PHYSICO-CHEMICAL AND GC-MS ANALYSIS OF *Gossypium hirsutum (Cotton Seed)* OIL

ABSTRACT

Background and objective: Nutritional value and industrial utilization of cotton seed oil can be derived by having glance at fatty acids profile which carries different carbon chain lengths and degrees of unsaturation. It provides essential amino acids like lipase, phytase, and lecithin. It has prolonged shelf life than other seed oils due to higher amount of natural antioxidants and alpha-tocopherols The aim of this study is carry out physicochemical evaluation of *Gossypium hirsutum* oil and characterized the oil using GC-MS analysis.

Materials and Methods: The cotton seed oil was extracted using soxhlet extraction method. The physicochemical properties such as Acid value, Free Fatty acids content, Iodine value, Saponification value, Peroxide value, Viscosity(40°C), Refractive Index, Moisture content, Specific gravity (30°C), and colour index were determined by standard procedures described by AOAC , while the ester value was determined by baltes method. The oil was characterized using gas chromatography mass spectrophotometry (GC-MS) analysis.

Results:The physicochemical parameters showed that the acid value, free fatty acids content, iodine value, Saponification value, ester value, Peroxide value, viscosity, refractive index, moisture content, Specific gravity of the oil were 0.94±0.020(mgKOH/gOil), 0.34±0.016%, 75.70±0.150gI/100g, 210.9±0.023millieqv/g, 209.4±0.027mgKOH/gOil, 8.82±0.010meq/kg, 4.41±0.113cSt, 1.383±0.003, 0.22±0.010%, 0.915±0.001 respectively. While the the oil was found to be Dark red in color. The GC-MS analysis of cotton seed oil shows the presence of twenty five (25) chemical compound ,in which six (6) where found to have biological activity related to Antibacterial activity, Anticancer Drug, Antiseborrhoeic , Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic Hepatoprotective, Antiandrogenic, Nematicide, Antihistaminic, Cytoprotective activity and Anti-inflammatory this includes 4,8-Diaza-2,9-dibenzoyl-5, 6-diphenyl-2,8-decadienedioic acid tridecan-7-ol, Undecanoic acid, octadecanoic acid, 9,12-Octadecadienoic acid(Z,Z)-, (*E*)-hexadec-7-enal and (Z)-7-Hexadecenoic acid respectively.

Conclusion: The physicochemical parameters of cotton seed oil were found to be within the NAFDAC and Cordex standard, liquids in nature, degree of unsaturated as well slow to oxidation and rancidity. hence suitable for consumption. The GC-MS analysis of cotton seed oil shows the presence of twenty five (25) chemical compounds , in which six (6) where found to have biological activity that can be employed for industrial, foods, additive and other pharmaceutical formulation.

Keywords: Cotton seed, oil, soxhlet, physicochemical, GC-MS

1. Introduction

Nutritional value and industrial utilization of cotton seed oil can be derived by having glance at fatty acids profile which carries different carbon chain lengths and degrees of unsaturation. It is reported that consumption of one tablespoon of cotton seed oil provides 120 calories, 3.5 g saturated fatty acid along with vitamin A, K, and antioxidants [1] . It provides essential amino acids like lipase, phytase, and lecithin. It has prolonged shelf life than other seed oils due to higher amount of natural antioxidants and alpha-tocopherols (35 mg 100 g–1) , which promotes vitamin E activity . It is a good source of phosphoros (1%). It contains the modest level of cyclopropenoid fatty acids (0.5–1%) which are regarded anti-nutritional [2].

Cotton seed oil consists of 65–70% unsaturated fatty acids while saturated fatty acids are 26-35%. Among unsaturated fatty acids, a major proportion (55%) is contributed by linoleic acid followed by oleic acid (15%) and linolenic acid less than 1%. The saturated fatty acids carry palmitic acid (26%) and stearic acid (2%) .In addition to major fatty acids, cotton seed oil also contains little amount of several fatty acids (0.1–1% each) like myristic, lignoceric, arachidic, cis-vaccenic, sterculic, malvalic, palmitoleic, behenic, and α-linolenic[3].

