# IDENTIFICATION OF FATTY ACID ESTERS OF COCONUT (*Cocos nucifera*) OIL FROM KUMO METROPOLIS OF GOMBE STATE NIGERIA

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**Abstract:** Coconut (Cocos nucifera) oil was extracted with nHexane using Soxhlet apparatus. The Oil recovery was 51.60%. The oil was having a Pale-yellow coloration at room temperature, with a pleasant smell, with a temperature of $25^0$C, Specific Gravity of 0.917, moisture content of 0.2 and refractive index of 1.4458 respectively. The FTIR analysis shows absorption around 2922 $cm^{-1}$ which indicates the C-H stretch for alkyl. The absorption around 1746 $cm^{-1}$ indicates the presence of C=O for an aliphatic hydrocarbon. Two absorption bands around 1230 $cm^{-1}$ and 1159 $cm^{-1}$ indicate the C-O stretch. The absorption around 3600 – 2550 $cm^{-1}$ shows the presence of O-H. From the information obtained from the spectrum which shows that the functional groups present is that of esters (Aliphatic). The GCMS identified; 9-Octadecene and Dodecane, Pentadecanoic acid, Hexadecenoic acid, 10,13-Octadecadienoic acid, 9,12-Octadecadienoic acid, trans-13-Octadecenoic acid, 11-Octadecenoic acid, 12-Octadecenoic acid, cis-13-Eicosenoic acid, cis-11-Eicosenoic acid and 11-Eicosenoic acid.

**Key words:** Coconut, Virgin coconut oil (VCO), fatty acid, Extraction

## Introduction

Coconut (Cocos nucifera) oil that is extracted from fresh coconut flesh is known as virgin coconut oil (VCO). The extraction involves a process that does not use thermal treatment or food preservatives. Coconut oil obtained from copra, dried coconut, has no taste or fragrance, due to the refining process, whereas, VCO has the fragrance and taste of coconut (Zuknik et al., 2016). The absence of heating and chemical treatment in the oil makes it tasty and healthy. The antioxidant activity of VCO is superior to that of regular coconut oil, which is extracted from copra, and also of groundnut oil (Nevin and Rajamohan, 2005). The existing production process of VCO is basically conducted through oil separation from coconut. Coconut milk can be obtained by either pressing of fresh coconut flesh without additional water or grating the coconut flesh followed by extraction of the water-oil emulsion with water. The oil can be separated from the emulsion by means of fermentation, enzymatic extraction, refrigeration or mechanical centrifuge (Marina et al., 2009). Separation of the oil from the water-oil emulsion can also be accomplished by breaking the emulsion and creating an oil-oil emulsion, in which pure coconut oil must be added to the coconut milk to extract the oil from the emulsion, and then the oil must be separated from the water and protein with decantation. The process requires 24 to 48 hours and produces an oil yield of about 40% of the oil available in the coconut (Sukartin and Sitanggang, 2005).

Coconut oil is an edible vegetable oil derived from the fruit of the Cocos nucifera L. tree belongs to the Aracaceae family. Copra is the source of oil, the dried coconut meat (endosperm) part that contains about 65-75%. Coconut oil has a natural sweet taste and carries high percentage of saturated fatty acids in the type of triglycerides (90%). In addition, it is composed for medium chain fatty acid (approximately 60% of total composition). Eighty percent of the world production of coconut oil is used for food, whereas approximately 14% is for nonfood uses as pharmaceuticals and cosmetics like insect repellant and skin moisturizer, as well effective against viruses (Pizzo, 2019). The fatty acid present in the coconut oil is responsible for the antiplaque, antiprotozoal, healing, antiobesity effects. Medium chain fatty acid reduces the threat of atherosclerosis and to supply energy for metabolism without increase the blood sugar level (Calder, 2012). Coconut oil got prevalence as of late after the roles of the medium chain fatty acids have been uncovered. Medium chain fatty acids have noteworthy job in human wellbeing as antibiotics, particularly as antiviral and source of quick vitality without upsetting the glucose in the body (MacDonald et al., 2018). Coconut oil contains a large proportion of lauric acid a saturated fat that arises total blood cholesterol levels by increasing both the amount of high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol (Rong et al., 2022). Although this may create a more favorable total blood cholesterol profile this does not exclude the possibility that persistent consumption of coconut oil may increase the risk of cardiovascular disease through other mechanisms, particularly via the marked increase of blood cholesterol induced by lauric acid (Nomikos et al., 2023). Because the majority of saturated fat in coconut oil is lauric acid, coconut oil may be preferred over partially hydrogenated vegetable oil when solid fats are used in the diet. Due to its high content of saturated fat with corresponding high caloric burden, regular use of coconut oil in food preparation may promote weight gain. Various fractions of coconut oil are used as drugs. Butyric acid is used to treat cancer, while lauric acid is effective in treating viral infections (Famurewa et al., 2017).

