is mostly composed of long chain fatty acids of both saturated and unsaturated carbon skeleton which is housed by the triglyceride (Sánchez-Zapata, Fernández-López and Angel 2012). The triglyceride has oleic acid as its most abundant fatty acid which plays a major role in ameliorating an individual's risk of developing diseases including diabetes, asthma and cancer (Lunn and Theobald 2006). Also, the presence of oleic acids in tiger nut tuber oil makes it a good moisturizer, and a number of cosmetic companies add it to lotions and soap in order to boost their ability to nourish the skin. The triglyceride contains other fatty acids including linoleic fatty acids (belonging to the omega-3 family of fatty acids) and alpha linolenic fatty acid (belonging to the omega family of fatty acids) which are considered essential as they cannot be synthesized by mammals and must be obtained from food. Also, the oil of tiger nuts contains antioxidant activity of fatty hydroxamic acid (Adewuyi *et al*., 2015). Its properties have been exploited in a number of studies including its suitability as biodiesel (Barminas *et al*, 2001). It is also useful for culinary, medicinal, cosmetic, feedstock (for industrial polymers) and many other industrial purposes. Therefore, tiger nuts' utilizations could be scaled-up through innovative methods of applying their constituents (such as its oil) in both food and non-food fields. This study is aimed at extracting and characterizing oil from tiger nut (*Cyperus esculentus* L) tuber using gas chromatography flame ionization detector (GC-FID) and utilizing it in the synthesis of fatty acid amides.

# MATERIALS AND METHODS

**Materials:** The tiger nut (*Cyperus esculentus*) tubers (used for the study) were obtained from Crop Science and Technology farm, Federal University of Technology, Owerri. These tiger nut tubers (raw materials) were stored (under dry condition and ambient room temperature of $27^0$C) in the Laboratory of Chemistry Department, Federal University of Technology, Owerri, Imo State, Nigeria.

**Methods**

***Preparation of sample:*** The tubers of the tiger nuts were manually sorted to remove unwholesome ones and all extraneous materials. They were washed with portable water and efficiently milled to a uniform thickness with an attrition mill (9FC-36, China). The milled sample (tiger nut flour) was weighed and dried in the oven (Gallekamp, England) at a temperature of 110°C for 1 h. Dried sample was hermetically stored in air-tight polythene bags (Ziplock, China) at room temperature ($27^0$C) in readiness for further analyses.

***Oil extraction from the Tiger nut Tuber flour sample:*** Oil was extracted from ground sample of tiger nut tuber using the method outlined by Warra *et al*. (2017) with slight modification. Dried ground sample of tiger nut tuber (50 g) was put into a porous thimble and placed in a Soxhlet extractor, using 150 cm$^3$ of n-hexane (with boiling point of 40-60°C) as extracting solvent for 6 hours repeatedly until required quantity was obtained. The oil was obtained after evaporation using water bath at 70°C to remove the excess solvent from the extracted oil. The oil was then cooled in a desiccator and stored in refrigerator prior to further analysis. The percentage oil content was calculated as follows:

$$\% \text{ Oil Content} = \left(\frac{\text{weight of oil}}{\text{weight of sample}}\right) \times 100$$

***Gas Chromatography Flame Ionization Detector (GC-FID) Analysis of the Tiger nut Tuber oil:*** Conventional GC-FID was performed using an Agilent 7680 gas chromatograph equipped with a split/splitless auto-injector (7683B series) and a flame ionization detector (FID) (Agilent, Palo Alto, CA) as reported by Forsythe *et al.* (2017). The GC was fitted with a 20 m × 0.18 mm ID fused silica capillary column coated with a 0.4 µm thick film of 100% dimethylpolysiloxane (Rtx-1, Restek, Bellefonte, PA). Ultra-high purity helium was used as a carrier gas with a constant flow rate of 0.8 mL min$^{-1}$. Tiger nut tuber oil sample (0.1 g) was injected at 300°C with a split ratio of 25:1 using a HP 6890 series autosampler (Agilent, Palo Alto, CA). The GC oven was programmed from 35°C to 335°C at 2°C min$^{-1}$ with a final hold time of 10 min. Data from the FID was collected at 100 Hz and reference standards were used to identify peaks.

***Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the Tiger nut Tuber oil*:** The Fourier Transform Infrared (FTIR) Spectroscopy was used to quantify the amount of free fatty acid present in the tiger nut tuber oil sample, as well as investigate the possible functional groups in the tiger nut tuber oil (Warra *et al*., 2019). It was also used to monitor and detect the quantity of fatty acid methyl esters in tiger nut tuber oil. Fourier Transform Infrared Spectrometer Nicolet 8400S equipped with a detector of deuterated triglycine sulphate (DTGS) and connected to software of OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FT-IR spectrum of the tiger nut tuber oil sample. The oil sample was placed in contact with KBr disc and FT-IR spectrum was collected in frequency of 4500-400 cm$^{-1}$ by co-adding 32 scans and at resolution of 4 cm$^{-1}$. The spectrum was rationed against a background spectrum. In each scan, a new reference background spectrum was detected. The spectrum was recorded as absorbance value at the data point in triplicate.

**Synthesis of fatty acid amides from the Tiger nut tuber oil:** The method described by Bentacourt-Jimenez *et al*. (2020) was used to synthesize fatty acid amides from the tiger nut tuber oil sample. Tiger nut tuber oil (4.0 g; ~14 mmol) was reacted with 3 times molar excess diethylamine (~126 mmol) and 20 mg of DABCO (~0.2 mmol). The reaction was carried out at 60 °C for 72 h with magnetic stirring to produce fatty acid amides. The fatty acid amides produced were rinsed twice with deionized water and recovered using a separatory funnel. Lastly, the fatty acid amides were subjected to high vacuum until the pressure stabilized (approximately at 300 mTorr) to evaporate any remaining water and unreacted amine.

# RESULTS AND DISCUSSION

**RESULTS**

% Oil content of Tiger nut tuber = 4.72%

### GC-FID Analysis of Tiger nut oil

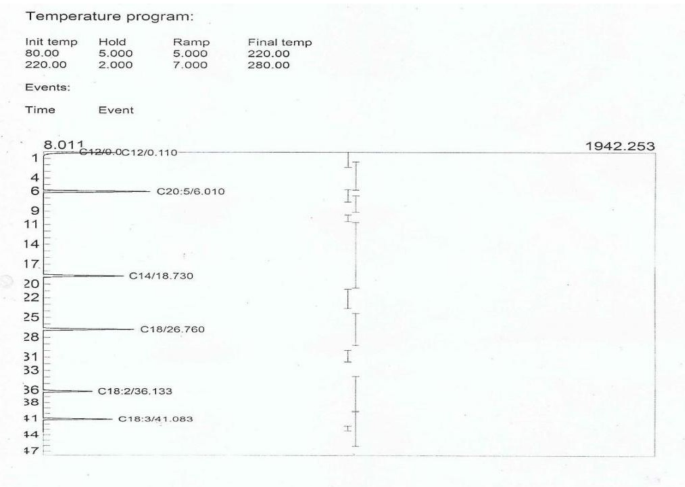



**Figure 1. The GC-FID Spectrum of the Tiger nut oil**

**Table 1. GC-FID Spectrum of the Tiger Nut Oil**

| Component | Retention | Area | Height | External Units |
|---|---|---|---|---|
| C12:0 | 0.000 | 117.5658 | 105.230 | 0.2076 |
| C12:0 | 0.110 | 1376.6016 | 164.556 | 2.4314 |
| C20:5 | 6.010 | 4476.2198 | 351.878 | 0.2787 |
| C14:0 | 18.730 | 3350.7662 | 263.663 | 2.0854 |
| C18:0 | 26.780 | 3823.2398 | 300.724 | 5.2227 |
| C18:2 | 36.133 | 2981.1352 | 234.487 | 1.3687 |
| C18:3 | 41.083 | 3130.5531 | 246.239 | 3.0408 |

**Note**: Table 1 above is a Chromatographic pattern comparison based on relative peak areas of the fatty acid components present in the Tiger nut oil sample.