The unsaturated fatty acids are beneficial for health but deep frying for longer period convert them into short chain hydroperoxide, aldehydes, and keto derivatives responsible for off type flavor[4]. Presence of higher percentage of saturated fatty acid (palmitic acids) is responsible for cottonseed oil oxidative stability during frying which compensates the instability of unsaturated fatty acids.Therefore, partial hydrogenation is used to increases oil stability by converting polyunsaturated fatty acids into

monounsaturated and statured fats by keeping the oil in liquid state[3] . Partial hydrogenation has its own side effects particularly produces trans fatty acids which uplift the level of LDL-cholesterol and reduce the HDL-cholesterol in blood serum [5]. Monounsaturated fatty acid (oleic acid) is comparatively stable towards oxidative decomposition at high temperature. High oleic acid containing oils offer improved cooking stability for deep frying and are relatively more resistant to oxidative deterioration. Therefore, it could be increased at the cost of polyunsaturated fatty acids for improving quality. Saturated fatty acids do not cause health risk by themselves but production of trans fatty acids as byproduct during the process of vegetable oils hydrogenation have significant cholesterol-raising properties. Cotton seed oil having elevated levels of palmitic acid is undesirable due to associated health risks [6]. On the other side, enhancement in palmitic acid contents is necessary for oxidative stability of oil to make margarine, shortening, and confectionery products.

Cotton seed which remains after the cotton ginned is used to produce cotton seed oil. The plant has been found to possess several ethno medicinal uses. Cotton seeds feature in traditional medicine; in various forms, they are taken internally and applied externally to treat a range of conditions.

Preparations containing cotton are notably used as a therapy for skin problems and injuries. For headaches, a drink is made from powdered cotton seeds and mixed with milk. Dysentery is also treated with an infusion of seeds. Spots and other skin conditions are treated using cotton seed or extracts from the leaves. In Western medicine, cotton is put to use in the form of dressings, bandages, swabs and cotton wool. Scientific investigations have shown that cotton seeds contain certain compounds that may be beneficial to the health, potentially for treating Cancer and HIV.

These compounds have various activities such as anti-microbial, antibacterial, hemolytic and foaming activity [7]. Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives [8].

The important component of cotton seed oil is tocopherols, natural antioxidants. However, amount of tocopherols that present in oil declines significantly during the refining process. Therefore crude cotton seed oil when compared to refined cotton seed oil and soybean oil is rich in terms of amount of tocopherol and, more resistant to oxidation [9] . Fatty acid composition of cotton seed oil is the one of important properties . Cotton seed oil has a 2:1 ratio of polyunsaturated to saturated fatty acid. It is described as naturally hydrogenated because its fatty acid profile generally consists of 70% unsaturated fatty acids, including 18% mono-unsaturated (oleic) and 52% poly-unsaturated (linoleic), and 26% saturated (primarily palmitic and stearic) acids. These make the oil stable for frying without the need for additional processing or the formation of trans-fatty acids [10]. As with other vegetable oils quality of cotton seed oil usually comes from fatty acid composition and unsaponifiable matters mentioned. Their amount and oil yield varies depending on genotype, ecological conditions of region process and storage conditions [10] . Cotton, from the family *Malvaceae* and the genus *Gossypium*, has a number of species such as *G.hirsutum, G. barbadense, G. arboreum and G. herbaceum* of commercial importance and food value [11]. Many varieties of cotton have been developed with improved crop yield and productivity . *Gossypium hirsutum* being a native to tropical and subtropical regions, is the most important fiber/food crop in the world. It is also considered as one of the best source of plant (vegetable) protein after soybean and the fifth prominent seed oil crop after soybean, palm, canola and sunflower . Cotton is regarded as one of the important conventional oilseed crops with potential to bridge the existing gap between the supply and domestic demand of vegetable oils . Cotton as a major crop covers about 2.5% of the world's cultivated lands and is also recognized to be a dual-purpose crop, used both for its natural fiber [12], as well as contributes to almost 4% of the world's vegetable oil production. The aim of this research is to evaluate the physicochemical property of cotton seed oil and characterized the oil using GC-MS analysis

2.Materials and Methods

2.1 Collection of cotton seeds sample .

Cotton seeds were brought from Dawanau Market in Dawakin Tofa Local Government of Kano State. The seed sample was identified and authenticated at Department of Agricultural Science, Kano University of Science and technology, Wudil with identification NO KUSTAGRIC-CS456. The seeds sample were obtained by removing/ breaking/ external cover mechanically using mortar and pestle. The experiment was carried out at the Department of Food Science and Technology, Kano University of Science and Technology Wudil, Kano.

