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EXTRACTION AND ANALYSIS OF PHOENIX DACTYLIFERA L. (DATE SEED) OIL

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ABSTRACT

Date palm seeds (DPS) are the small, oval-shaped seeds found within the fruit of the date palm tree (Phoenix dactylifera). While often discarded, they possess several potential benefits and applications. The study's main focus was to extract oil from DPS and to determine its yield and physiochemical characteristics (density, specific gravity, viscosity, moisture content, acid value and saponification value), as well as the functional groups and fatty acid compositions. Oil was extracted from dust or powdered (0.15 mm size) and coarse or lump (1.4 mm size) DPS particles using n-hexane solvent extraction method. The physicochemical characteristics of the DPS oil (DPSO) were determined using the standard procedure of the Association of Official and Analytical Chemists. Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) were used to determine the functional groups and the fatty acid compositions, respectively. The oil yields were determined as 30.8% for dust samples and 26.5% for lump samples. Physicochemical characterization showed the DPSO has a moisture content of 6.77%, acid value of 1.083 mg KOH/g, and saponification value of 189 mg KOH/g, with specific gravity of 0.9016, density of 0.9177 g/cm^3, and viscosity of 24 centipoise for both samples. FTIR spectra indicated the presence OH, C-H, C=C, C=O, and N-H. functional groups in DPSO while the GC-MS revealed the dominant presence of lauric acid followed by stearic acid and oleic acid in the DPSO. Thus, DPSO has a versatile potential to be used as feedstock for industrial soap, cosmetic and biodiesel productions.

Keywords: *Phoenix Dactylifera L. Soxhlet Extraction, Date seed oil, physiochemical properties, FTIR, GC-MS*

INTRODUCTION

In recent years, the use of Date Palm Seed Oil (DPSO) has grown around the world, particularly in the cosmetics manufacturing and pharmaceutical industries (Niazi, *et al.,* 2017). This oil is classified among the precious vegetable oils owing to its richness in fatty acids and phenolic and antioxidant compounds. Furthermore, DPSO has more than one benefit for human health (Al-Shahib *et al.,* 2003). Besbes, *et al.* (2004) have stated that in contrast with olive oil, DPSO has greater oxidative stability. Other studies have revealed that DPSO is a suitable source of α-tocotrienol (Nehdi, *et al.,* 2010), which is pronounced as a high-quality compound to decrease breast cancer risk (Delgado, *et al.,* 2020; Marinova, *et al.,* 2008), low-density lipoprotein and cholesterol in humans (Yuen, *et al.,* 2011).

Two approaches had been oftentimes used for oil extraction from date palm seeds. The first approach involves utilizing the pressing method permitting the acquisition of very pure oil containing no foreign chemical substances. This approach results in very low oil yield (≈5.5%) (Fakhfakh *et al.,* 2019). Nevertheless, the oil obtained through this method contains minor bioactive compounds that have a good effect on human fitness and also help to prolong the shelf life of the cold-pressed oil by

amplifying the oxidative stability of the oil (Bozdoğan *et al.,* 2020).

The second approach entails the usage of an organic solvent, which is recovered by way of a range of methods (Jemni *et al.,* 2019). Jemni *et al.* (2019) have discussed the use of several solvents (polar and nonpolar solvents) in the extraction of oil from date palm seed. These authors concluded that nonpolar solvents such as toluene, chloroform, and hexane gave exceptionally high yields. DPSO is among the precious vegetable oils with low yield, whose extraction is commonly done with organic solvents. This study aimed to extract oil from date palm seeds using the solvent extraction method and to determine its physiochemical characteristics, as well as the functional groups and fatty acid compositions.

