https://dx.doi.org/10.4314/wojast.v16i1.68

ISSN: 2141 – 3290
www.wojast.com

QUANTIFICATION BY GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GC-MS) AND PHYSICOCHEMICAL PROPERTIES OF *Theobroma cacao* SEED OIL

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ABSTRACT

The physicochemical properties and bioactive compounds of *Theobroma cacao* (cocoa butter) seed oil were evaluated using standard methods and Gas Chromatography-Mass Spectrometry (GC-MS). Key properties, including melting point (34.2°C), yield (14.13%), acid value (4.70 mg KOH/g), iodine value (44.95 g/100g), and peroxide value (1.085 meq O2/kg), were within acceptable industrial limits, except for moisture content (3.11%), which exceeded the permissible range. GC-MS analysis identified 71 compounds, with major bioactive components such as p-Xylene, n-Hexadecanoic acid, and γ-Sitosterol. These compounds suggest antibacterial, antioxidant, anti-inflammatory, and hypocholesterolemic potentials, supporting the oil's applications in food, pharmaceutical, and cosmetic industries. Findings highlight the potential of Nigerian *Theobroma cacao* oil as a bioactive-rich material for industrial use

KEYWORDS: *Theobroma cacao*, p-Cymene, D-Limonene, Saponification value, Peroxide value

INTRODUCTION

Vegetable fats and oils are lipid materials obtained from plants, which are solids and liquids at room temperature, respectively. These include palm oil, cotton seed oil, ground oil, sunflower oil etc. (Adebayo *et al.*, 2012). Edible oils from plant sources are of interest in various food applications and industries because they provide characteristic flavours and textures to foods as integral diet components (Odoemelam, 2005). Nowadays, oils from plant origin are largely preferred to animal fat because of its low or complete absence of cholesterol, presence of unsaturated free fatty acids, complex carbohydrates and fat soluble vitamins like as A, D, E, and K (Kostik *et al.*, 2013; Wilcox, 2006).

Theobroma oil, commonly known as Cocoa butter, is a common fat are obtained from cocoa seeds. *Theobroma cacao*, the fruit tree where cocoa butter is extracted from is commonly cultivated in equatorial areas, mainly in western Africa, South America, Central America and eastern Asia (Shukla, 2006). Cocoa beans yield about 50 - 55% of the cocoa butter which is mainly used for food processing, cosmetics, pharmaceutical and chemical industries because of its physicochemical properties (Mounjouenpou *et al.*, 2018). The composition of cocoa bean varies according to their geographical origin (Torres-Moreno *et al*., 2015). However, the dominant types of fatty acids in cocoa beans are palmitic, stearic, and oleic acids (Ristanti *et al*.,2016; Torres-Moreno *et al.*, 2015; Vieira *et al*.,2015) with levels> 25% (palmitate),> 33% (stearic), and> 34% (oleate) (Torres-Moreno *et al.*, 2015). The dominant triglycerides found in cocoa fat include 1, 3 dipalmitin-2-oleate glycerol (POP), 1-palmito, 2-olein, 3-stearin glycerol (POS), and 1, 3 distearin-2-oleate glycerol (SOS) (Lipp and Anklam, 1998). Aside from the neutral lipids, Cocoa is also known to be a rich source of phenolic phytochemicals (611 mg of gallic acid equivalents), flavonoids (564 mg of epicatechin equivalents) as well as natural sources of antioxidants such as tocopherols. (Medina, 2017; Erickson *et al.*, 1973; Lee *et al.*, 2003). Additionally, Triacylglycerol (TAG) composition in cocoa butter is responsible for the physical properties of processed cocoa products, such as shiny appearance, hardness or ease of melting, and taste (Sirbu, *et al*., 2018).