Cocos *Nucifera* (Coconut) Oil and its derivatives, Coconut Acid, Hydrogenated Coconut Acid, and Hydrogenated

Coconut Oil, are used by industry as a convenient source of lower chain length fatty acids. A safety assessment for these ingredients was published in 1986 with the conclusion from the Cosmetic Ingredient Review (CIR) Expert Panel that these ingredients are "safe for use as cosmetic ingredients" (Elder 1986). Coconut oil is edible oil that has been consumed in tropical countries for thousands of years. As it has a long shelf life and a melting point of 76 °F, it is used in baking industries (Canja et. al, 2015). A negative campaign against saturated fats in general, and the tropical oils in particular, led to most food manufacturers abandoning coconut oil in recent years in favor of hydrogenated polyunsaturated oils, particularly soy, which contain trans fatty acids. Studies done on populations consuming diets high in coconut oil show no adverse effects on the health of the population (Deen et al., 2021).

Coconut oil has >90% saturated fatty acids, hence is less attractive to consumers. Saturated fat is one that has no unsaturation or double bonds and tends to be solid at room temperature. Coconut oil is rich in short and medium chain fatty acids. Shorter chain length allows fatty acids to be metabolized without use of the carnitine transport system (Folayan et al., 2019). Various fractions of coconut oil are used as drugs. Butyric acid is used to treat cancer, while lauric acid is effective in treating viral infections (Famurewa et al., 2017). Hence, the literature is reviewed in the context of increase of fat related disorders diseases through consumption of highly unsaturated oils.

**Materials and Methods**

**Table 1: Materials used for the Study.**

| S. No | Instruments | Manufactured by |
|---|---|---|
| 1 | Gas chromatograph* | Perkin Elmer |
| 2 | Analytical weighing balance | Shimadzu |
| 3 | Hot air oven | Narang scientific works |
| 4 | Probe sonicator | Sonics Vibra cell |
| 5 | Electric sonicater | Sonica |
| 6 | Abbe refractometer | |

*Perkin Elmer-Clarus 590 Gas chromatograph with Elite-5 capillary column, (5 % phenyl, 95% dimethyl polysiloxane) 30 m x 0.25 mm, 0.25 µm and Flame ionization detector.

**List of solvents and chemicals used in the study include;**

- Hexane
- Methanol
- Ethanol
- Ether
- Conc. HCl
- Sodium hydroxide
- Potassium Hydroxide
- Iodine
- Phenolphthalein
- Sodium thiosulphate
- Carbon tetra chlorid**Methodology**

***Extraction of Coconut Oil***

***Extraction of coconut oil using Hexane***

25g of coconut flakes taken in a 250 ml beaker and 100 ml of hexane was added to it. The mixture was sonicated for 20 minutes in a probe sonicator. After optimization, the amplitude of sonication set to 70% and the time interval set to 10:10. The temperature was maintained at 35 ± 1°C. After sonication, the solvent layer was filtered out using Whatmann's filter paper. The coconut oil was then collected by natural evaporation method. This procedure was repeated for 10 times and 70% yield was calculated for 250g of coconut flakes (Aytac 2022).

***Physical Examination***

***Determination of Colour***

Colour of the samples was determined by visual comparison method.10mls of each water sample was poured into beaker and carefully viewed for any alteration in color (Mansor et al., 2012).

