Quality Mapping of Tigernut Oil and the Extraction Efficiency Between n-Hexane and Petroleum Ether Solvents

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Abstract

Edible Tigernut (*Cyperus* esculentus) oil samples were separately extracted using n-hexane and petroleum ether solvents. This study aimed at comparing the extraction efficiencies of both solvents and the quality and colour of oil produced with a view for large scale industrial application. The quality parameters tested included moisture content, saponification value (SV), iodine value (IV), peroxy value (PV), free fatty acid content (FFA), unsaponifiable matter, total fatty matter (TFM), titre (ºC), impurity, and colour.. Results showed that n-hexane extraction efficiency was 86% and significantly higher ($p<0.05$) than that of petroleum ether which gave 75%. n-Hexane produced 17.10g or 19.01 ± 0.11cm³ of oil yield from 20g of powdered tigernut seed, while petroleum ether produced 15.05g or 16.72 ± 0.12 cm³ of oil from 20g of powdered tigernut seed. The mean SG of oil samples was 0.8917 ± 0.011 at 40ºC. The appearance of oil extracted with n-hexane was brighter and golden yellow, when compared to that of pet ether which gave a brownish-yellow tint. The colour on Lovibond Scale (R*Y*B*N) using $5^{1}/_{4}$" cell, showed n-hexane extracted oil to be 0.9R, 10Y, 0B, 0N, while the pet ether extracted oil gave 1.2R 12Y, 0.5B, 0N. There was no significant difference in moisture content of both oil samples. The mean values of chemical quality parameters for both oil samples gave saponification value 209.33 ± 0.58, iodine value 91.33 ± 0.58 , peroxide value 1.06 ± 0.01, ester value 208.33 ± 1.16, moisture content 0.23 ± 0.021%, free fatty acid content 0.24 ± 0.0058%, impurity content 0.013 ± 0.0058%, unsaponifiable matter 0.11 ± 0.01% and total fatty matter 95.03 ± 0.027%. GC-MS analysis gave seven major fatty acids of the oil samples after conversion to fatty acid methyl esters (FAMEs). Five of these fatty acids were saturated (SFA) and include   lauric acid (1.32 ± 0.08%); myristic acid (31 ± 1.0%); palmitic acid (14.6 ± 0.1%); stearic acid (3.37 ± 0.15%); crotonic acid (0.53 ± 0.068%), giving a total SFA content of 44.77 ± 0.29%. The other two were unsaturated fatty acids (UFA), and included oleic acid (45.9 ± 1.83%) and linoleic acid (9.37 ± 0.15%), giving a total of (55.17 ± 0.76%) UFA content. Therefore, the ratio of UFA:SFA gave 1.18 ± 0.0058, suggesting that the oil may be categorized as an ω-6 edible oil. The quality parameters tested, established the quality mapping for quality control, standardization and regulatory activities of future tigernut oil industry in Nigeria.

Keywords: Tigernut, Oil quality, extraction efficiency, fatty acid, ω-6 edible oil.

1.   Introduction

Tiger nut (*Cyperus* esculentus) has always been in use as food, eaten raw, roasted, dried, baked, or made into beverages (Adejuyitan, et al 2009). It is an underutilised crop and to boost its utilization means finding other innovative methods of applying constituents   of tiger nut, its oil being one of such constituents. This tiger nut is really not a nut but a small tuber of the family Cyperaceae which produces rhizomes from the base and tubers that are spherical, the size of a pea nut (Mohammed et al 2011). Literature is replete of information on the nuts because of its high nutritional value. On the other hand, tiger nut oil is not finding use in food industries when compared with soya bean oil, olive oil or peanut oil even when research has shown they share same physical properties (Ezeh et al 2014). Oils, a natural product widely distributed in both plants and animals are principally classified as lipids. These lipids are heterogeneous group of substances (biomolecules) which share the property of being relatively water-insoluble (hydrophobic) and soluble in non-polar organic solvents like chloroform, benzene, ether e.t.c (Pamela & Harvey, 1994). Lipids could also be described as fatty acids and their derivatives thus, the term refers to the fatty acids, sterols and similar chemicals often found in the oils produced from living things, (Wikipedia), thus referred to as organic oils. Elaborate studies on tiger nut oil constituent and environmentally friendly or cost effective extraction methods need to be explored in other to expose the economic potentials of this oil and by extension tiger nut expanded use.

Lipid extraction from oil- bearing materials can either by mechanical method or solvent extraction. Mechanical process of oil extraction is as old as history and usually result in achieving less, 6% -14% residual oil. On the other hand , solvent extraction methods recovery has about 0.5- 0.7 % residual oil in the raw material (http://www.oilgae.com/ref/glos/hexane solvent oil extraction. html). N-hexane and petroleum ether are low boiling solvents [$(67/157)^{O}$ C for n-hexane and $(60/140)^{O}$ C for petroleum ether]. Both are non-polar solvents high in solubility of oils and fat and have wide application as commercial techniques in recovering oil from oilseeds.