2.2 Extraction Of Oil From Gossypium Hirsutum Seeds

The seeds were crushed and placed in paper bags. The sample placed in a Pyrex glass Soxhlet extractor, attached with a water condenser and a Pyrex round bottomed flask (500 mL capacity). Extraction was carried out using a water bath with n-hexane as extraction solvent. The crude fat of cotton seed flour was determined using AOAC-2000 method [13] . Ninety grams (90g) of cotton seed flour were weighed using thimble and covered by purified cotton. Then 200 ml of n-hexane as solvent was added. The sample with the solvent was placed in the soxhlet extractor for the cycle was allowed to repeat many times for about 8 hours. After 8 hours the remaining solvent was evaporated using oven dry method and the extracted fat was cooled in a desiccator and weighed.

After the oil extraction, the solvent was removed under vacuum in a rotary evaporator machine (EYELA, N. N. Series fitted with an Aspirator and a Digital Water Bath SB-651, Japan) at 45°C. The solvent (hexane) and oil were separated using distillation at a temperature of slightly higher than the boiling temperature of hexane, which is recovered again for further extraction with fresh hexane. The oil was stored in the Food Science and Technology laboratory room for physico-chemical properties and GC-MS analysis. All the physicochemical analysis was conducted under laboratory condition. The data were recorded on as percentage yield/crude fat(oill contents), acid value and saponification value, Iodine value, refractive index, specific gravity/density, ester value, Peroxide value, free fatty acid, Viscosity and moisture content.

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2.3 Determination of Percentage Yield

$$\text{Percentage yield} = \frac{\text{Net weight of oil(g)} \times 100}{\text{Total weight of ground seed}}$$

2.4 Determination of Acid Value (AV)

The acid value was determined using the method described by Bamgboye and Adejumo [14]. Equal volumes (25 ml) of diethylether and ethanol were mixed together and 1 ml of 1% phenolphthalein indicator solution was added and then neutralized with 0.1 M potassium hydroxide solution.

Procedure

Five grams of oil sample was dissolved in the neutralized solvent mixture and titrated with 0.1 M potassium hydroxide solution with constant shaking until a pink color which persists for 15 seconds is obtained.

$$\text{Acid Value (AV)} = \frac{\text{Titrate value(ml)} \times 56.1}{\text{Weight of sample used(g)}}$$

Titre value= Blank titre value (B) - Real titre value (R)

2.5 Determination of saponification value (SpV)

Determination of saponification value was carried out using the method described by AOAC [14]

Procedure

Two grams of the oil sample was added to a flask with 30 cm^3 of ethanolic potassium hydroxide solution and was then attached to a reflux condenser and heated on a water bath for 1 hour with occasional shaking to ensure the sample was fully dissolved. After the sample cooled, 1cm^3 of phenolphthalein indicator was added and titrated with 0.5M hydrochloric acid until a pink endpoint was reached. A blank determination

was also carried out omitting the oil under the same condition and saponification value was calculated using the equation:

$$\text{Saponification Value(SpV)} = \frac{(b-a)x\ M\ x\ 56.1}{\text{Sample weight(g)}}$$

Titre value= Blank titre value (B) - Real titre value (R)

2.6 Determination of ester value

The ester value was determined by subtracting acid value from saponification value[15] .

Ester value = Saponification value - Acid value

2.7 Determination of Refractive Index (RI)

Refractive Index (RI) was determined following method. Melt the sample if it is not already liquid and filter through a filter paper to remove impurities and traces of moisture. Make sure sample is completely dry Circulate stream of water through the instrument. Adjust the temperature of the refractometer to the desired temperature. Ensure that the prisms are clean and dry.

Procedure

Place a few drops of the sample on the prism. Close the prisms and allow standing for 1-2 min. Adjust the instrument and lighting to obtain the most distinct reading possible and determining the refractive index or butyrorefractometer number as the case may be [16] .

2.8 Determination of Specific gravity (SG)

Determination of Specific gravity (SG) was conducted using the following method.

Procedure

The pycnometer was filled with the prepared sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. Insert the stopper, immerse in water bath at 300°C and hold for 30 minutes. Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side arm and quickly weigh ensuring that the temperature does not fall below 30°C [17].

$$\text{Specific Gravity at 30 degree C / 30 degree C} = \frac{A - B}{C - D}$$

A = weight in gm of specific gravity bottle with oil at 30°C

B = weight in gm of specific gravity bottle at 30°C

C = weight in gm of specific gravity bottle with water at 30°C

2.9 Determination of peroxide value

Peroxide value was determined by [18].

Procedure

Exactly two grams (2g) of the oil sample was weighed in ground neck flask and 10ml of chloroform was added to dissolve the butter. This was followed by addition 15ml acetic acid and 1ml 50% of KI solution. The mixture was shaked and keep it in the dark for 5minutes. After which, 25ml of water was added and titrated with 0.002M sodium thiosulphate.