***Determination of Temperature***

Temperatures of the sample were taken at the point of sampling with the use of mercury in glass thermometer. Thermometer was immersed in the oil sample up to the mark specified by the manufacturer and read directly from the thermometer (Mansor et al., 2012).

***Determination of pH***

pH of the oil was measured using pH meter, the pH meter was calibrated by adjusting the reading to match the pH of the buffer solution (buffer 7 solution) neutral, which is in accordance with the applicable general procedure and standard. After which 10 ml of oil sample was poured into a beaker and the pH probe inserted into the sample and the reading taken directly from the meter screen (Mansor et al., 2012).

***Specific gravity***

Dry specific gravity bottle was filled with the oil in such a manner to prevent entrapment of air bubbles. Inserted the stopper and weighed the oil at 30°C ± 0.2°C. In a pre-weighed specific gravity bottle, weight of water at 30°C ± 0.2°C was weighed.

$$Specific\ Gavity@\ 30°C\ = \frac{A-B}{C-B}$$

Where, A = Weight of specific gravity bottle with oil (g)

B = Weight of empty specific gravity bottle (g)

C = Weight of specific gravity bottle with water (g)

***Refractive index***

After cleaning the Nicol prisms, few drops of oil was placed on the prism. Closed the prisms and allowed to stand for 1-2 min. Turned on the light source. The eye piece micrometer screw was adjusted to focus the boundary between the bright and dark regions. Refractometer scale adjusted to place the cross wire of the telescope exactly on the boundary between the bright and dark regions. Read the index of refraction using the telescope scale.

***Moisture content***

Weighed in a previously dried and tared dish about 5-10 g of oil which was mixed thoroughly by stirring. Loosed the lid of the dish and heated, in an oven at 105 ± 1°C for 1 hour. The dish was removed from the oven and closed the lid. Cooled the dish in a desiccator containing phosphorus pentoxide or equivalent desiccant and weighed. The oil was heated in the oven for a further period of 1 h, cooled and weighed. Repeated this process until change in weight between two successive observations does not exceed 1 mg.

$$Moisture\ Content = \frac{W1 * 100}{W}$$

Where, W1 = Loss in weight of the material on drying (g) W = Weight of coconut oil used for the analysis (g)

**Table 2: Physical parameters and specification for coconut oil**

| Parameters | Specification as per FSSAI / IS 542 |
|---|---|
| Specific gravity | 0.915-0.920 |
| Refractive index | 1.4480-1.4490 |
| Moisture content | Maximum 1% |

***Extraction Of Fatty Acids***

***Base catalyzed derivatization method***

Fifty mg of coconut oil was weighed in a screwed cap test tubes (10 mL) and 400 uL of 5M methanolic NaOH was added. The mixture was then heated at 50°C for 20 seconds. One mL of hexane was added to the mixture and vortexed for 30 seconds. After settling, the top layer was collected in a clean tube and to that solution 400 µL of (2 N) methanolic HCl was added which was then mixed using vortex. The same top layer was used for the chromatogram analysis Destaillats and Angers (2002).

***The Analysis of Fatty Acid Using Gas Chromatography-Mass Spectroscopy (Gcms)***

***Optimization of chromatographic conditions Selection of column***

The selection of the proper capillary column should be based on four significant factors which are stationary phase, column internal diameter, film thickness, and column length. The differences in the chemical and physical properties of injected organic compounds and their interactions with stationary phase are the basis of separation process. When strength of the analyte-phase interactions differs significantly for two compounds, one is retained longer than the other. 30 metres capillary column (Internal diameter 0.25 mm, 0.25 µm) coated internally with 5 % phenyl, 95% dimethyl polysiloxane.

***Selection of initial separation condition***

Different chromatographic conditions are applied for the optimization. Particularly by changing the stationary phase, mobile phase, column oven temperature initial oven programming.