Quality of oils is usually classified by its iodine value which is a measure of unsaturation; free fatty acid- a measure of free acid content; saponification value; peroxide value- a measure of the amount of oxygen

chemically bound to an oil/ fat[ISO 3960:2007]; Ester value- the relative amount of ester present in an oil/fat; Moisture value and percentage impurity. Extraction of tiger nut oil and evaluating the oil quality is an under researched area, considered a widow for exposing an expanded utilization potentials of this underutilised tuber.

2 Material and Methods
2.1 Sample preparation
Fresh tubers were purchased from a Hausa hawker residing in Ogoja, Cross River State, Nigeria. They were washed with water to eliminate adhering soil particles, foreign materials and bad nuts that may affect quality of oil extract. Then, 5 kg was sun-dried for 60 days to remove the humidity and finally oven dried at $70\,^{O}C$ for 1hour within which the weight was constant. The dried nuts were ground into flour and stored at less than $25\,^{O}C$ in a refrigerator.

2.2 Tiger nut oil Extraction using Petroleum Ether and n-Hexane
5 portions of 400g powdered sample each were used for each extraction per solvent using a soxhlet extractor, 500ml volumetric flash containing the solvent , heated with an electronic meter at $70\,^{O}C$ for hexane and $60\,^{O}C$ for petroleum ether. Oil/solvent extracts were distilled off using, distillation apparatus at $75\,^{O}C$ for n-hexane and $65\,^{O}C$ for petroleum ether.

% Composition = $\dfrac{X}{Y}$ x 100

Where

X= mean value

Y= weight of sample

2.3 Determination for fatty acid distribution of Oil
Fatty acid composition of oil was determined using Gas chromatography and distribution pattern by fatty acid methyl esters (FAMEs) method, AOAC 1991. 5g oil sample was heated with 5ml methanol using a magnetic stirrer on a hot plate at $55\,^{O}C$ for 5 minutes, allowed to cool and filtered. 1ml filtrate injected into the FID pod of the Hewlett Packcard ' mass selective detector' GC (Model 68779 USA) and allowed run for 15 minutes.

2.4 Saponification value determination
The oil's saponification value was determined by ASTM D1962 Specification.   Into three 250 ml ground neck Erlenmeyer flasks was added 25ml 0.5N alcoholic KOH, 5ml deionised (DI) water and boiling stones . And 2g tiger nut oil put in 2 of the 3 flasks. The third served as the blank. A condenser loop was inserted inside each of the flasks and refluxed on a hot plate for 3 hours to complete saponification, thereafter allowed to cool to $60\,^{O}C$. The condenser, ground glass and flasks neck were rinsed with 5ml DI water each . Contents of each of the 3 flasks were then titrated with 0.5 N H_2SO_4 using 0.5 ml phenolphthalein indicator. At end point the pink solution became colourless. Saponification value (SV) calculated as,

SV = $\dfrac{[\,(\,B\text{-}A)\,N \times 56.1]}{C}$

A = ml of H_2SO_4 required for titration of sample

B = ml of H_2SO_4 required for titration of blank

N = normality of H_2SO_4

C = Weight (g) of sample used

2.5 Determination of iodine value
Iodine value determination was by Wijs method (Sharma, 2006). Into a 250 ml stopper flask was added 0.1 g oil extract, 10 ml CCl_4 , 25 ml Wijs solution and swirling the flask. This was allowed to stand (incubation) for 30 minutes at $27\,^{O}C$. Thereafter, 20 ml KI solution followed by 100 ml distilled water were added to the flask. Obtained solution was gradually titrated with 0.1 N sodium thiosulphate $Na_2S_2O_2$ solution which gave yellow coloration until the colour disappears. Then , 1 ml starch solution indicator was further added and this gave a blue colour. Titration continued until sudden disappearance of blue colour. A reaction blank containing all ingredients except the oil sample ran simultaneously.

Iodine value IV = $\dfrac{[\,(\,B\text{-}S) \times N \times 12.692]}{W}$

B = ml of $Na_2S_2O_2$ required by blank titration

S = ml of $Na_2S_2O_2$ required by sample titration

N = normality of $Na_2S_2O_2$

W = weight (g) of sample.

2. 6. Determination of peroxide value

Peroxide value was obtained by AOCS iodometric official method Cd 8-53 (Crowe and White, 2011). Unto 5g oil sample weighed in a 250ml glass stoppered Erlenmeyer was added 12ml acetic acid- chloroform solution. The flask was carefully warmed and swirled until the oil sample was completely dissolved. With a 1ml Mohr pipette, 0.5ml saturated potassium iodide(KI) solution was added. The stopper flask swirled for 1 minute, 20ml distilled water added and shaken vigorously to liberate iodine from the chloroform layer. Resulting solution was slowly titrated with 0.1N sodium thiosulphate $Na_2S_2O_3$ solution until colour change to amber, then 1ml starch solution further added as indicator. Titration continued until disappearance of blue gray colour. A blank titration also conducted and peroxide value of oil determined using the formula below.