2.10 Determination of Free fatty acid (FFA)

Procedure

Determination of the FFA in Oil sample was carryout by potentiometric titration in Ethanol/Diethyl ether (1:1, V/V) as solvent with NaOH in Isopropyl alcohol.4500 ml absolute Ethanol and 500ml Diethyl ether were mixed in a bottle. Five grams (5gm) of sample was weighed into a 150 ml beaker and dissolved in 70 ml of the solvent. The mixture was heated gently to increase the solubility of the oil. After a complete

dissolution the sample was titrated with 0.1N NaOH. using phenolphthalein as indicator until the pink colour was formed for approximately 30 seconds.

$$\%FFA = \frac{\text{Conc. Of consumption titrant as first equiv.–B x T x M xF1}}{\text{Weight of the sample(g)x conversion factor}}$$

2.11 Determination of Iodine value by Wij's method

Principal

Iodine value give the degree of unsaturated fatty acid of oil or fat and It is the relative measure of the unsaturated bonds present in the oil or fat. Iodine value expressed in grams of iodine absorbed by 100g of oil or fat. Unsaturated compounds absorbed iodine (in suitable form) and saturated compounds. The amount of iodine absorbed in compounds is the measure of unsaturated of the oil.

Procedure

Iodine value was determined according to titre metric method of Pearson, [19]. The oil sample (2g) weighed into a dry 500ml conical flask and 10ml of carbon tetrachloride was added to the oil. Exactly 20ml of Wij's solution was added and allowed to stand in the dark for 30minutes, 15ml of (10%) potassium iodine and 100ml of distilled water was added and then titrated with 0.1N sodium Thiosulphate solution using starch as indicator before the end point. a blank was also prepared alongside the oil sample. The iodine value was calculated using the formula below:

$$\text{Iodine value (Wij's)} = \frac{\text{V2-V1 x1.269}}{\text{Weight of sample}}$$

Where, V1=volume of sodium thiosulphate required for sample, V2 =volume of sodium thiosulphate required for blank.

2.12 Determination of Moisture content

Moisture content of cotton seed was determined according to Association of Official Analytical Chemistry [13] (AOAC, 2000) using the official method 925.09 by oven drying method.

Procedure

A crucible was cleaned and dried in an oven at 105°C for 1 hour and placed in desiccators to protect moisture absorption. Weight of crucible (W1) was determined. 5 gm sample of cotton seed flour was weighed in the dry crucible (W2) dried at 105°C for 3 hours and after cooling the sample in desiccator to room temperature it was weighed again (W3). The moisture content of cotton seed flour was calculated using the formula below :

$$\%Mo = \frac{W2 - W3}{W2 - W1} \times 100$$

%MO = percentages of moisture content

W2= weight of the crucible plus weight of fresh sample

W1= weight of the empty crucible

W3= weight of the crucible plus weight of the sample after oven dried.

2.13 Determination of oil Viscosity

The standard determination of kinematic viscosity generally employs a glass u-tube viscometer with a capillary tube build into one leg. This procedure was described in ASTM D445 and ISO 3104.

Procedure

A certain amount of cotton seed crude oil sample was poured into a beaker then transferred to the viscometer. The viscometer have been cleansed with a non toxic solvent and dried. The viscometer, containing the crude, was inserted, into the water bath at the temperature of 40°C. The pump was used to raise the level of the crude to the starting mark on the left hand limp of the viscometer; another finger

used to close the other limp to avoid the flow of oil due to air. The finger is removed to allow his flow of oil down the capillary at that point, the time at which the oil flow down is taken and recorded. The viscosity then is obtained by multiplying the constant of the viscometer by the time obtained from the equation below [20] .

$$V = Ct + B/t \text{ [cSt]}$$

where C = the instrument calibration constant,
B = the instrument type constant depending on the capillary diameter,
t = efflux time in seconds.

2.14 Colour Detection of the oil

The color of the cotton seed oil was analyzed by physical observation.