Nigeria is known to grow cocoa in relatively large quantities especially in the South-Western part of the country, producing about 328.263 metric tonnes annually (ICCO, 2021). Cocoa is currently an import agricultural export crop in Nigeria with Nigeria producing about 5% of total world production (FAO, 2011). The characterization of oils is important because it helps in determining the properties intrinsic in each oil as well as its suitability or otherwise for consumption and industrial purposes (Bezerra *et al.*, 2014). This study aims at evaluating the physicochemical properties and chemical composition of *Theobroma* oil originating from Akwa Ibom state, Nigeria since it has a wide range of applications and uses in almost all facets of life.

MATERIALS AND METHODS

Sample Collection and Preparation

Freshly harvested *Theobroma cacao* pods were obtained from Ini Local Government Area, Akwa Ibom State, Nigeria. The pods were authenticated at the Department of Botany, University of Uyo. Seeds were manually extracted, washed, and oven-dried at 50°C for 12 hours. Dried seeds were pulverized, stored in airtight containers, and used for further analyses.

Oil Extraction

Oil extraction was performed using the Soxhlet method with hexane as a solvent. Extracted oil was concentrated using a rotary evaporator, dried at 75°C, and stored in dark brown glass bottles.

Physicochemical Analysis

Standard methods (AOAC, 1984; AOAC, 1990) were used to determine the following properties:

- **Moisture Content:** Gravimetric method.
- **Acid Value, Peroxide Value, and Iodine Value:** Titrimetric methods.

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- **Saponification Value:** Hydrolysis with ethanolic KOH followed by titration.
- **Specific Gravity:** Measured using a density bottle.

GC-MS Analysis

GC-MS was performed on a Varian Factor Four VF-5ms column using helium as the carrier gas. The temperature program ranged from 140°C to 280°C. Data were collected for m/z 40–650 to identify bioactive compounds.

RESULTS AND DISCUSSION

Table 1: Physico-chemical screening result of *Theobroma cacao* oil

Physicochemical properties	values
Melting point	34.2
Percentage yield %	14.13
Acid value (mg KOH g^{-1}oil)	4.70
Iodine Value (g iodine 100 $g^{-1}$of oil)	44.95
Peroxide value (Meq KOH/g)	1.085
Free Fatty Acid (meq/g)	0.76
Saponification value (mg KOH/goil)	185.56
Specific gravity at 25 °C (g mL^{-1})	0.94
Colour	Pale yellow
Unsaponifiable matter %	5.845
moisture	3.11

The result of physicochemical screening of *Theobroma oil* is presented in Table 1. The oil had a characteristic pale yellow coloration and it was semi solid at ambient temperature. The percentage yield of *Theobroma* oil in this study (14.13%) was relatively low when compared to yields from *Theobroma* oil gotten from Taiwan and southern Nigeria (Ying and Youk 2022; Chinaka *et al*.2018). This may depend on the different methods of extraction, geographical areas of cultivation and their different climate conditions, especially the temperature (Gunstone and Harwood, 2007; Tucci *et al.,* 1996).

Specific gravity is the ratio of the density of a respective substance to the density of water at 4°C (Bamgboye and Adejumo, 2010). The density of vegetable oils is dependent on their fatty acid composition, minor components and temperature (Fakhri *et al* 2011). According to Pearson (1976), specific gravity of cocoa butter should not exceed 1. The result obtained in this research indicated that *Theobroma* oil had a specific gravity of 0.94, showing that the oil was less dense than water. It was also within the range reported by Anietie and Ajayi (2015) and Chinaka and Awemu (2018) for *Theobroma* oil produced in Nigeria. The specific gravity obtained in this study may be attributed to the presence of polyunsaturated and unsaturated fatty acids such as linoleic acid, which is a major factor for the increase in relative density of this oil (Mohammed and Ali, 2015).

Moisture content of any oil is very important because excessive moisture in the oil may lead to degradation and decomposition. The permissible limit for moisture content in oil is 0.2%. The result obtained in this study indicated that the moisture content in *Theobroma* oil was far above the permissible limit, which may be attributed to improper treatment during oil processing. This may encourage microbial growth, rancidity and reduces shelf –life of the oil.