Peroxide Value, $PV = \dfrac{[(S-B) \times N \times 1000]}{W}$ (mEq/kg)

Where,

       S= ml of $Na_2S_2O_3$ for titration of sample

        B= ml of $Na_2S_2O_3$ for titration of blank

      N = normality of $Na_2S_2O_3$

      W = weight (g) of sample used.

2. 7. Determination of free fatty acid value

Determination of free fatty acid was by International Standard FIL-IDF 6A: 1969 Method. Into 250ml conical flask containing 50ml 95% ethanol previously neutralised with 0.5ml 0.1N phenolphthalein was added 5g oil sample. Flask was heated on a hot plate to boiling stage and shaken vigorously to liberate the free fatty acid present in the oil. Resulting solution was cooled and then titrated with 0.1N alcoholic KOH solution using phenolphthalein indicator. Free fatty acid content expressed as acid value:

          Acid Value, $AV = \dfrac{Y \times N \times 5.61}{W}$

Where,

       Y = ml of KOH solution used for titration

       N = normality of KOH solution

       W = weight(g) of test sample.

2.8 Determination of unsaponifiable fatty matter

Unsaponifiable fatty matter of tiger nut oil was determined by AOAC Method 933.08(2000) as adopted by FSSAI (2012), Vol. 2. Saponificaton of oil samsple involved boiling under reflux air condenser for an hour of 5gm oil and 50ml alcoholic KOH in a 250ml conical flask. The condenser washed with 10ml ethyl alcohol, saponified mixture while still warm was transferred into a separating funnel. washing the saponification flask with some ethyl alcohol, cold water in the first instance, it was again rinsed with 50ml water, 50ml petroleum ether and shaken vigorously. In the separating funnel, lower soap layer was transferred into another separating funnel. The ether extraction repeated 3 times to completely remove unsaponifiable matter and combined ether extract also washed 3 times with 25ml aqueous alcohol then water to ensure ether extract was free of alkali. Ether solution in separator transferred into 250ml beaker and separating funnel rinsed with ether, on a hotplate, beaker content reduced to about 5ml. Quantity was pipetted into a 50ml previously dried and weighed 50ml Erlenmeyer flask. While heating, the ether evaporated and 2ml acetone added. The solvent was removed under gentle air and finally dried at $100\ ^O C$ for 30 minutes to remove all traces of ether to obtain a constant weight. Residue was thereafter dissolved in 50ml warm ethanol (which had been previously neutralised to phenolphthalein end point) and titrated with 0.02N NaOH solution. Weight (g) of free fatty acid in the extract=0.285VN. Where ,

   V = volume in ml of standard NaOH solution and N = Normality of standard NaOH solution. The unsaponifiable matter was then determined as follows :

      Unsaponifiable fatty matter $= \dfrac{(A-B) \times 100}{W}$

Where,

      A = Weight (g) of the residue

      B = Weight (g) of the free fatty acids in the extract

      C = Weight (g) of sample

2.9 Determination of total fatty matter

Total fatty matter (TFM) was determined after Chloroform- Methanol extraction using simplified Gravimetric method. Into 5g tiger nut oil in a polypropylene centrifuge bottle was added sodium acetate and the volume made up to 30ml. Aliquots of methanol and chloroform were added, bottle capped and shaken for 1 hour. Another aliquot of chloroform added and bottle again shaken for 30 minutes, after which aliquot of water was added and shaken for another 30 minutes. A centrifuge tube, dried and weighed, holding 20ml aliquot of chloroform layer was centrifuged at 1800 rpm for 10 minutes, allowed to set in 25° C water bath for 15 minutes. The sample was then evaporated to dryness under a nitrogen blanket, dried in an oven for 30 minutes and cooled in a desiccator for another 30 minutes and weighed.

$$\text{TFM (g/100)} = \frac{(W_2 - W_1) \times V_c \times 100}{V_A \times S_w}$$

Where,

W_2 = Weight of glass tube and dried extract(g)

W_1 = Weight of empty dried glass tube (g)

V_C = Total volume of chloroform (ml)

V_A = Volume of dried extract (ml)

S_w = Weight of oil sample assayed (g)

2. 10. Determination of ester value

Tigernut Ester Value is determined as the difference between saponification value and acid value.

Ester Value, EV = [(Saponification) value – (Acid) value]

2.11 Determination of moisture value

Moisture content of tigernut oil was determined by the AOAC Official method 933.08 (2000) as adopted by the FSSAI (2012), Vol.2. Into a dried, tared and weighed moisture dish was added 5g tiger nut oil. This was heated in an oven(Memmert, Germany) at 105^OC for 1 hour, cooled in a desiccator containing phosphorus peroxide and weighed. This was repeated until a constant weight obtained.

$$\% \text{ Moisture} = \frac{\text{Loss in mass on drying}}{\text{Weight of test sample}} \times 100$$

$$= \frac{M_s - M_h}{M_s - M_t}$$

Where,

M_s = Weight of moisture dish + Sample (g)

M_h = Weight of moisture dish +sample after heating(g)