2.15 Gas Chromatography – Mass Spectroscopy (Gc-Ms) Analysis of cotton seed oil

Gas chromatography-mass spectrometry (GC-MS) was performed with GCSM (QP2010plus Shimadzu, Japan). The analysis was conducted by gas chromatography with flame ionization detection (GC/FID) and mass spectrometric detection (GC/MS). In the first instance, gas chromatograph (model HP-5890 Series II) equipped with a split-splitless injector, an HP-5 capillary column (25 mm x 0.32 mm, film thickness 0.52µm) and a flame ionization detector was employed. Hydrogen was used as the carrier gas (1 mL/min). The injector was heated at 250^{o}C, the detector maintained at 300^{o}C, while the column temperature will be linearly programmed from 80-280^{o}C (10o/min and held at 80oC (1min), 200^{o}C (4min) and 280^{o}C (5min). The GC-MS analysis was performed, using an HP G 1800C Series II GCD analytical system, equipped with an HP-5MS column (30m x 0.25mm x 0.25µm). Helium was used as a carrier gas (0.9mL/min). The transfer line was heated at 260^{o}C. The EI mass spectra (70eV) was acquired in the scan mode in the mix range 40-600. In each case 1µL of sample solution in methanol (10µL/mL) was injected in the split mode (1:30). Identification of constituents were carryout by matching their mass spectra and retention indices with those obtained from authentic samples and/or NSIT/Wiley spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data [21]. The percentage compositions were obtained from electronic integration measurements using flame ionization detection.

2.16 Statistical Analysis

The data was statistically analyzed at P-value ($p<0.05$) significantly accepted and a comparison between the groups was performed using one-way analysis of variance (ANOVA) by Graphpad instat3 software (2000) version 3.05 by Graphpad Inc. The data are given as the mean ± standard deviation.

3. Results and Discussion

3.1 Percentage oil yield

The percentage yield of n-hexane-extracted oil of cotton seed was found to be (19.98±0.005%), (table 1). This may be considered to be appreciable yield levels, similar finding showed that the oil content of cotton seeds from different varieties, ranged from 15.85–19.49%. Nangbes, *et al* [22] reported the oil content in different genotypes of cotton was ranged from 15.84–21.35%, respectively. The difference of oil yield may be related to different Geographical location and genetic variability. The cotton varieties with high level of oil, protein and low level of moisture and carbohydrate, makes the variety potential source of edible oil. The oil content of *Gossypium hirsutum* seed obtained is comparable to those well known seed oil such as linseed (33.33), soybean (18.35). e.t.c.

Table (1): Percentage yield of *Gossypium hirsutum* oil using soxhlet extraction

Method	% Yield
Soxhlet	19.98±0.005

Value is a mean ± SD n=5

3.2 Physicochemical properties of Gossypium hirsitum Oil.

The physicochemical analysis showed that the average value of Saponification value of the oil was (210.9±0.023millieqv/g), the iodine Value of the *Gossypium hirsutum* oil was (75.70±0.150), peroxide Value (8.82±0.010millieqv/g oil), The Viscosity(40°C) of the oil was (4.41±0.113), Specific gravity (0.915±0.001 at 30°C), Refrective index(30°C) of (1.383±0.003), free fatty acids content (0.34±0.016%).The acid value was (0.642±0.020mgKOH/gOil), Moisture content (0.22±0.010%), and the colour was observed to be dark red (table 2).

Table(2): Physicochemical properties of *Gossypium hirsitum* Oil

Properties	Values	Standard
Saponification Value(mgKOH/g oil)	210.9±0.02	185-265
Iodine Value(gI/ 100g)	75.70±0.150	Below 90-90
Ester value	135.2±0.127	_
Peroxide Value(meq/Kg)	8.82±0.010	10
Viscosity (cSt)(40°C)	4.41±70.113	_
Specific Gravity(30°C)	0.915±0.001	0.0-1.0
Refractive Index(30°C)	1.383±0.003	_
Free Fatty acids(%)	0.34±0.016	0.3%max
Acid Value(mg KOH/g oil)	0.642±0.020	0.6
Moisture content	0.22±0.010	0.2
Colour index	Dark red	_

All values are the average of three (3) replicates presented as mean±Standard deviation

The Physicochemical analysis of oil is mainly made for the stand point of the oil edible use as well as industrial uses. From the Table 1, Analysis of oil seed residues showed a good agreement compared with some conventional oil seed crops, the protein content of the cotton seed oil in this study was similar to that of safflower (20–22%), sunflower (16.5–19.6%) and cotton seed (19.40%) as reported in the literature [22]. The moisture content of the cotton seed oil was 0.22±0.010% . Moisture content in the seeds depends upon the maturity and quality of seeds. The moisture contents of seed determine the ability of seeds to be stored moisture. [22].

Saponification value is defined as the amount of potassium hydroxide (KOH) in milligrams required to saponify one gram of fat or oil under the conditions specified by (AOCS Method Cd 3–25 and AOCS Method Cd 3c–91). Saponification value analyzed was 210.9±0.023 mgKOH/g oil. The saponification value of the cotton seed oil is in line with those given in the literature for cotton seed oils and several other conventional seed oils [23] .The value obtained was in line with the standard guidelines set by NAFDAC and CODEX as well as some other literatures. Studies show that the high saponification values of cotton seed oil indicate that the oils have normal triglycerides and can be use in soap production [24].

