

# Synthesis and Characterization of Watermelon Seed Oil (*Citrullus lanatus*) as Biodiesel Feedstock

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## Abstract

Watermelon Seeds oil was extracted using two techniques: chemical Soxhlet extraction and mechanical pressing. The percentage yields were found to be 37.3% and 26.0%, respectively. The physicochemical properties of the oil were determined, and the following values were obtained: Peroxide value 6.78 Meq O<sub>2</sub>/kg, Saponification value 191.10 mg KOH/g, and iodine value 104.22/100g. According to the fatty acid profile, the oil contains approximately 70.5% unsaturated fatty acids, mainly represented in Linoleic acid and oleic acid, with percentages of 50.31% and 19.62%, respectively, and 29.5% saturated fatty acids.

**Keywords:** Watermelon, Soxhlet Extraction, Mechanical Pressing, Saponification, Fatty Acids.

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## INTRODUCTION

*Citrullus lanatus* is an annual plant belonging to the Cucurbitaceae family, grown in warm climates around the world. *Citrullus lanatus* is known as a watermelon because it has a high-water content, which is around 93% of its weight (Maoto *et al.*, 2019). It is a climbing summer plant, and the Leaves are generally broad and lobed. Flowers are large and yellow and appear at the nodes, which produce spherical or cylindrical fruits of light green or dark green color, with a sweet red pulp (Gabriel *et al.*, 2018). Watermelons play a very important role in Africa as they are used as a thirst quencher when water is scarce (Erhirhie and Ekene, 2013). Watermelon is a good source of vitamins B, C, and E, as well as minerals such as phosphorus, magnesium, calcium, and iron (Maoto *et al.*, 2019). The sweetness of watermelon is mainly owing to a mixture of sucrose, glucose, and fructose. Sucrose and glucose account for 20–40% and fructose for 30–50% of total sugars (Bianchi *et al.*, 2018).

Watermelon seeds oil is described to be a good source of essential fatty acids, carotenoids, tocopherols, thiamine, flavonoid, riboflavin, and other phenolic substances, quantities of which differ depending on watermelon variety and extraction method (Ouassor *et al.*, 2020). Linoleic acid is the most abundant fatty acid

found in watermelon seeds oil and usually accounts for 50-70% of the total fatty acid composition, unsaturated fatty acids are known for their beneficial effects on skin nutrition and cardiovascular health. Watermelon seed oil shows a similar composition to other widely used oils, such as sunflower oils and soybeans (Ziyada and Elhussien, 2008; Mahla *et al.*, 2018).

Watermelon seeds oil plays an essential role in several industrial uses. It is applied in the production of combinations and Nano-emulsion paints and helps as an important component in the production of biodiesel (Vinhas *et al.*, 2021, Petchsomrit *et al.*, 2020).

There are several ways to extract oils from seeds. One of them is mechanical pressing extraction. Mechanical extraction is considered to be the best option. In this extraction, hydraulic presses are used to remove oil from the seeds. This method is generally preferred because of its lower initial and operational costs and because it can be easily operated. It produces relatively good-quality oil as compared to the solvent extraction process. However, a disadvantage of mechanical extraction is the lower oil recovery compared to solvent extraction (Subroto *et al.*, 2015), on the other hand, solvent extraction is widely used for its high efficiency, achieving 90–98% oil recovery (Tesfaye and Tefera, 2017). The objective of this study is to extract oil from

watermelon seeds and analyze its physicochemical properties and fatty acid composition.

## MATERIALS AND METHOD

### Materials

- Watermelon (*Citrullus lanatus*) seeds were obtained from Kadugli South Kordofan state in Sudan as shown in Fig. 1.
- n-Hexane from Sigma Aldrich was used as an extraction solvent.



Figure 1: Watermelon seed

### Methods

#### Oil Extraction

The watermelon seeds oil was extracted using two techniques: chemical soxhlet extraction and mechanical pressing.

In the soxhlet extraction method; 200g of the watermelon seeds were packed in a muslin cloth and inserted into the soxhlet extractor, and n-hexane was used as the extraction solvent for eight hours. At the end of the period, the mixture was transferred to the rotatory evaporator to separate the remaining solvent from the oil. The extracted oil and the yield % of extracted oil were calculated (Handa *et al.*, 2008).