Acid value is an indicator of the presence and extent of hydrolysis by lipolytic enzymes and oxidation (Gordon, 1993). The acid value represents free fatty acid content due to enzymatic activity and is usually indicative of spoilage as rancidity. The acid value in this research was 4.70 mg KOH g^{-1}oil, which was within the value (1-6 mg KOH/ g) accepted for most fresh fats and oils (Olaniyi and Ogungbamila 1998). Low acid value in oil suggests low levels of hydrolytic and lipolytic activities in the oil, hence making it suitable for consumption and industrial usage.

The iodine value of oil can be defined as the number of grams of iodine absorbed by 100 grams of oil. It also indicates the degree of unsaturation of the oil (Ononogbu 2002). According to Codex Alimentarius (1999), lipids are classified as drying and non-drying according to standard based on iodine value. Lipids with iodine values lower than 100gI_2/100 g oil are referred to as non-drying oil. The iodine value of *Theobroma cacao* oil from this study was 44.95 g/100 g, indicating that it is non- drying oil. The iodine value in this study was within the range reported by Chinaka and Awemu (2018) but slightly higher than that reported by Anietie and Ajayi (2015) and for *Theobroma* oil produced in Nigeria. Aremu *et al.* (2006) reported that the lower the iodine value the lesser the number of unsaturated bonds; thus the lower the susceptibility of such oil to oxidative rancidity.

Peroxide value is used as a measure of the extent to which rancidity reactions have occurred during storage and it is used as a good criterion for the prediction of the quality and stability of oils (Nangbes *et al.,* 2013). The maximum peroxide value specified for cocoa butter by Standard Organization of Nigeria (2003) is 2.0 meq/kg. The peroxide value obtained in this study (1.085 Meq KOH/g) was below the permissible limit, indicating that *Theobroma* oil may not be easily susceptible to deterioration. (Izuagie *et al*., 2008).

Free fatty acid is the Percentage by weight of a specified fatty acid such as percent oleic acid in oil. Free fatty acid (FFA) values are used to indicate the level of rancidity and edibility of oils (Amos *et al.,* 2013). The Standard Organization of Nigeria (SON) specified that the percentage FFA for cocoa butter should not be more than 1.75%. Equally, the range recommended for oil by both FAO/WHO and Ethiopian Standards (ES) (1.0- 3.0%). The free fatty acid value in this study (0.76meq/g) was below the permissible limits of SON, FAO/WHO and ES, indicating that *Theobroma* oil is edible and has a long shelf life.

Saponification value is the number of mg of KOH required to neutralize the fatty acids obtained by complete hydrolysis of 1 g of the oil. It is a measure of the total fatty acids (free and combined) present in the sample. The saponification value in this study was 185.56 mg KOH/g According to AOAC (1990), lipids with saponification value within the standard value ≥180mgKOH/g are considered as lipids good for soap making. According Muhammad *et al.,* (2011), oil with higher saponification values contains high proportion of lower fatty acid.

Unsaponifiable matters which include hydrocarbons, sterols, vitamins and pigments compounds usually play crucial roles in the oil stability (Umezuruike *et al*, 2016). The high unsaponifiable matter of *Theobroma* oil suggest that the oil

may contains natural antioxidants such as sterols, tocopherols, phenolics, lycopene and carotene (Ikram *et al.*, 2015).

https://dx.doi.org/10.4314/wojast.v16i1.68

Table 2. Results of GC-MS analyses of *Theobroma* oil.