Saponification is only of interest if the oil is for industrial purposes, as it has no nutritional significance. But due to the fact that each fat has within the limits of biological variation, and a constant fatty acid composition, determination of the saponification value can served as a means of characterizing and identification of the fat [25].

Acid value is the mass of potassium hydroxide in milligrams that is required to neutralize one gram of chemical substance. The acid number is a measure of the number of carboxylic acid groups in a fatty acid, or in a mixture of compounds.The acid value of cotton seed oil was founds to be (0.642±0.020mg KOH/g) which is within the normal range, According to Othman and Ngassapa [25], WHO reported that the Acid value of oil suitable for edible purposes should not exceed 4mg/g as stated by many literatures. However, the finding of this research was below the stated ranges of reported by [22]. Low level of acidity is referring to suitable quality of oil [26]. Low acid value in oil indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. This could be attributed to presence of natural antioxidants as well as other possible phytochemical like flavonoids. Acid value is used as an indicator for edibility of an oil and suitability for use in the paint and soap industries .High acid value in oil showed that the oil may not be suitable for use in cooking (edibility), but however, can be used for production of paints, liquid soap and shampoos [26].

The refractive Index(RI) value of cotton seed was found to be 1.383±0.003 which is in line with the values of refractive Index findings by Nagaraj, [27]. WHO reported RI of 1.470 at 32°C for cotton oil. and also findings of Rossell and Pritchard [22] who reported the Refractive index was ranged from 1.4590–1.468 at 30°C. Density and refractive indices of investigated oils in the present analysis were in close agreement with some other oil seed crops . The refractive index of the oil contained some double bond in fatty acid composition, that refractive index increase as the double bond increases [28] .

The free fatty acids (FFA), which reflect the extent of enzymatic or chemical hydrolytic products in oil. The free fatty acid value of cotton seed oil was 0.34±0.016% which was less than the results reported by Anhwange *et al.,* [29]. High concentrations of free fatty acids are undesirable in vegetable oils because they can reduce the palatability and the shelf-life of the oil [30].

The Specific gravity for cotton seed oil was found to be (0.915±0.001 g/cm3) at 830°C. This value is similar with the report of [22]. The Viscosity of the cotton seed oil was 4.41±80.113. and is similar to the finding of [31].

The iodine value (or iodine adsorption value or iodine number or iodine index, commonly abbreviated as IV) is the mass of iodine in grams that is consumed by 100 grams of a chemical substance. Iodine numbers are often used to determine the amount of unsaturation in fats, oils and waxes. The Iodine value obtained for cotton seed oil was 75.70±0.150 which is similar with the findings of Anigo *et al.*,[31]. Refractive index was found to be 1.383±0.003 which was less than the findings of [32].

The color of the cotton seed oil observed was dark red. The intensity of the color of vegetable oils is mainly due to occurrence of coloring pigments, such as carotenoids and chlorophyll which have to be removed during oil bleaching. Vegetable oils with least color intensity are recognized to be more appealing from commercial view-point[33] . The vegetable oils with low color values are better for edible and domestic applications [34]. Cotton seed oil is also exceptional for the presence of a toxic polyphenolic component named gossypol. This pigment gives a dark red color to crude cotton seed oil. Most parts of the gossypol are removed during neutralization. The ester value was obtained by subtracting acid value from saponification value The ester value analyzed was(135.2±0.127)which is similar with some studies [35].

3.3 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) ANLYSIS OF COTTON SEED OIL

The GC-MS analysis of cotton seed oil shows the presence of twenty five (25) chemical compounds ,in which six (6) where found to have biological activity related to Antibacterial activity, Anticancer Drug, Antiseborrhoeic , Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic Hepatoprotective, Antiandrogenic, Nematicide, Antihistaminic, Cytoprotective activity and Anti-inflammatory this includes 4,8-Diaza-2,9-dibenzoyl-5, 6-diphenyl-2,8-decadienedioic acid tridecan-7-ol, Undecanoic acid, octadecanoic acid, 9,12-Octadecadienoic acid(Z,Z)-, (*E*)-hexadec-7-enal and (Z)-7-Hexadecenoic acid respectively. While the remaining 19 compounds yet no activity was reported (Figure 1)(Table 3).The presence of this various chemical compounds indicated that cotton seed oil is very important source of chemical compounds needed in various industries like chemical industries, food industries, pharmaceutical industries etc. and also provide more light on production of cheapest traditional medicines.