**Table 3: Selection of initial separation condition**

| **Rate (**°C /min) | **Temperature (**°C) | **Hold (Min)** |
|---|---|---|
| | 50 | 1 |
| 8 | 240°C | 1 |
| **Rate (**°C /min) | **Temperature (**°C) | **Hold (Min)** |
| | 50 | 1 |
| 5 | 140 | 1 |
| 10 | 270 | 1 |

***Selection of injection volume***

The reproducibility of the amount of sample injected is important to ensure the reproducibility of results. A sample can be injected manually into the system or by using an auto sampler system. Derivatized Coconut oil was injected manually into the injection port. 1µl of sample injected

***Selection of split mode***

The temperature of injector is used to rapidly vaporize the liquid sample into gaseous phase that can be carried to the column for separation. In capillary gas chromatography, there are four primary techniques for vaporizing a sample and transferring it onto the inlet of the analytical column: split, split less, direct, and on-column injections. Of these, split and split less injections are the most commonly used techniques. Split Injector was selected for analysis of sample with high concentration levels. In the split injection mode, only a fraction of the vaporized sample is transferred onto the head of the column. The remainder of the vaporized sample is removed from the injection port via the split vent line. Split injections should be used only when sample concentrations are high enough to allow a portion of the sample to be discarded during the injection process, while still maintaining a sufficient concentration of analytes at the detector to produce a signal.

***Splitless mode***
***Selection of oven temperature***

The column resides in an oven, and temperature, which greatly affects the effectiveness of the chromatographic separation. In many cases, isothermal is not the most effective temperature mode for sample separation; in such cases, a temperature program can be used. Most GC temperature program has initial temperature, a ramp (degree increase per minute) and a final temperature. Using a linear temperature program as a starting point if previous analysis information is not available to use as a guide, the first program development step is to try a simple, linear temperature program. To improve the resolution of earlier eluting peaks, decrease the initial temperature or increase the initial hold time. Decreasing the initial temperature usually results in the largest resolution improvement, but analysis times are substantially increased. The resolution of peaks eluting in the middle of the chromatogram can be altered by change in ramp rate. If there is excessive peak resolution, the ramp rate can be increased to reduce resolution and the analysis time. If there is insufficient resolution, decrease the ramp rate, but there will be an increase in the analysis time. Better resolution of later eluting peaks often occurs when decreasing the ramp rate. Another option to alter resolution of peaks in the middle of a chromatogram is to use a mid-ramp hold. A mid ramp hold is a several minute isothermal portion somewhere during a temperature ramp.

**Table 4: Selection of oven temperature**

| **Rate (**°C /min) | **Temperature (**°C) | **Hold (Min)** |
|---|---|---|
| | 50 | 1 |
| 10 | 140 | 1 |
| 10 | 270 | 1 |

***Selection of carrier gas***

Nitrogen, Hydrogen, Helium and Argon are the gases used as mobile phase in GC. Each carrier gas has its benefits and selection is based on the detectors sued and the nature of the sample to be analyzed. The gas used as mobile phase in gas chromatography is should have the following requirements

- Inert
- Dry
- Free from oxygen
- Safe
- Less expensive
- Freely Available

**Nitrogen gas** was used as mobile phase for the analysis of coconut oil.

Selection of detector

The detector used should have the following desirable features

- High sensitivity
- Reproducibility of response to changes in composition of eluting gas
- Large linearity of response
- Less peak broadening
- Non-destructive

Detector temperatures and the relative flow rates of carrier gas, hydrogen and air into the detector are the key operating parameters. A series of standards is defined for evaluation of detector parameters such as drift, noise, sensitivity, linear range, dynamic range etc. The variation in detector response with flow rate depends on whether the detector is concentration or mass flow dependent. For concentration dependent detectors a decrease in the flowrate does not affect the peak height, which remains approximately constant.

In this study, Flame ionization detector is used for the analysis coconut oil, since it responds to compounds that contain carbon and hydrogen.

**Result and Discussion**

**Result**

The results of the extraction carried out on the Cocos nucifera is represented on Table 5, which indicates the percentage oil recovery, colour, texture, odour, solvent used and other properties.