MATERIALS AND METHOD

Materials

Chemicals

The chemicals used for this work include n-hexane (99% purity, Aldrich, Germany), potassium hydroxide, anhydrous sodium sulfate (Sigma-Aldrich, UK), phenolphthalein, potassium iodide (98.5%, England), and starch (99.9%, England) and fatty acid standards (99%, Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Apparatus and Equipment

The glass wares that were used in this research include; beakers, round–bottom flasks, conical flasks, hygrometers, petri dishes, measuring cylinders and burettes. The equipment used for this study includes blender/grinder, Soxhlet apparatus, weighing balance (Mettler Toledo model AB54), digital weighing balance, rotary evaporator (Heidolph Instrument, and Co. KG, Germany), Abbe refractometer (Kirkland, WA, USA), and heating mantle (capable of measuring temperatures up to 150 ^{o}C), Agilent gas chromatograph equipped with a mass spectrometer detector and Agilent automatic injector spectrometer (Agilent Technologies, 7890A GC–MS, USA).

Collection of Samples

Date palm seed was acquired from Aritalin in Yenagoa town, Yenagoa LGA, Bayelsa state. The samples were checked and thoroughly cleaned to remove any defective ones and guaranteed that they were dirt-free.

Methods: Preparation of Samples

The date palm seeds were manually crushed, washed to remove skins, and then sun-dried for two days before being ground to fine particle sizes of 0.15 and 1.4 mm, respectively using a blender. The powdered or coarse-grinded samples were placed in plastic bottles and refrigerated for subsequent analysis.

Extraction of Oil from Date Palm Seeds

The extraction was carried out in a Soxhlet apparatus. The apparatus consisted of a round bottom flask, extraction chamber, heating mantle, condenser, thimble, and siphon. The extraction process was carried out according to the flow diagram presented in Figure 1 (Olowokere *et al*., 2019). Twenty (20) grams of the finely ground date palm seeds (with particle size 0.15 mm) were weighed and put into the thimble and the thimble was placed inside the extraction chamber. Then, 200 ml of n-hexane solvent was added into the round bottom flask and it was heated at a temperature of 30 °C and continuously circulated through the extraction chamber. The extraction process was carried out for 15 mins. The extracted oil-solvent mixture was filtered using filter paper to separate the solvent-oil mixture from any remaining solid particles.

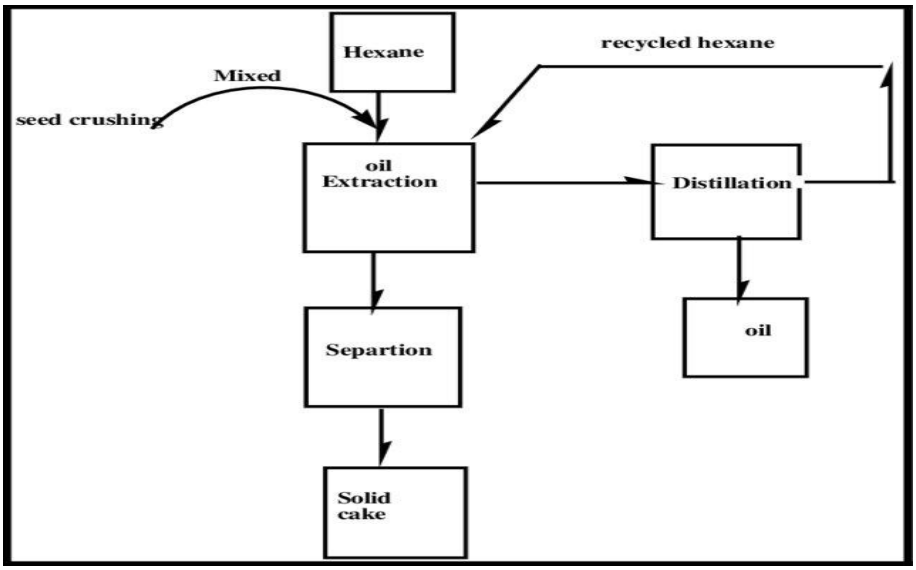


Figure 1: Flow diagram for solvent-oil extraction from date palm seeds

The filtered solvent-oil mixture was then passed through a rotary evaporator to evaporate the hexane solvent, leaving behind the extracted oil (i.e., DPSO). This procedure was repeated for a particle size of 4 mm. The DPSO was thereafter stored in an airtight container that protected it from light and heat until further use. The percentage oil yield was calculated using Equation (1).

$$Extracted\ oil\ yeild\ (\%) = \frac{Weight\ in\ grams\ of\ extracted\ oil}{Weight\ in\ grams\ of\ seed\ powder\ sample}\ x\ 100 \quad (1)$$

Characterization of DPSO

The physicochemical characterization (moisture content, specific gravity, refractive index, density, viscosity, acid value, and saponification value) of DPSO was conducted based on the standard procedures of AOAC (1990).