**Table 2. Result of the Gas Chromatography Flame Ionization Detection (GCFID) Analysis of the Tiger Nut Tuber Oil**

| Lipid Numbers of the fatty acid | Name of the fatty acid | Chemical formula of the fatty acid | Retention time (Minutes) | Concentration (ppm) | Percentage composition of the fatty acid (%) |
|---|---|---|---|---|---|
| C12:0 | Lauric acid | $C_{12}H_{24}0_2$ | 0 | 0.2076 | 1.42 |
| C12:0 | Lauric acid | $C_{12}H_{24}0_2$ | 0.11 | 2.4314 | 16.61 |
| C20:5 | Eicosapentaenoic acid | $C_{20}H_{30}O_2$ | 6.01 | 0.2787 | 1.90 |
| C14:0 | Myristic acid | $C_{14}H_{28}0_2$ | 18.73 | 2.0854 | 14.25 |
| C18:0 | Oleic acid | $C_{18}H_{34}O_2$ | 26.76 | 5.2227 | 35.69 |
| C18:2 | Linoleic acid | $C_{18}H_{32}O_2$ | 36.133 | 1.3687 | 9.35 |
| C18:3 | Linolenic acid | $C_{18}H_{30}O_2$ | 41.083 | 3.0408 | 20.78 |

Note: Oleic acid > Linolenic acid > Lauric acid > Myristic acid > Linoleic acid >Eicosapentaenoic acid

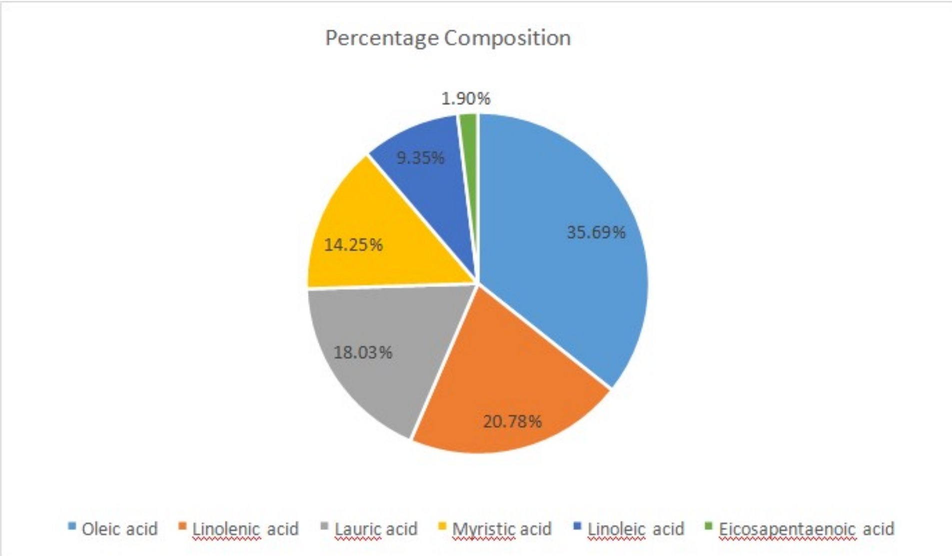
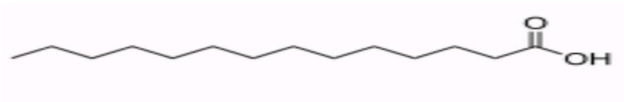



**Figure 2. The percentage composition of fatty acids in Tiger nut tuber oil**

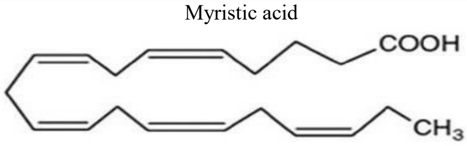
*Chemical structures of the fatty acids*