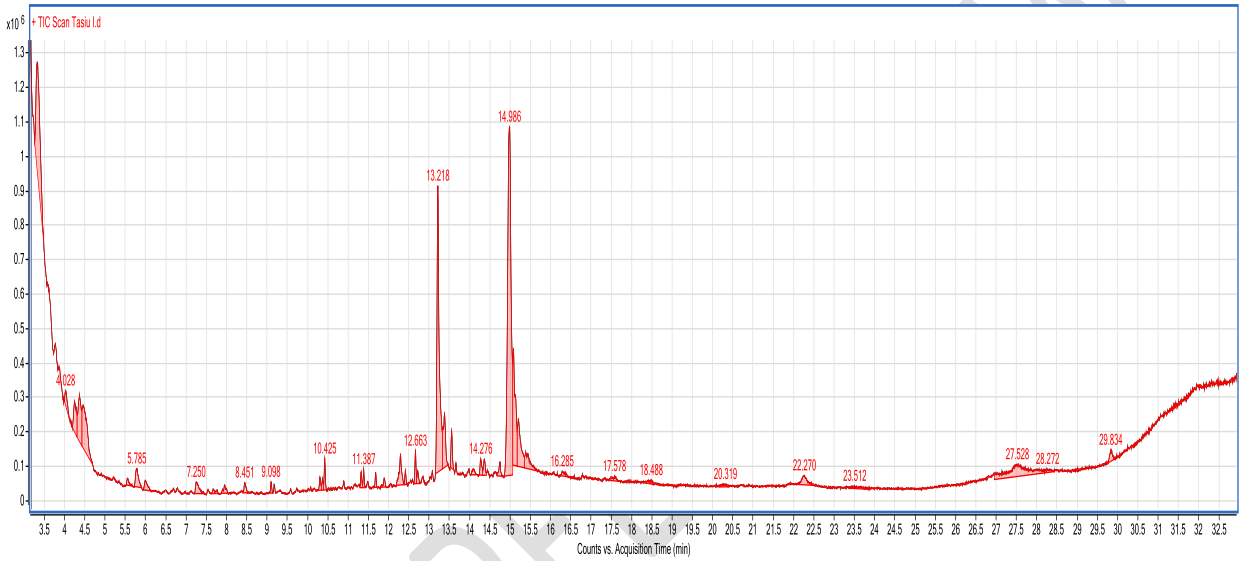
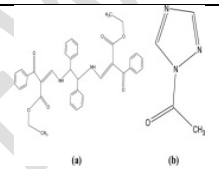
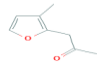
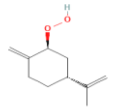
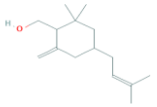
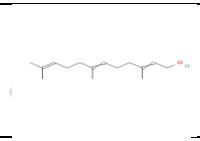
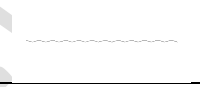
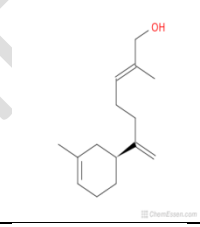
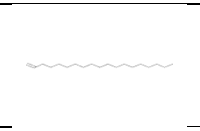
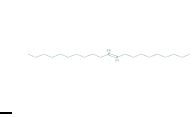
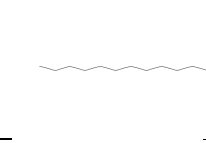
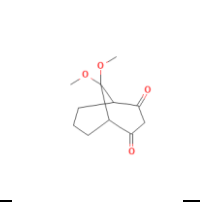
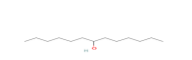
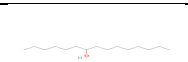
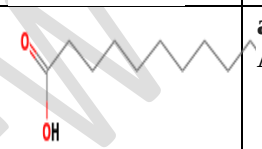
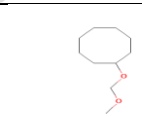
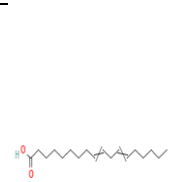
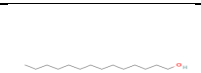
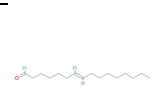


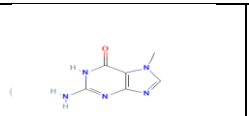
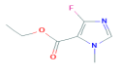
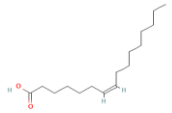
Figure 1: Chromatogram of GC-MS of cotton seed oil