Determination of moisture content

Three (3) grams of DPSO sample was weighed into a moisture dish of 5 cm diameter and 2 cm deep with tight-fit-slip-over cover. The moisture dish was placed in the oven set at a temperature of 125 ^{o}C above the boiling point of water and a working pressure of 95 mmHg. The sample was weighed at 30-minute intervals and the weight of the sample was noted until a constant weight was achieved when there was no additional loss of 0.05 %, The moisture content was calculated using Equation (2).

$$Moisture\ Content\ (\%) = \frac{initial\ weight\ of\ the\ sample - Final\ weight\ of\ dried\ sample}{initial\ weight\ of\ the\ sample} \quad (2)$$

Determination of specific gravity

The specific gravity of DPSO sample was determined using the specific gravity bottle. The bottle was filled with distilled water and the weight was noted. After a while, the bottle was also filled with DPSO and the weight was recorded. The specific gravity was obtained using Equation 3.

$$Specific\ gravity = \frac{weight\ of\ the\ oil\ measured}{weight\ of\ an\ equal\ volume\ of\ water} \quad (3)$$

Determination of viscosity

A viscometer glass tube which was held in a vertical direction was used. The DPSO sample was drawn into a capillary tube via suction to a marked volume. The sample was then allowed to flow down into the

lower bulb and the time taken to pass through the marks was noted. The kinematic viscosity was calculated using Equation (4).

$$Kinematic\ viscosity = time\ taken\ x\ viscometer\ factor \quad (4)$$

Determination of refractive index

The refractive index of a material is a measure of how much a wave velocity is reduced inside that material. In this study, the Abbe refractometer (VEE GEE Model C10, Thomas Scientific, USA) was employed for determining the refractive index.

Determination of acid value

The acid value was determined by dissolving 3 g of DPSO sample in a hot mixture of 25 ml (95 %v/v) diethyl ether and 2.5 ml ethanol in a 250 ml flask. The hot solution was then neutralized with 0.1 M potassium hydroxide solution. Thereafter, three drops of phenolphthalein as an indicator were added to the neutralized solution. The acid value was calculated using Equation (5).

$$Acid\ Value\ (mgKOH/gOil) = \frac{V\ x\ N}{w} x\ 100 \quad (5)$$

Where V = volume of KOH used during titration, N = Normality of KOH and W = Weight of DPSO.

Determination of saponification value

DPSO sample of 2 g was weighed into a 250 ml Pyrex flask with 25 ml of ethanol and 0.1 M potassium hydroxide added. The content was constantly stirred and was allowed to boil gently for 60 min, with a reflux condenser placed on the flask to achieve uniform temperature. Two drops of phenolphthalein indicator were added to the warmed soap solution and then titrated with 0.5 M HCI to the endpoint until the pink colour of the indicator just disappeared. The saponification value was calculated using Equation (6).

$$S.V\ (mgKOH/gOil) = 28.05\ X\ \frac{Volume\ of\ HCl\ required\ by\ blank - vol.\ of\ 0.5\ MHCl}{weight\ of\ sampling} \quad (6)$$

Determination of functional groups

Fourier-Transform Infrared Spectroscopy (FTIR) was used to determine the functional groups present in the DPSO sample. The FT-IR spectra of DPSO sample were recorded at 400–4000 cm using a Spectrum 65, PerkinElmer model FTIR spectrophotometer.