S/N	PK	RT	Area Pct	Library/ID
1	1	5.1973	2.0155	Cyclohexane, 1,1,3-trimethyl-
2	2	5.4672	2.0007	Benzene, 1,3-dimethyl-
3	3	5.5289	0.9152	Cyclohexane, 1,2,4-trimethyl-, (1.alpha.,2.beta.,4.beta.)-
4	4	5.7483	11.6079	p-Xylene
5	5	5.8202	0.4432	Pentalene, octahydro-
6	6	5.9174	2.1132	Octane, 2-methyl-
7	7	6.0741	2.1491	Heptyl isobutyl carbonate
8	8	6.2388	3.9772	p-Xylene
9	9	6.4005	1.9649	1-Ethyl-4-methylcyclohexane
10	10	6.4526	0.9575	Cyclohexane, 1-ethyl-4-methyl-, cis-
11	11	6.6809	0.2279	Cyclohexene,1-propyl-
12	12	6.8511	8.9097	Nonane
13	13	6.9115	0.9229	3,5-Dimethyl-3-heptene
14	14	6.9606	0.3455	Cyclohexane, 1-ethyl-4-methyl-, cis-
15	15	7.0507	0.4686	Benzene, (1-methylethyl)-
16	16	7.1482	0.9972	Bicyclo[3.3.1]nonane
17	17	7.1998	0.7205	Cyclohexane, (1-methylethyl)-
18	18	7.2952	0.7965	3-Octyne, 6-methyl-
19	19	7.422	0.1864	Cyclohexane, 1-ethyl-2-methyl-, cis-
20	20	7.496	2.457	Cyclohexane, propyl-
21	21	7.6	0.7631	Octane, 2,7-dimethyl-
22	22	7.7585	2.0852	Nonane, 3-methyl-
23	23	7.8583	1.6577	Benzene, propyl-
24	24	7.9453	0.9422	Heptane, 3-ethyl-2-methyl-
25	25	8.0187	0.2538	Cyclopropane, 1,1-dimethyl-2-(2-methyl-2-propenyl)-
26	26	8.1029	3.228	Benzene, 1-ethyl-2-methyl-
27	27	8.1497	1.0457	Benzene, 1-ethyl-3-methyl-
28	28	8.2473	0.5756	Cyclohexane, 1,1,2,3-tetramethyl-
29	29	8.3471	4.1474	Benzene, 1,2,4-trimethyl-
30	30	8.4362	0.365	Cyclohexane, 1-ethyl-2,3-dimethyl-
31	31	8.4936	0.2369	Nonane, 5-methyl-
32	32	8.5509	1.8287	Nonane, 4-methyl-
33	33	8.6295	1.3533	Nonane, 2-methyl-
34	34	8.703	0.4513	Cyclopentene, 4,4-dimethyl-
35	35	8.7445	0.4665	Carbonic acid, allylcyclohexylmethyl ester
36	36	8.8054	1.294	Nonane, 3-methyl-
37	37	9.0645	5.7987	Benzene, 1,2,4-trimethyl-
38	38	9.1474	0.9001	Cyclohexane, 1,4-dimethyl-, trans-
39	39	9.2418	0.37	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)-
40	40	9.4327	0.5985	Benzene, (2-methylpropyl)-
41	41	9.5242	0.556	Benzene, (1-methylpropyl)-
42	42	9.7237	6.5874	Decane

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43	43	9.8249	1.1866	Benzene, 1,2,4-trimethyl-
44	44	9.8823	0.4467	p-Cymene
45	45	9.9533	0.3924	p-Cymene
46	46	10.098	0.3893	Indane
47	47	10.2546	2.0199	D-Limonene
48	48	10.389	1.1854	Decane, 4-methyl-
49	49	10.4771	0.8227	Cyclohexane, butyl-
50	50	10.6046	0.253	Cyclopentane, hexyl-
51	51	10.7312	0.6679	Benzene, 1-methyl-3-propyl-
52	52	10.8462	0.6536	2-Tolyloxirane
53	53	10.9565	0.5384	Benzene, 4-ethyl-1,2-dimethyl-
54	54	11.0452	0.7975	Naphthalene, decahydro-, trans-
55	55	11.4081	0.2226	Decane, 5-methyl-
56	56	11.4995	0.628	Benzene, 2-ethyl-1,4-dimethyl-
57	57	11.6181	0.4321	Decane, 2-methyl-
58	58	11.7344	0.2521	Benzene, 4-ethyl-1,2-dimethyl-
59	59	11.8011	0.2464	Decane, 3-methyl-
60	60	12.7232	2.0344	Undecane
61	61	12.7874	0.3059	Naphthalene, decahydro-2-methyl-
62	62	13.2704	0.3859	Naphthalene, decahydro-2-methyl-
63	63	15.7269	0.3059	Dodecane
64	64	31.3473	0.2249	Hexadecanoic acid, methyl ester
65	65	31.7252	1.7013	n-Hexadecanoic acid
66	66	32.657	0.3792	9,12-Octadecadienoic acid, methyl ester
67	67	32.72	0.6044	9-Octadecenoic acid (Z)-, methyl ester
68	68	32.916	0.2693	Methyl stearate
69	69	33.0107	1.0992	cis-Vaccenic acid
70	70	33.188	0.936	Octadecanoic acid
71	71	35.7438	0.9372	.gamma.-Sitosterol