M_t = Weight of Tare/moisture dish(g)

2. 12. Determination of % impurity in tiger nut oil

10g tigernut oil in already weighed 250ml Erlenmeyer flash was added 100ml light petroleum ether, stirred vigorously and allowed to stand for 30 minutes. Obtained solution filtered using Wattman no 4 paper and residue washed with 50ml ether to remove all oil. The filter paper with residue dried at 105° C until a constant weight. Amount of impurities present in the oil increased weight of filter paper. Percent impurities is expressed as :

$$\% \text{ impurities} = \frac{(W_R - W_P)}{W_S} \times 100$$

Where,

W_R = weight in g of dried residue +filter paper

W_P = weight in g of filter paper

W_S = weight in g of test sample

3. Discussion

Conventionally, tiger nut oil production involves slowly squeezing the flour with big presses, making it sweat in a first cold extraction of the oil and then, filtered with reinforced filters. This production method gives oil quality that has been highly recommended for cosmetic, massages, bio-diesel and culinary use. Currently known of tiger nut oil is that, it is a stable oil, high in monounsaturated fatty acids and sold as cold pressed oils (Ezeh et al 2014). A report on analysis of oil obtained through cold press method gave acidity 0.40; acidity index 1.10; peroxide value 3.80 meg O_2/kg; saponification index 192,50 and 15% yield. A fatty acid profile of miristic acid 0.08 : palmitic acid 15.50 : oleic acid 68.83 : linoleic acid 12.70 . www.tigernut.com These values compare closely with those for tiger nut oil obtained through solvent extraction (Fig 2 and Fig 3). Other attributes of cold

pressed tiger nut oil are that it remained in a uniform liquid form at refrigeration temperature, has high oleic acid content, low polyunsaturated fatty acids, low acidity, high oxidative stability due to presence of polyunsaturated fatty acids (Osagie et al, 1986; Okladnikov et al, 1977; Ezebor et al ,2005) . Solvent extracted tiger nut oil had total unsaturated fatty acids more than the total saturated fatty acids (Fig 4 and Fig 5) and monounsaturated fatty acids were more abundant than the poly-unsaturated fatty acids in the case under consideration(Fig 6). The implication being comparable oil quality based on fatty acid profile despite extraction procedure. Research reports also show that oils with high saponification values have corresponding large proportion of lower fatty acids(Sharma, 2006; Ekeanyanwu & Onogogbu 2010). This position agrees with result obtained from analysis of oil quality obtained using solvent extractions (Fig5 and Fig 7). Furthermore, oil extraction from tiger nut using supercritical carbon dioxide has also been reported (Ola and Sambo 2012). The highest yield was 26.28g/100g after 210minutes of extraction i.e 26.28%. With solvent extraction, the highest oil yield was 19.09cm^3 /80g (23.86%) Fig 1. On the other hand,   the supercritical carbon dioxide extraction indicated marked variation in fatty acid composition and viscosity of oil when compared with established values from cold press extraction oil. On the contrary, solvent extraction compared favourably in extraction efficiency with supercritical carbon dioxide extraction and the oil obtained also showed no marked difference in quality from that of conventional cold press method.

4. Conclusion

Efficiency of oil extraction was higher for n-hexane compared to petroleum ether and quality of extracted oil based on chemical parameters showed suitability for human nutrition as it was graded an w-6/w-9 oil. Large scale production of tiger nut oil using solvent extraction would give same oil quality as the conventional cold press oil and extraction efficiency comparable with supercritical carbon dioxide. Thus, a suitable alternative for exploiting mass production of tiger nut oil rather than importation of foreign ones which drains the national economy of Nigeria. Finally, food studies that expose high quality of underexploited foods is a window for negotiating extinction and underutilization of indigenous crops in rural farming systems.