Determination of fatty acid composition

The gas chromatography-mass spectrometer (GC-MS) was used for determining the fatty acid composition. The procedure was followed according to that of Hagos et al. (2023). The GC-MS analytical separation was carried out at a flow rate of 1 ml/min and a pressure of 8-psi on a DB-1701 micro-column that has a length, internal diameter and film thickness of 30 m, 0.25 mm, and 0.25 µm, respectively. At constant low mode, helium with high purity was used as the carrier gas. At a total run time of 16.67 min, using a split-less injection mode, the Agilent G4567A auto sampler was utilized to inject 1.0 µL of the DPSO sample into the inlet that has been heated to 275 °C. The starting column temperature was set to 100 °C while the oven temperature was set to 100 °C for 2 min. The column temperature was raised to 220 °C at a rate of 15 °C/min and further increased at a rate of 3 °C/min until the temperature increased to 240 °C. The ion source and transfer line temperatures were 230 °C and 280 °C, respectively. The electron energy was set at 70 eV. Ions with a mass-to-charge ratio of 40 to 650 were collected. Identification of the fatty acids was done by comparing the standard mixture's retention times to the fatty acids' retention times, as well as by comparing with NIST spectral

library. Area normalization was applied to calculate the individual fatty acid's relative content.

RESULTS AND DISCUSSION

Oil Yield of Date Palm Seeds

The DPSO yield obtained from the dust or powdered date seed particles and coarse or lump date seed particles was 30.8% and 26.5%, respectively. This indicates that ground seeds with smaller particle sizes give rise to higher oil yield than ground seeds with bigger or greater particle sizes. This is so because smaller particle sizes possess a larger surface area such that it provides a larger contact area for the extracting solvent and consequently there is a greater mass transfer of oil from the solid phase to the liquid phase. A similar observation was reported by Reddy et al. (2017). Furthermore, the Food and Agricultural Organization (FAO) has said that any seed that yields more than 17% oil can be considered an oil seed and can be utilized as a biodiesel production feedstock (Hagos *et al*., 2023). Thus, date palm seeds can be employed for industrial vegetable oil processing. Nonetheless, the oil yield obtained in this study is comparably higher than the oil yield range of 2.63 – 9.8% achieved from date palm seeds as previously reported by other researchers (Olowokere *et al*., 2019; Reddy *et al*., 2017; Hamza *et al*., 2021; Halabi *et al*., 2022). The variation in this oil yield could be due to several variables such as the extraction solvent, extraction procedure and the environment in which the date palm was grown. Moreover, when compared to the oil yield of other seeds, DPSO was higher than soybean oil yield (18–22%) and cottonseed oil yield (22–24%), however, lower than rapeseed oil yield (40–48%) and pumpkin seed oil yield (43.6%) (Hagos et al., 2023).

Physicochemical Characteristics of DPSO

Table 1 shows the results of the physicochemical characterization of DPSO.

Table 1: Physicochemical Characteristics of DPSO

Characteristics	Value	
	Dust (Powdered particles)	Lumps (Coarse particles)
Physical characteristics		
Colour	Pale yellow	Pale yellow
Odour	Pleasant	Pleasant
Texture	Viscous	Viscous
Density	0.9177 g/cm^2	0.9177 g/cm^2
Moisture content	6.77 %	6.77 %
Viscosity	24cp	24cp
Refractive index	1.44	1.44
Specific gravity	0.9016	0.9016
Chemical characteristics		
Acid value	1.083 mgKOH/g	1.083 mgKOH/g
Saponification value	189 mgKOH/g	189 mgKOH/g

As observed from Table 1, the results show that the DPSO has a pale-yellow colour with a pleasant odour and a viscous texture with a viscosity value of 24 centipoises. The specific gravity and density of DPSO were found to be 0.9016 and 0.9177 g/cm^3, respectively. These values are comparable with the values of 0.91 - that had been reported for DPSO by other researchers (Abdalla *et al*., 2012) as well as 0.89 – 0.92 for other seed oils like pumpkin seed oil (Hagos *et al*., 2023), The refractive index was

obtained to be 1.44 while the moisture content was 6.77%. The refractive index obtained in this study is found to be adequately comparable to the values of 1.43 -1.47 that have been obtained for other seed oils such as cashew seed oil (Hayford *et al*., 2023) and pumpkin seed oil (Hagos *et al*., 2023).