**Table 5: Some Characteristics of *Cocos nucifera* oil extracted**

| **CHARACTERISTICS** | **REMARKS** |
|---|---|
| Solvent used for extraction | Hexane |
| Odour | Pleasant smell |
| Colour at room at temperature | Pale yellow |
| Cake texture | Coarse powder |
| Cake Colour | Whitish brown |
| Oil yield | 51.60% |
| Temperature | $25^0$C |
| Specific Gravity | 0.917 |
| Moisture Content | 0.2 |
| Refractive index | 1.4482 |

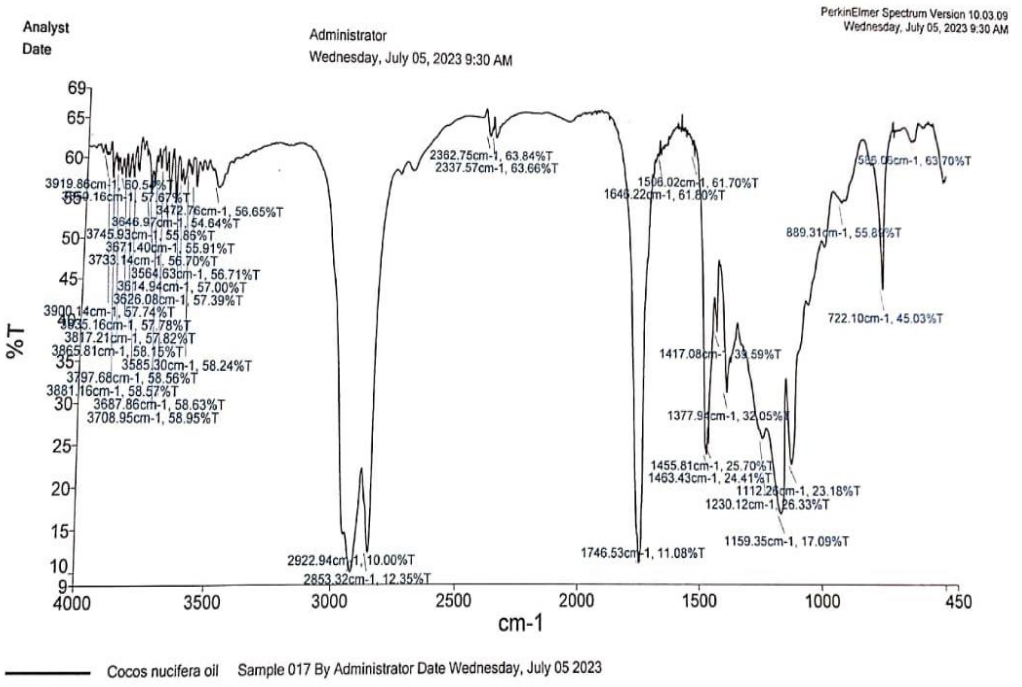


**Figure 1**: FTIR Analysis Spectrum of Cocos nucifera oil

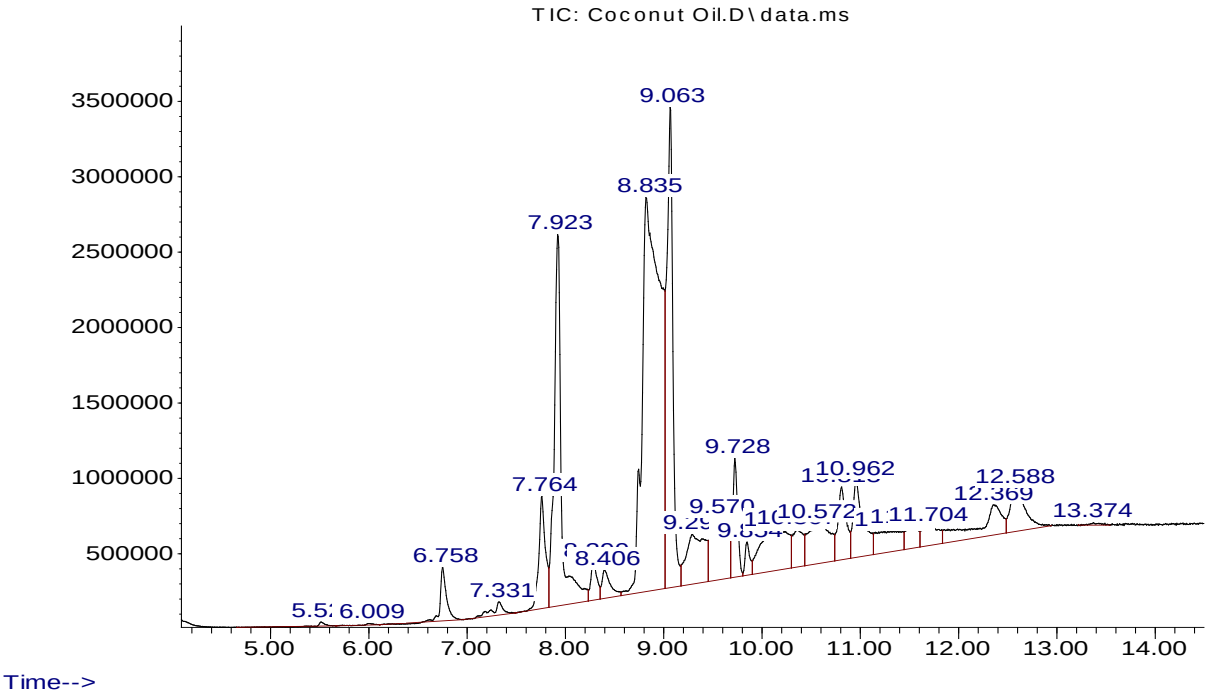


**Figure 2**: GCMS Analysis Spectrum of Cocos nucifera oil

**Discussion**

The FTIR analysis in Figure 1, shows absorption around 2922 $cm^{-1}$ which indicates the C-H stretch for alkyl. The absorption around 1746 $cm^{-1}$ indicates the presence of C=O for an aliphatic hydrocarbon. The two absorption bands around 1230 $cm^{-1}$ and 1159 $cm^{-1}$ indicate the C-O stretch. The absorption around 3600 – 2550 $cm^{-1}$ indicates the presence of O-H. From the information obtained from the spectrum, it shows that the functional group present is esters (Aliphatic).

The GCMS analysis was successfully carried out on *Cocos nucifera* oil as showed in Figure 2 above. From the summary of the GCMS analysis, the following fatty acids identities are proposed based on Chemistry lab library search report**:**

Component 1 with retention time of 6.758 and base peak area of 0.05 may be 9-Octadecene and Dodecane. Component 2 with retention time of 7.764 and base peak area of 0.58 may be Pentadecanoic acid. Component 3 with retention time of7.921 and base peak area of 11.42 may be Hexadeconoic acid. Component 