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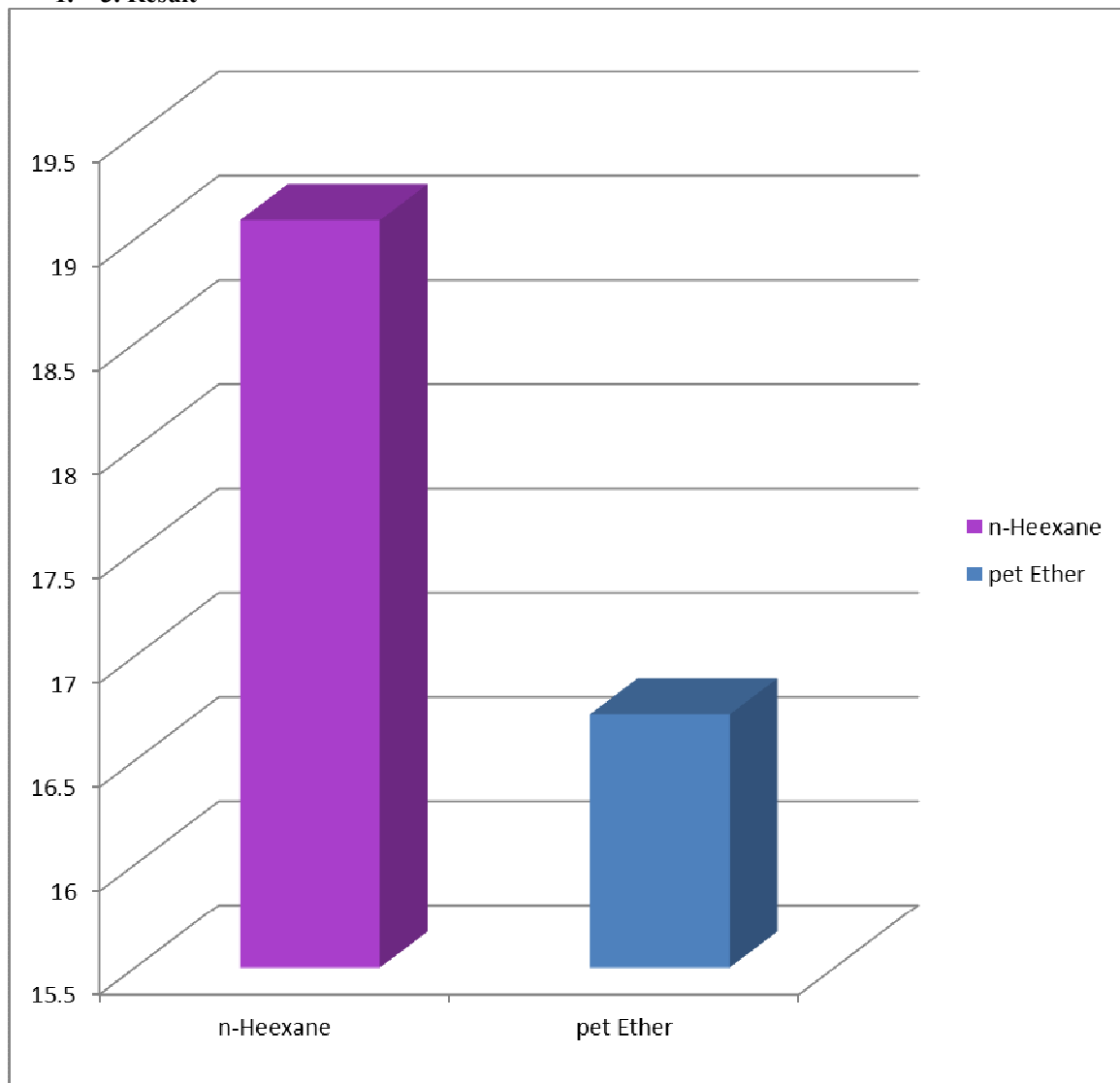Tigernuts Traders S. L. www.tigernut.com Profile of tigernut oil.