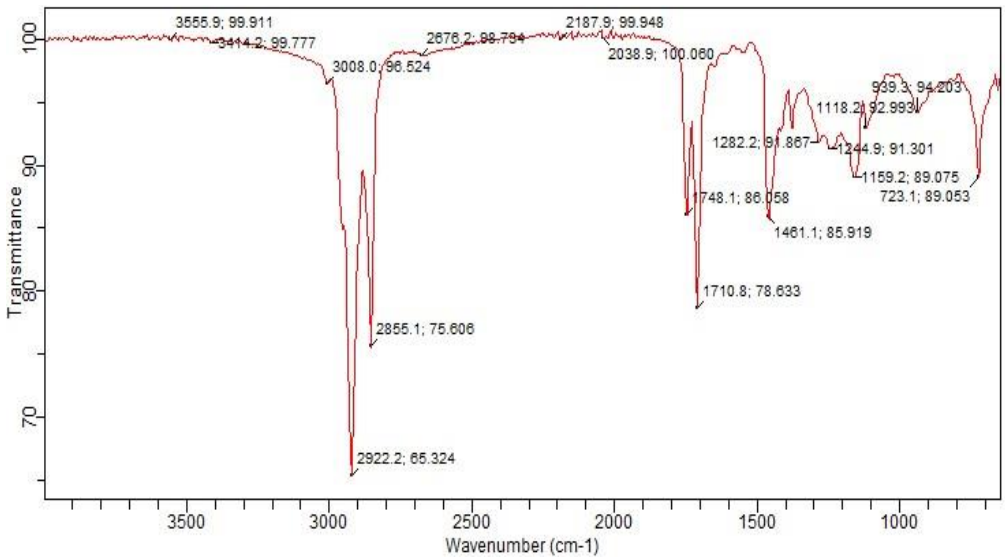
The acid value or number indicates a measure of how much potassium hydroxide is required for the neutralization of the free acids present in one gram of oil or fat (Hagos *et al*., 2023). Table 1 revealed that the acid value obtained for DPSO is 1.083 mg KOH/g. This acidity value is relatively lower than the previously reported values of 1.77 and 2.55 mg KOH/g for DPSO by Olowokere et al. (2019) and Abdalla et al. (2012), respectively. In comparison with other seed oils, the acid value achieved for the DPSO in this study is equally lower than the values of 4.75, 5.75, 6.36, 9.86, 12.57, and 32 mg KOH/g obtained for cottonseed oil, coconut oil, soybean oil, cashew nut oil, and rubber seed oil, respectively (Hayford *et al*., 2023; Kukeera *et al*., 2015; Hagos *et al*., 2023; Salim *et al*., 2023; Maliki and Ifijen, 2020). Thus, an oil sample that possesses low acid value indicates that there are few free acids, thereby minimizing the risk of the oil going rancid (Shimamoto *et al*., 2016; Hagos *et al*., 2023) and hence, possessing the potential for lengthy shelf life (Onoja *et al*., 2023).

The saponification value provides the required information on the average molecular weight of all the fatty acids present in the oil sample. This implies that when the saponification value of the oil is high, then the molecular weight of all fatty acids present in the oil will be low, and vice versa (Yusuf *et al*., 2021; Hagos *et al*., 2023). The saponification value was obtained to be 189 mg KOH/g (Table 1). The saponification value of DPSO in this study was found to be lower than the value of 213.3 mg KOH/g reported by Olowoekere *et al*. (2019) for DPSO but consistent with the values of 185.3 mg KOH/g attained for rubber seed oil (Maliki *et al*., 2020), 189 mg KOH/g obtained for sesame seed oil (Saeed et al., 2015), 190.4 mg KOH/g achieved for melon seed oil (Saeed *et al*., 2015) and 191 mg KOH/g obtained for pumpkin seed oil (Hagos *et al*., 2023). The saponification value of DPSO in this study was found to be higher than the saponification values reported by Saeed *et al*. (2015) for other seed oils such as cashew seed oil (161 mg KOH/g) and moringa seed oil (171.9 mg KOH/g) but lower than the values of 246 mg KOH/g reported for coconut oil. The Saponification value of 189 mg KOH/g attained for DPSO in this study indicates that the oil contains a high concentration of triglycerides, which is a desirable characteristic and thus implies that DPSO can be utilized as feedstock for biodiesel, liquid soaps and shampoo productions (Reddy *et al.*, 2017).