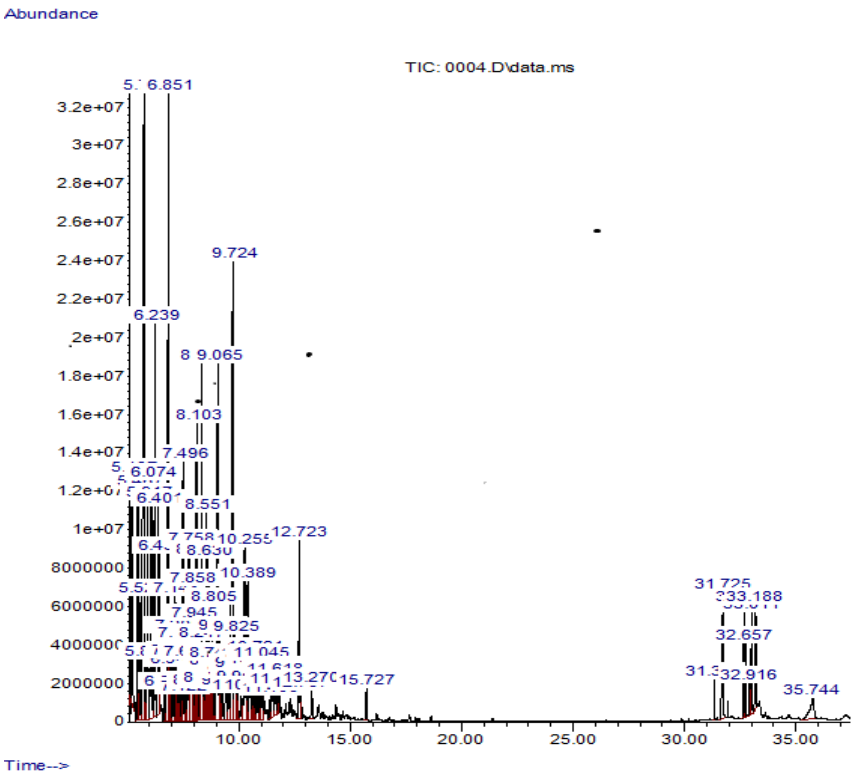

Fig. 1: Chromatogram of chemical components in *Theobroma* oil

The bioactive compounds present in *Theobroma* oil were identified by GC–MS and results presented in Table 2 and Figure1. The identified compounds, their retention indexes and percentage composition of each compound were given in the Table 2. The results obtained indicated that seventy one (71) compounds were identified. The identified bioactive compounds consist of alkanes, alkenes, alcohols, terpenoids, saturated and unsaturated fatty acids, fatty acid esters and aromatic hydrocarbons. Some of the major compounds found in the seed oil of *T.cacao* were; p-Xylene, nonane, benzene derivatives, decane, Carbonic acid, , p-Cymene, Indane, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid D-Limonene, 2-Tolyloxirane, 9, 12, octadecadienoic acid, 9-Octadecenoic acid, octadecanoic acid, methyl stearate, (Z)-,cis-Vaccenic acid and .gamma.-Sitosterol.

Alkanes, alkenes and aromatic compounds are secondary metabolites with antimicrobial, antioxidant, antiviral, anticancer and anti-inflammatory properties (Bakkali *et. al.*, 2008; Zhang *et. al*., 2015).