1.   3. Result

Figure 1: Extraction Efficiency of n-Hexane & Pet Ether.
The Ratio of Efficiency of n-Hexane Extracted Oil to Petroleum Extracted Oil gave 11:10. Values Are Express As Mean $\pm$ SEM, n=5
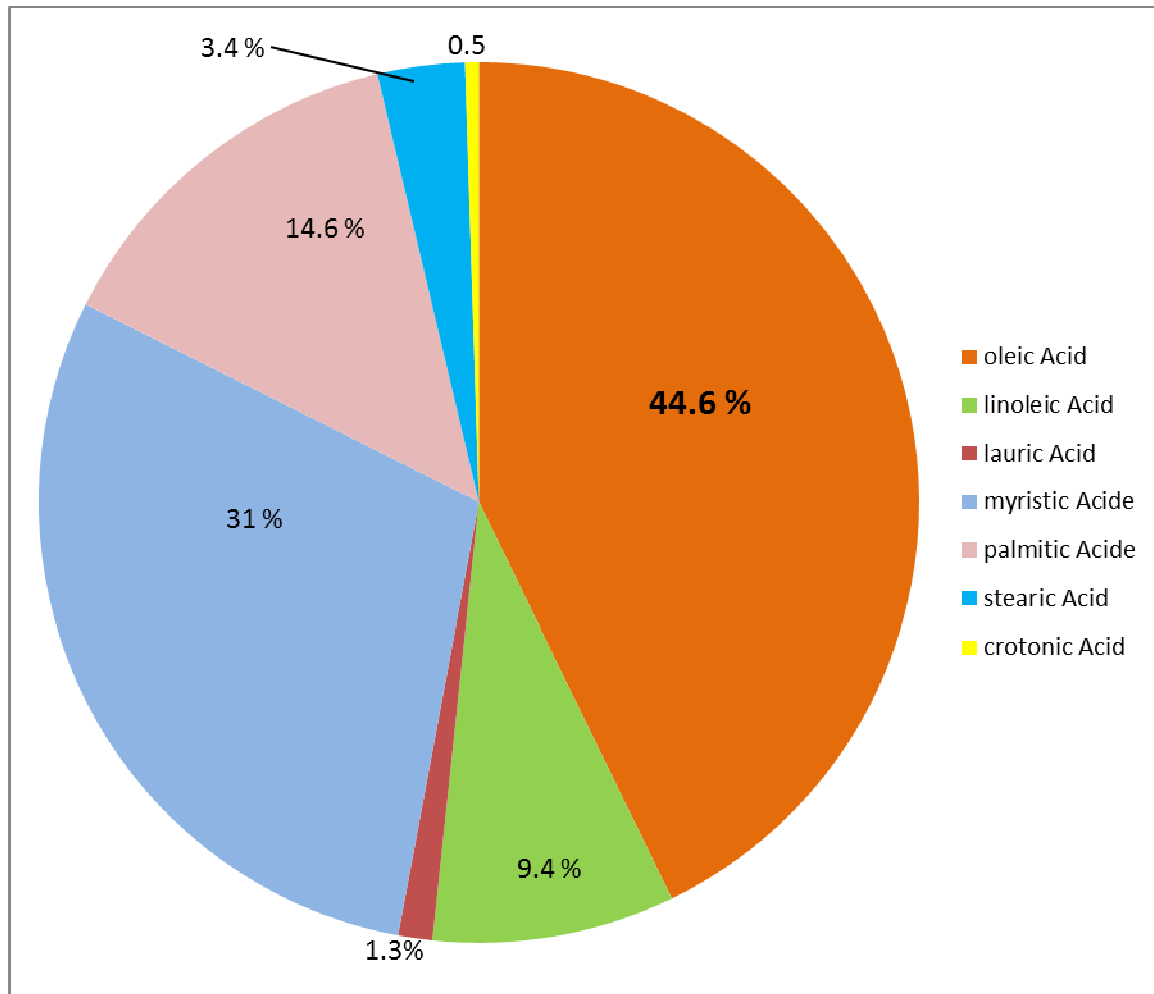
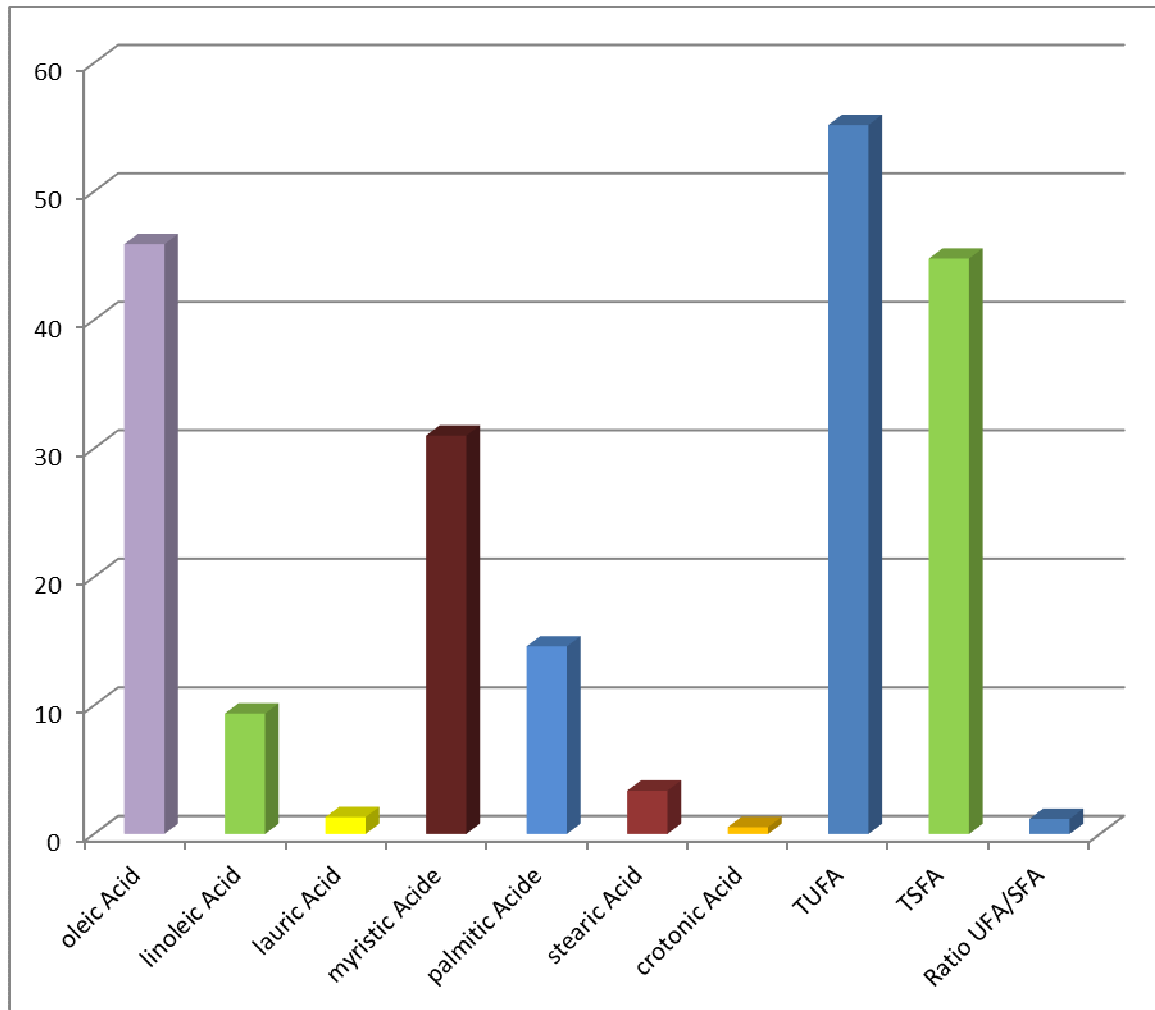**Figure 2: Fatty Acid Distribution of tiger nut oil, Expressed in Abundance (%), as mean $\pm$ SEM, n=3**