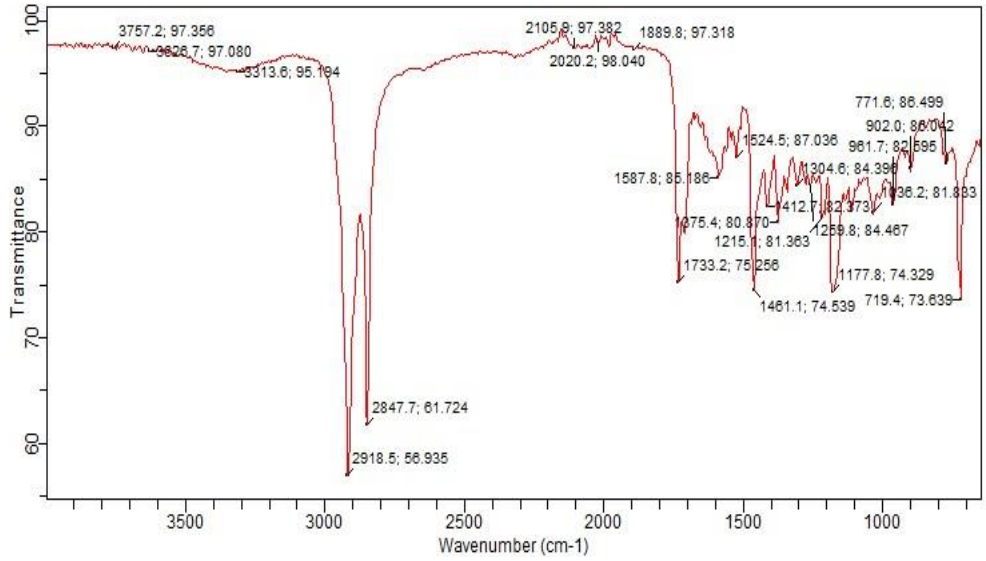
FTIR Analysis of Functional Groups

The purpose of FTIR analysis is to determine the types and number of functional groups present in the DPSO. This will provide information on the area of application or use of DPSO. Figure 2 shows the functional groups found in DPSO obtained from coarse or lumps' particle size and dust or powdered particle size.

Figure 2(a) shows the FTIR spectra of the functional groups found in DPSO obtained from lump or coarse particle size, The FTIR spectra revealed the presence of seventeen (17) peaks. Six (6) of these 17 peaks were recorded on the single bond stretch spectrum, two (2) peaks on the triple bond spectrum, two (2) peaks on the double bond stretch vibration, and seven (7) peaks on the fingerprint region skeletal vibration where C-O, C-N, and C-C groups are located. . On the other hand, Figure 2(b) shows the FTIR spectra of the functional groups found in DPSO obtained from dust or powdered particle size.



(a)



(b)

Figure 2. (a) FTIR spectra of DPSO obtained from ground date palm seeds with coarse or lump particles (1.4 mm). (b) FTIR spectra of DPSO obtained from ground date palm seeds with powdered particles (0.15 mm).