Oleic acid


Lauric acid




Myristic acid




Eicosapentaenoic acid


Linoleic acid


Linolenic acid

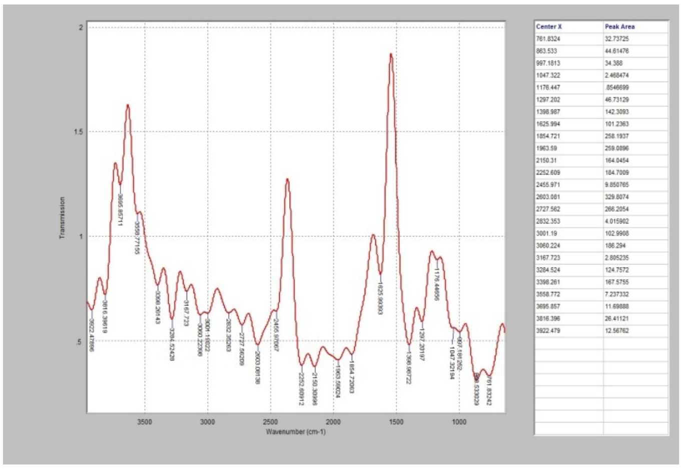



**Figure 3. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of the tiger nut tuber oil**

**Table 3. Functional groups of compounds absorbed from Tiger nut tuber oil in the Infrared spectrum**

| Frequency (cm$^{-1}$) | Peak Area | Functional Group |
|---|---|---|
| 761.8324 | 32.73725 | C-H |
| 863.533 | 44.61476 | C-H |
| 997.1813 | 34.388 | =C-H |
| 1047.322 | 2.468487 | C-O |
| 1176.447 | 0.8546699 | C-H Wag |
| 1297.202 | 46.73129 | C-O |
| 1398.98722 | 142.3093 | C-C |
| 1625.994 | 101.2363 | Aromatics Overtones |
| 1854.721 | 258.1937 | Aromatics Overtones |
| 1963.59 | 209.0896 | Aromatics Overtones |
| 2150.31 | 164.0454 | -C≡C |
| 2252.609 | 184.7009 | -C≡C |
| 2455.971 | 9.850765 | C=C |
| 2603.081 | 329.8074 | O-H |
| 2727.562 | 266.2054 | C-H |
| 2832.353 | 4.015902 | C-H |
| 3001.19 | 102.9908 | C-H |
| 3060.224 | 186.294 | O-H |
| 3167.723 | 2.805235 | O-H |
| 3284.524 | 124.7572 | N-H |
| 3398.261 | 167.5755 | N-H |
| 3558.772 | 7.237332 | O-H |
| 3695.857 | 11.69888 | O-H |
| 3816.396 | 26.41121 | O-H |
| 3922.479 | 12.56762 | O-H |

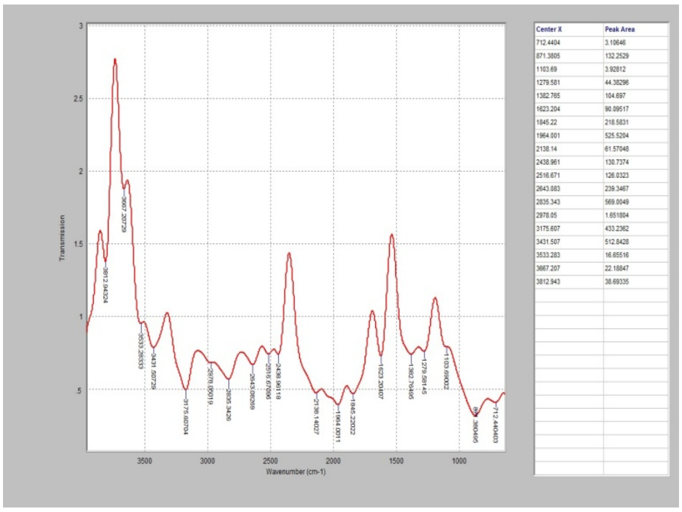



**Figure 4. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of the aminated fatty acids**

**Table 4. Absorption of compounds present in aminated fatty acids in the Infrared (IR) spectrum**

| Frequency (cm$^{-1}$) | Peak Area | Functional Group |
|---|---|---|
| 712.4404 | 3.10646 | C-H |
| 871.3805 | 132.2529 | C-H |
| 1103.69 | 3.92812 | C-O |
| 1279.581 | 44.38296 | C-O |
| 1382.765 | 104.697 | O-H |
| 1623.204 | 90.09517 | C-C |
| 1845.22 | 218.5831 | Aromatics Overtones |
| 1964. 001 | 525.5204 | Aromatics Overtones |
| 2138.14 | 61.57048 | -C≡C |
| 2438.961 | 130.7374 | -C≡C |
| 2516.671 | 126.0323 | O-H |
| 2643.083 | 239.3467 | O-H |
| 2835.343 | 569.0049 | O-H |
| 2978.05 | 1.651804 | C-H |
| 3175.607 | 433.2362 | N-H |
| 3431.507 | 512.8425 | O-H |
| 3533.283 | 16.65516 | O-H |
| 3667.207 | 22.18847 | O-H |
| 3812.943 | 38.69335 | O-H |