4 with retention time of 8.835 and base peak of 12.90 may be 10,13-Octadecadienoic acid and 9,12-Octadecadienoic acid. Component 5 with retention time of 9.063 and base peak area of 12.90 may be trans-13-Octadecenoic acid, 11-Octadecenoic acid and 12-Octadecenoic acid. Component 6 with retention time of 9.728 and base peak area of 2.59 may be cis-13-Eicosenoic acid, cis-11-Eicosenoic acid and 11-Eicosenoic acid.

**Conclusion**

In Summary, the result of FTIR and that of GCMS spectrums shows and identified the presence of fatty acid esters which include: 9-Octadecene and Dodecane; Pentadecanoic acid; Hexadeconoic acid; 10,13-Octadecadienoic acid and 9,12-Octadecadienoic acid; 10,13-Octadecadienoic acid and 9,12-Octadecadienoic acid; trans-13-Octadecenoic acid, 11-Octadecenoic acid and 12-Octadecenoic acid; cis-13-Eicosenoic acid, cis-11-Eicosenoic acid and 11-Eicosenoic acid respectively.

The oil yield of *Cocos nucifera* (51.60%) is an indication that is highly economical as a good oil source if utilized. Other properties of the oil show that it can be a good substitute in soap production and cosmetics. The oil can also be used pharmaceutical and food industries due to low fatty acid content. Based on the research findings, there is a need to assess the safety of *Cocos nucifera* oil extracted for human consumption. The residual cake obtained after extraction should be utilized and transformed into animal feeds and other important values. Cocos nucifera should be planted in this part of the country which will help fighting deforestation, and it will provide jobs to the unemployed youths of this country.

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