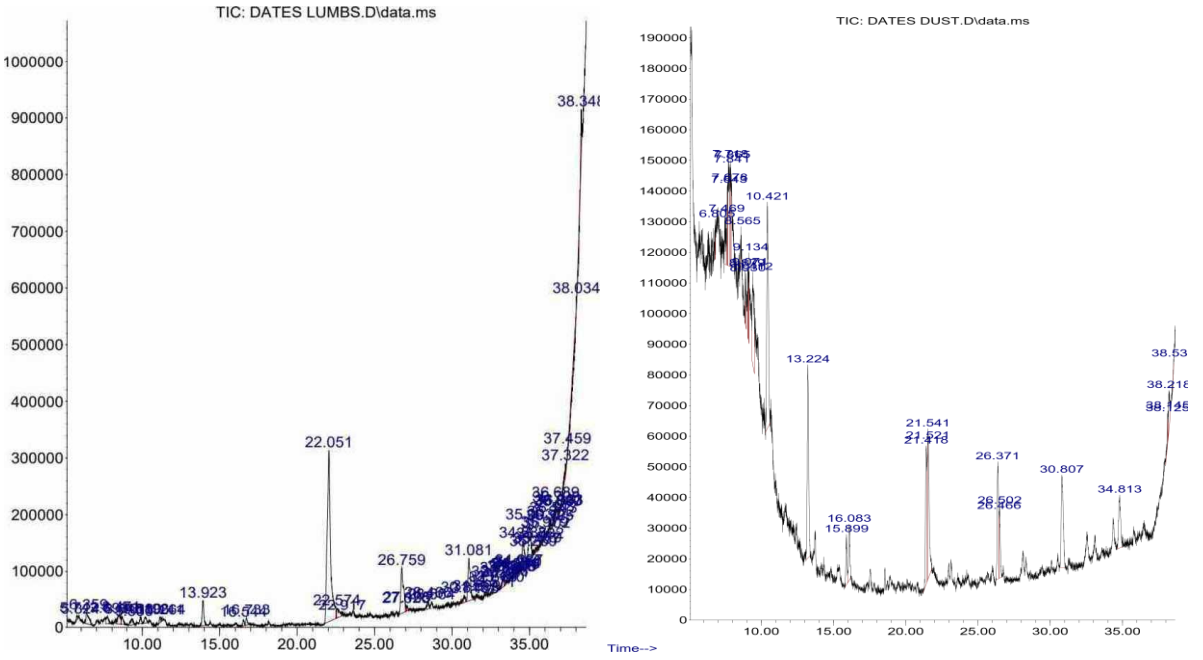
In this case, the spectra revealed twenty-three (23) peaks with five (5) of the peaks recorded on the single bond stretch spectrum, two (2) peaks on the triple bond spectrum, four (4) on the double bond stretch vibration, and twelve (12) on the fingerprint region skeletal vibration where C-O, C-N, and C-C groups are located.

A peak of 3555.9 cm^{-1} (Figure 2(a)) and 3757.2 - 3626.7 cm^{-1} (Figure 2(b)) indicates the existence of a free hydroxyl (OH) functional group for alcohols and phenols. The peak in the 3414.2 cm^{-1} region (Figure 2(a)) and 3313.6 cm^{-1} region (Figure 2(b)) demonstrates N-H stretch with a functional group for amines and amides. The presence of the functional group for carboxylic acid on the O-H band stretch is indicated by the peaks in the 3008.0 - 2855.1 cm^{-1} range (Figure 2(a)) and by the peaks in the 2918.5 - 2847.7 cm^{-1} range (Figure 2(b)). The peak of 2676.2cm^{-1} corresponds to C-H stretching with a functional group aldehyde (Figure 2(a)). The

peak of 2187.9cm^{-1} (Figure 2(a)) and the peak at 2105.9cm^{-1} (Figure 2(b)) corresponds to C≡C stretching with a functional group alkyne. The peaks in the region carbonyl C=O stretching is responsible for the peak at 1748.1 cm^{-1} – 1710.8 cm^{-1} (Figure 2(a)) and the peak at 1733.2 cm^{-1} (Figure 2 (b)) for carboxylic esters and ketone. The N-H bend with a functional group for primary and secondary amines and amides coincides with the peak at 1587.8 cm^{-1} (Figure 2(b)). The N=O asymmetric stretch of nitro compounds is represented by the peak at 1524.5 cm^{-1}. (Figure 2(b)). C-H bending methylene group is responsible for the peak at 1461.1 cm^{-1} (Figure 2(a)) and the peak at 1461.1 cm^{-1} and 1375 cm^{-1} (Figure 2(b)). Peaks in the 1282.2 cm^{-1} – 1118.2 cm^{-1} (Figure 2(a)) correspond to the bending vibration of C-O carboxylic acid, alcohols, esters, ethers and anhydrides. The peak at 939.3 cm^{-1} (Figure 2(a)) and the peak at 961.7 cm^{-1} (Figure 2(b)) is attributed to a C-H out-of-plane bend alkene, and the peaks from 723.1 cm^{-1} (Figure 2(a)) is attributed to C-H out-of-plane bend aromatic while the peaks from 771.6 to 719.4.95 cm^{-1} (Figure 2(b)) are attributed to C-X Halides for Chloride vibration This finding is comparable to those of (Dabai *et al.*, 2018) and (Olowokere *et al.*, 2019) for waste cooking palm oil and African locust bean seed oil, respectively.