Table 3: GC-MS analytical results

Peak No	Retention Time	Area	Height	Molecular weight (g/mol)	IUPAC Name	Molecular Formula	Structural Formula	Nature and Medical Important
1	4.028	181893.93	44020.94	616	4,8-Diaza-2,9-dibenzoyl-5,6-diphenyl-2,8-decadienedioic acid ester	$C_{38}H_{36}N_2O_6$		It has Antibacterial activity [36]
2	4.246	434527.18	88485.81	134.22	Benzene,2-ethyl-1,4-dimethyl	$C_{10}H_{14}$		No activity reported
3	4.372	76310.4	136139.19	224.38	5,10-Pentadecadien-1-ol, (Z,Z)	$C_{15}H_{28}O$		No activity reported
4	5.562	72318.2	19972.445 3285.69	138.16	3-Methyl-2-(2-oxopropyl)furan	$C_8H_{10}O_2$		No activity reported
5	5.997	151948.54	28581.97	142	(1*E*)-1-ethylideneindene	$C_{11}H_{10}$		No activity reported
6	7.25	295422.35	33680.03	222.37	2S,4R-p-Mentha-1(7),8-diene-2-hydroperoxide	$C_{15}H_{26}O$		No activity reported
7	7.667	65523.02	13198.97	168.23	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)	$C_{10}H_{16}O_2$		No activity reported

8	7.953	105320.31	24225.4	222.37	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl	$C_{15}H_{26}O$		No activity reported
9	8.451	98551.23	296502.05	338.7	Tetracosane	$C_{24}H_{50}$		No activity reported
10	9,098	65658	32505.81	220.35046	(2E)-2-methyl-6-(3-methylcyclohex-3-en-1-yl)hepta-2,6-dien-1-ol	$C_{15}H_{24}O$		No activity reported
11	10,305	86474.77	37503.31	266.5	nonadec-1-ene	$C_{19}H_{38}$		No activity reported
12	11.387	927729.09	53276.21	294.6	(*E*)-henicos-10-ene	$C_{21}H_{42}$		No activity reported
13	11.678	77718.47	42435.41	268.5	Nonadecane	$C_{19}H_{40}$		No activity reported
14	11.89	98204.11	25762.11	212.24	9,9-dimethoxybicyclo[3.3.1]nonane-2,4-dione	$C_{11}H_{16}O_4$		No activity reported
15	12.296	374520.9	89013.66	200.36	tridecan-7-ol	$C_{13}H_{28}O$		Its use as Anticancer Drug[37]

16	13.218	3391493.88	831914.39	228.41	pentadecan-7-ol	$C_{15}H_{32}O$		No activity reported
17	13.384	664405.41	157120.99	186.2912	Undecanoic acid	$C_{11}H_{22}O_2$		**antifungal agent and** Antiseborrhoeic [38]
18	13.555	248757.23	108813.99	284.5	octadecanoic acid	$C_{18}H_{36}O_2$		anti-inflammatory lipid [39]
19	14.093	72366.06	16333.58	172.26	methoxymethoxycyclooctane	$C_{10}H_{20}O_2$		No activity reported
20	15.083	1464719.73	337713.12	280.4	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$		**Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti coronary, antimicrobial** [40]
21	20.319	73665.97	7836.08	214.39	tetradecan-1-o	$C_{14}H_{30}O$		No activity reported
22	22.27	265710.12	27270.4	238.41	(*E*)-hexadec-7-enal	$C_{16}H_{30}O$		Cytoprotective activity[41]

23	23.512	119139.76	97488.9	165.15	2-amino-7-methyl-1*H*-purin-6-one	$C_6H_7N_5O$		No activity reported
24	28.272	81460.26	10158.79	172.16	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	$C_7H_9FN_2O_2$		No activity reported
25	29.834	146114.54	33146.47	254.41	(Z)-7-Hexadecenoic acid	$C_{16}H_{30}O_2$		Anti-inflammatory[40]

Conclusion

The physicochemical parameters of cotton seed oil were found within the NAFDAC and Cordex standard, hence its non drying oil of low saturation, slow to oxidation and rancidity, and can remain liquid for a long time and is suitable for consumption. The GC-MS analysis of cotton seed oil shows the presence of twenty five (25) chemical compounds, in which six (6) where found to have biological activity that can be employed for industrial, foods, additive and other pharmaceutical formulation.

Ethical Approval

No ethical approval required.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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