# DISCUSSION

The results (Table 1) of the characterization of the Tiger nut tuber oil using gas chromatography with a flame ionization detector indicated the following fatty acid composition: lauric acid (18.03%), eicosapentaenoic acid (1.90%), myristic acid (14.25%), oleic acid (35.69%), linoleic acid (9.35%) and linolenic acid (20.78%). From the results obtained, oleic acid was highest in abundance while eicosapentaenoic acid was lowest. The results are in line with the reports of Ezebor *et al*. (2005) and Yu *et al*. (2022) but differ from some of the reports of (Ezeh, Gordon and Niranjan 2014). Generally, some reports on the fatty composition of tiger nut tuber oil from Egypt, East Mediterranean region, Ghana, South Korea and China had palmitic acid, palmitoleic acid, stearic acid and arachidonic acid in addition to the detected fatty acids observed in this study (Zhang *et al*., 1996; Coskuner *et al*., 2002; Kim, No and Yoon 2007; Arafat *et al*., 2009; Muhammad *et al*., 2011; Yeboah *et al*., 2012; Salama *et al*. 2013; Ezeh, Gordon and Niranjan 2014. For example, the fatty acid composition of tiger nut tuber oil reported in some literatures are: myristic acid (0.0 1.7%), palmitic acid (10.4-16.32%), palmitoleic acid (0.0-150%), stearic acid (0.3-5.33%), oleic acid (65.5-76.1%), linoleic acid (8.36-16.2%), α-linolenic acid (0.0-0.69), arachidonic acid (0.0-6.1%) and eicosaenoic acid (0.76%) (Salama *et al*., 2013; Ezeh, Gordon and Niranjan 2014; Yu *et al*., 2022).

These variations in the fatty acid composition of tiger nut tuber oil observed from other authors' reports could be attributed to differences in colour, variety, geographical locations, seasons of harvest, climatic condition and method of analysis (Ezeh, Gordon and Niranjan 2014). However, tiger nut tuber oil's fatty acid profile is similar to olive oil, avocado oil and hazelnut oil (Ezeh, Gordon and Niranjan 2014; Touria *et al*., 2022); and this makes it recommendable for human consumption and food applications due to its nutritional and health benefits (Barros *et al*., 2020; Nina *et al*., 2020; Ogori, Nina and Ukeyima 2021; Touria *et al*., 2022). It is a rich flavouring agent for ice cream and biscuits (Cantalejo 1997). The lower percentage of polyunsaturated fatty acids (Eicosapentaenoic acid, linoleic acid and linolenic acid) in tiger nut tuber oil will contribute to its increased stability against spoilage and high temperature applications. Also, the high contents of oleic acid and linolenic acid (omega-3 fatty acid) in tiger nut tuber oil makes it excellent for utilizations in production of cosmetics that are effective against skin alterations, premature ageing, formation of eczema and psoriasis, and other skin problems (Kamalu and Oghome, 2008) Zieli´nska and Nowak, 2014; Bialek *et al*., 2016). Of course, the presence of omega-3 and 6 fatty acids (linoleic acid, linolenic acid and eicosapentaenoic acid; which are essential fatty acids) in tiger nut tuber oil promotes skin cell renewal and lowers the bad cholesterol level, amidst many other nutritional and health benefits; thus, the tiger nut tuber oil has highly exploitable pharmaceutical and medicinal value. Infrared spectroscopy is a type of spectroscopic technique that involves the interaction of infrared radiation with matter; It is based on the absorption, emission, or scattering of infrared radiation by a sample, and is used to identify and analyze the chemical structure of a sample. Infrared (IR) spectroscopy can be very sensitive to determination of functional groups within a sample since different functional group absorbs different particular frequency of IR radiation. The infrared spectroscopy of the oil provided information about the measurement of the tiger nut tuber oil's absorbance of infrared light at various wavelengths which determined the molecular composition and structure of the organic molecules present in the oil as was shown by the vibration of different molecules at specific frequencies corresponding to different functional groups, absorbed infrared radiation at different absorption locations as shown in the infrared spectrum (Table 3). In this analysis, twenty-five functional groups were detected in the tiger nut tuber oil.