N- hexadecanoic acid commonly known as Palmitic acid is reported to have nematicide, pesticide, lubricant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant and hypo-cholesterolemic properties (Komansilan *et al* 2012; Sera *et al.,* 2021). Hexadecanoic acid, ethyl ester is a fatty acid ester with nematicide, pesticide, lubricant, anti-androgenic, flavor, and has hemolytic 5-alpha reductase inhibitor properties (Venkata-Raman *et al* 2012; Aneesh *et al* 2013).

Gamma.-Sitosterol, an epimer of beta Sitosterol has been reported to possess antihyperglycemic properties by increasing insulin secretion in response to glucose confirmed with immune histochemical study of pancreas. It also has anticancer and hepaprotective properties (Singh and Chaturvedi 2012; Balamurugan *et al*, 2011; Balamurugan *et al*. 2012).

Cis vaccenic acid is a kind of Trans -fatty acid (omega 7 fatty acid) with antibacterial, anticancer and hypolipdermic properties (Hamazaki *et al.,* 2016; Semwal *et al*., 2018; Pongprayoon *et al* 1992).

Limonene is reported in many studies to possess antioxidant, anti-inflammatory, antinociceptive, anticancer and insecticidal properties (Keinan *et al*., 2005; Golshani *et al;* 2004).

9, 12-Octadecadienoic acid, an ethyl ester, (also known as linoleic acid) belongs to omega 6-fatty acids used in the biosynthesis of arachidonic acid and thus some prostaglandins, thromboxane and leukotrienes collectively known as eicosanoids. It is a polyunsaturated fatty acid that plays a key role in support of heart vitality by lowering LDL cholesterol and reduces risk of developing heart disease (Farvid *et al*., 2014). Linoleic acid is reported to have antimicrobial, anticancer, hepatoprotective, antiarthritic, antiasthma and diuretic properties (Chandrasekaran *et al* 2011).

9- octadecenoic acid, a methyl ester possesses antimicrobial, anticancer, fungicidal activity, α-reductase inhibitor, antiautoimmune, antihypertensive, antidiabetic and anti-inflammatory properties (Kumar and Tanwar 2011; Bhattacharya et al 2012; Turgis, 2009).

Methyl stearate, a fatty acid ester also known as methyl octadecanoate has Gamma – aminobutyric acid (GABA) amino transferase and gastrin inhibitory properties. It is also reported to have anti- inflammatory, anthehelmintic and antnociceptive properties as well as act as intestinal lipid metabolism regulator (Chy *et al.*, 2019).

Octadecanioc acid (stearic acid), a saturated fatty acid has a hypocholesterolemic properties associated with low density lipoprotein (LDL) cholesterol levels (Mensink, 2005). It also has antioxidant, hepatoprotective, antihistaminic, antieczemic, anti-androgenic, dermatitigenic, anaemiagenic,, antiviral, antibacterial and insecticidal properties (Duke, 2018; Fadeyi *et al.,* 2015; Sudharsan *et al.*, 2010; Oladimeji *et al.,* 2013).

CONCLUSION

The physicochemical properties of *Theobroma* oil were determined to ascertain its suitability or otherwise for consumption and industrial uses. GC-MS chromatography was used to identify the bioactive compounds present in the oil. The result obtained indicated that *Theobroma cocoa* is a moderate oil yielding plant. It was further revealed that most of the physicochemical properties of *Theobroma* oil were within permissible limits except moisture content, which was slightly higher than the permissible limit. The results obtained from the identification and quantification of the bioactive compounds present in *Theobroma* oil showed that 71 compounds were identified such as saturated and unsaturated fatty acids, esters, hydrocarbons, terpenes, alcohols etc. The presence of these bioactive compounds indicates that *Theobroma* oil possesses potential antibacterial, anti-inflammatory and antioxidant properties, hence the need for further Isolation, purification, identification and structure elucidation of the phenolic phytochemical constituents.

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