GC-MS Analysis of Fatty Acid Composition

The GC-MS chromatogram results for DPSO obtained from coarse or lump particles and dust or powdered particles are presented in Figure 3. The composition of the compounds found in the DPSO is presented in Table 2.



(a) (b)

Figure 3. (a) GC-MS chromatogram of DPSO obtained from coarse or lump particle size. (b) GC-MS chromatogram of DPSO obtained from dust or powdered particle size.

Figure 3(a) and Table 2 show that some fatty acid molecules are present in relative abundance in the DPSO derived from the coarse or lump particles. These include vaccenic acid, lauric acid, oleic acid, and stearic acid. However, oleic acid is the sole monounsaturated fatty acid found in DPSO.

Table 2: Fatty acids detected in DPSO

Fatty acids found in DPSO derived from coarse or lump particle size				
Peak No	**Compounds**	**Retention Time**	**Area %**	**Saturation**
11	Capric acid	11.264	0.24	C10:0
15	Lauric acid	22.051	40.76	C12:0
26	Oleic acid	31.599	0.27	C18:1
Fatty acids found in DPSO derived from dust or powdered particle size				
1	Palmitic acid	6.805	0.42	C16:0
2	Oleic acid	7.469	2.32	C18:1
5	Stearic acid	7.718	6.08	C18:0
19	Nonanoic acid	21.521	3.67	$C_9H_{18}O_2$
27	Behenic acid	38.145	0.32	C22:0
20	Lauric acid	21.541	7.85	C12:0

Oleic acid is known to contain omega-9 fatty acids, which are also found in olive oil. According to research, it has medicinal characteristics that improve the health of the heart and brain by maintaining normal fluidity of cell membranes for the transport of minerals to fight pathogens in living cells. Also, Figure 3(b) and Table 2 show that some fatty acid molecules are present in relative abundance in the DPSO derived from dust or powdered particles. These fatty acids are behemic acids, palmitic acid, capric acid, oleic acid, and stearic acid. These analytical results are in agreement with the observations previously reported by Reddy et al. (2017) and Hamza et al. (2022).

CONCLUSIONS

Date palm seeds (*Phoenix dactylifera L.)* is a highly attractive source of oil for biodiesel production due to their exceptional oil content, ranging from 26.5% to 30.8%. This translates to a significant yield of oil extractable from the seeds. Furthermore, the oil exhibits moderate polarity, as evidenced by its saponification value (189 mg KOH/g) and acid value (1.083 mg KOH/g). These characteristics make date palm seed oil a particularly suitable feedstock for biodiesel conversion processes. Date palm seed oil shows promise as a biodiesel feedstock due to its similarity to other bio-oils in both chemical composition and basic fuel properties. This resemblance is further supported by the oil's low refractive index (1.44), indicating the presence of fatty acids with medium-short hydrocarbon chains. These shorter chains contribute to the low viscosity (24 cP) of date seed oil, making it one of the least viscous vegetable oils – a property similar to olive oil and favorable for biodiesel production.

DECLARATIONS

Author contribution statement

Blessing Amabogha developed, designed the experiments and wrote the paper, performed the experiment, analysed and interpreted the data.

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Data availability statement

Data included in article/supp. material/referenced in the article.

Declaration of Interest's statement

The author declares no conflict of interest.

Additional information

No additional information is available for this paper.

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