Figure 3: fatty Acid distribution of Tiger nut oil including the Total Unsaturated fatty Acids (TUFAs), the Total Saturated Fatty Acids (USFAs) to saturate Fatty Acid (SFAs). Values are expressed as mean $\pm$ SEM, n=3.
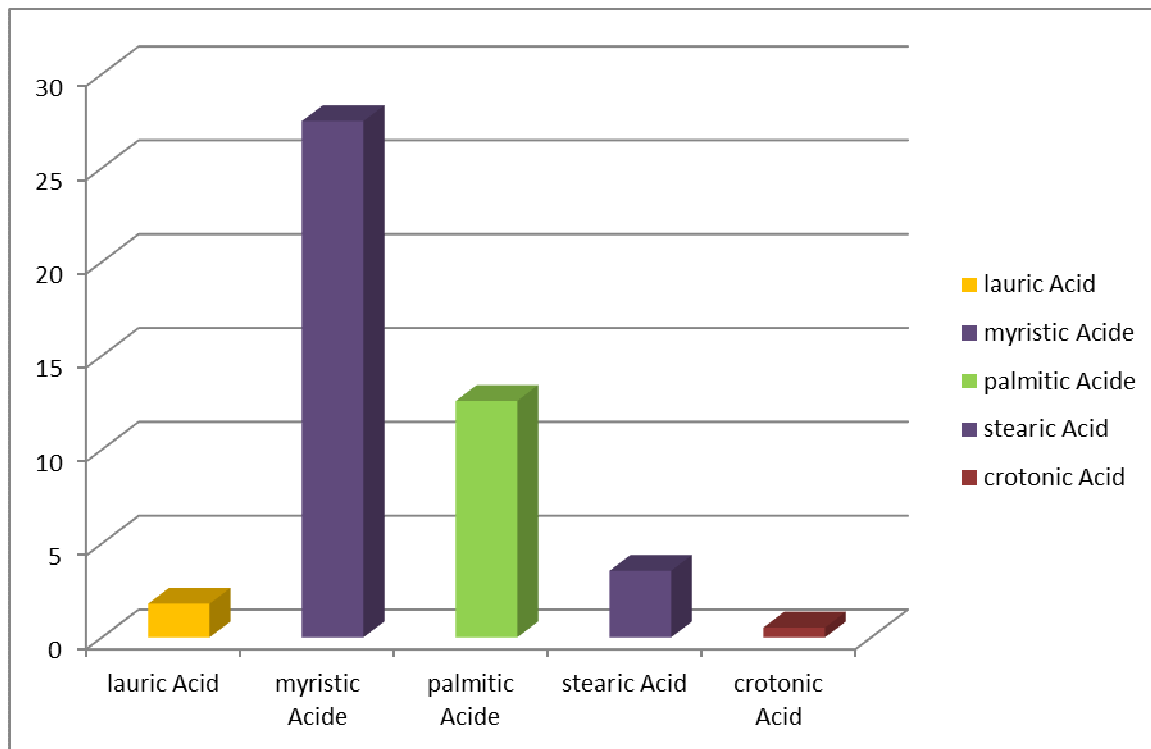
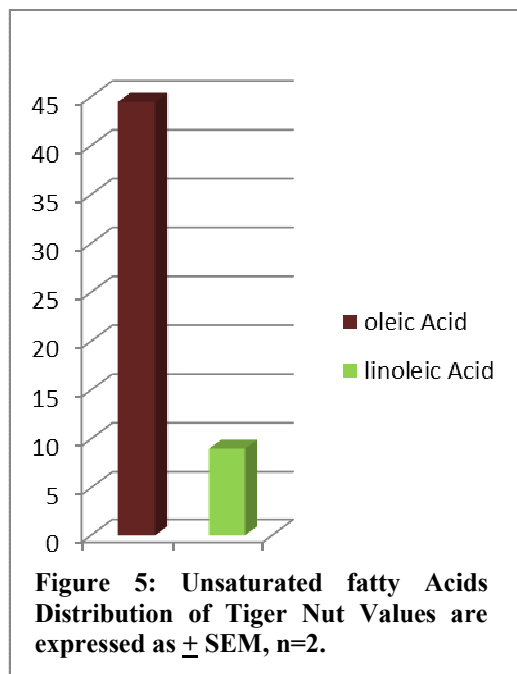**Figure 4: saturated fatty Acid distribution of Tiger nut oil. Values are expressed as mean $\pm$ SEM, n=3.**