Furthermore, fatty acid amides (FAAms) were successfully synthesized from tiger nut tuber oil (a vegetable oil) and alkyl amines, thereby indicating the potentials of tiger nut tuber oil in serving as key raw material for commercial synthesis of fatty acid amides. More so, infrared spectroscopy of the fatty acid amides derived from tiger nut tuber oil revealed the molecules present in them and their absorption locations as shown in the infrared spectrum (Figure 4) as presented in Table 4. The chemical structures of the samples of the fatty acid amides, analyzed through Fourier Transform Infrared Spectroscopy (FTIR) indicated possible composition of the fatty acid amides (derived from tiger nut tuber oil) of about 19 functional groups (Table 4). These results reveal that tiger nut tuber oil is a potential precursor of fatty acid amides such as linoleamide, oleamide, eicosapentaenoylethanolamide etc. Fatty acid amides (compounds containing long-chain fatty acid and amine group in their structures) have wide applications in food, polymer and pharmaceutical industries. For instance, oleamide is used in production of lubricants, slip agent, corrosive inhibitor and sleeping pills (Naumoska *et al*., 2020; Cyriac *et al*., 2021). Also, fatty acid amides play key roles in biological functions, such as cancer treatment, sleep induction, analgesic, anti-anxiety, anti-convulsion, fat hydrolysis, weight loss, anti-epilepsy activities, neuroprotection and regulation of blood sugar level (Huang and Jan 2001; Ishida *et al*., 2013; Xu *et al*., 2016; Nam *et al*., 2017; Hermes *et al*., 2018; Post *et al*., 2018; Yerlikaya *et al*., 2019; (Murkar, De Konnick and Merali 2021); Kobayashi*et al*, 2021; Li *et al*., 2022).

# CONCLUSION

Vegetable oil was extracted from tiger nut tubers (*Cyperus esculentus* L) and characterized using gas chromatography flame ionization detector (GC-FID); the tiger nut tuber oil was further utilized in the synthesis of fatty acid amides whose functional groups were determined using Fourier Transform Infrared Spectroscopy (FTIR). The fatty acid composition of the tiger nut tuber oil was: lauric acid (18.03%), eicosapentaenoic acid (1.90%), myristic acid (14.25%), oleic acid (35.69%), linoleic acid (9.35%) and linolenic acid (20.78%). This fatty acid profile of the tiger nut tuber oil and the derivatives of their fatty acids (fatty acid amides) is a pointer that tiger nut tuber oil has enormous exploitable potentials for utilizations in food, nutrition, cosmetics, pharmaceuticals, therapeutics, medicines, and even in polymer industries. Therefore, these detailed information about the industrial potentials of tiger nut tuber oil will reduce the relegation of tiger nut tubers in Nigeria and also add economic value to tiger nut tubers as well as boost the economy of Nigeria and other tiger nut-producing nations.

**Recommendations**

Tiger nut tuber oil has the deployable potentials to serve as a supplementary vegetable oil to the conventional vegetable oils that are far from meeting the increasing demand by food, therapeutics, plastics, pharmaceuticals, medicines, nutrition, health, cosmetics and many other industrial purposes. Nigeria (a major producer of tiger nut tubers) is under-exploiting the potentials of tiger nut tubers, thereby putting overbearing burden on the few indigenous oil seeds for vegetable oil production; there is need to expand sources of vegetable oil to tiger nut tubers. Such endeavour will encourage local agriculture, increase job opportunity and strengthen the fragile economy of Nigeria. Efforts should also be geared towards expanding the utilizations of all varieties of tiger nut tubers via their oil explorations, thereby transforming them from domestic crops to industrial crops. By the way, the pulp generated after oil extraction could be utilized in the formulation of animal feed.

***Conflict of interest:*** The authors declare that there are no conflicts of interest regarding the publication of this article.

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