Figure 5: Unsaturated fatty Acids Distribution of Tiger Nut Values are expressed as $\pm$ SEM, n=2.
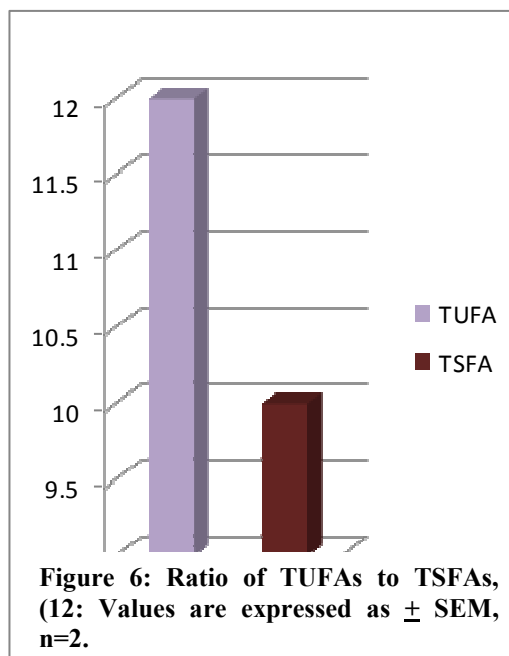

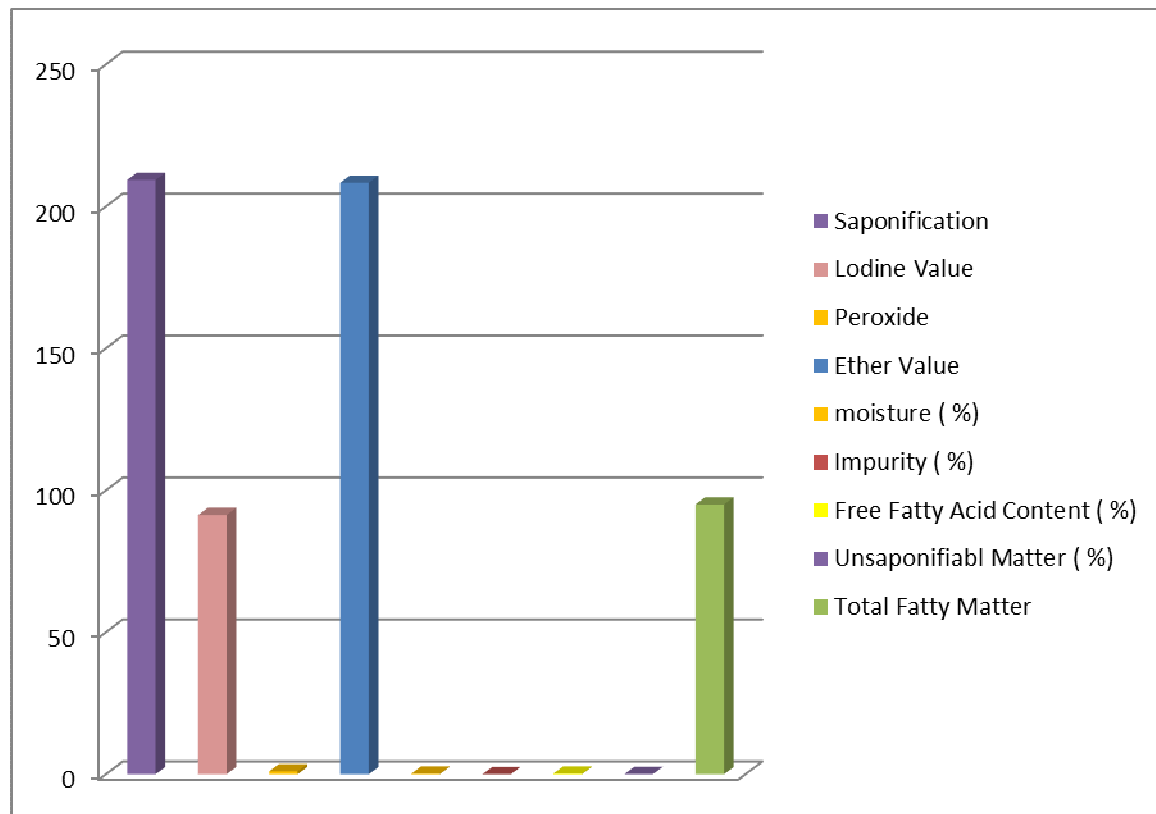**Figure 6: Ratio of TUFAs to TSFAs, (12: Values are expressed as $\pm$ SEM, n=2.**

Figure 7: Physicochemical Properties/Quality of Tiger Nut oil Value are express as mean $\pm$ SEM