In the mechanical pressing process, 500 grams of watermelon seeds were placed in the pressing machine to clean it, eliminate impurities, and prevent cross-contamination with other seeds. Subsequently, the oil obtained from the 500 grams of seeds was discarded. Afterward, an additional 5 kilograms of watermelon seeds were placed into the pressing machine, and the oil was collected in a plastic bottle. The extracted oil was then filtered to eliminate any remaining particles and impurities before being stored in a clean plastic container.

#### Fatty Acid Composition

The fatty acid composition of the oil was evaluated using the GC-2010 gas chromatograph (SHIMADZU). Capillary Column DB-1 (30m x 0.25mm x 0.25mm). The detector temperature was set to 300 °C, with a flow rate of 30 mL/min. The injection mode was

split up, the temperature was adjusted to 250° C, and Helium was used as the carrier gas. To identify the characteristics and composition of watermelon seed oil, retention times were compared to proper standards examined under similar conditions (Alrasheid *et al.*, 2018).

#### Sample Preparation

2 mL of the extracted crude oil was mixed in a separating funnel with 7 mL of alcoholic sodium hydroxide solution prepared by dissolving 2 g of NaOH in 100 mL of methanol, then 7 mL of alcoholic sulfuric acid prepared by adding 1 mL of H<sub>2</sub>SO<sub>4</sub> to 100 mL of methanol was added. The mixture was then shaken for 5 minutes. The mixture was allowed to stand overnight in the separatory funnel. Then, 1 mL of supersaturated sodium chloride (NaCl) was added, and the contents were shaken. 2 mL of normal hexane was added, and the contents were shaken thoroughly for three minutes. Then the lower layer of the mixture was drained off, and 5 µl of the upper layer of n-hexane was diluted with 5 mL of diethyl ether. The mixture was filtered using a 45-µm syringe filter and then dried using 1 g of anhydrous sodium sulfate as a drying agent. Then, 1 µl of the diluted sample was injected into the GC-MS instrument. (Alrasheid *et al.*, 2018).

### Determination of Physical and Chemical Properties of Watermelon Seed Oil

#### Physical Properties

Kinematic viscosity and density measurements were carried out following the ASTM D445 and ASTM D4052 methods. To determine the cloud point (CP) and pour point (PP), the ASTM D2500 and ASTM D97 protocols were employed. Water content was analyzed using the AOCS Ca 2e-84 method. Color and refractive index measurements were conducted according to ASTM D1500 and ASTM D1714, respectively.

#### Chemical Properties

##### Acid Value

The acid value of watermelon seeds oil was determined by dissolving 2 g of the oil sample in 50 ml of absolute ethanol. The above solution was heated for 3 minutes and titrated with 0.1 N KOH using phenolphthalein as an indicator. The titration was carried out until a faint pink color appeared. (AOAC 969.17) The total acid number was calculated as equation below:

$$\text{Total Acid Number, mgKOH/g} = \frac{(A - B) \times N \times 56.1}{W}$$

Where:

A: Volume of KOH required for the titration of the sample.

B: Volume of KOH required for the titration of the blank.

N: Normality of KOH.

W: Weight of the sample.

### Free Fatty Acid

The watermelon seeds oil was tested for free fatty acid using the American Oil Chemists' Society (AOCS) method Ca 5a-40.

7.05 g of crude oil was weighed in an Erlenmeyer flask, then 100 ml of 95% Ethyl alcohol was added to the sample and mixed well, then 2 ml of phenolphthalein indicator (1% in 95% alcohol) was added. The flask contents were titrated against 0.1M standardized Sodium Hydroxide solution until a faint pink color endpoint was reached.

The percentage of free fatty acid was expressed as Oleic acid as per the following equation:

$$\text{Free Fatty Acid as Oleic acid, \%FFA} = \frac{(A - B) \times M \times 28.2}{W}$$

Where:

A: Volume of NaOH required for the titration of the sample.

B: Volume of NaOH required for the titration of the blank.

M: Molarity of NaOH.

W: Weight of the sample.

### Saponification Value

A conical flask was filled with 2 g of watermelon seeds oil. Then, 25 ml of an alcoholic potassium hydroxide solution was added to the flask. The flask was heated for one hour at 60-70 °C while repeatedly was shaken in boiling water with a reflux condenser attached. Then 1 ml of 1% solution of phenolphthalein was added. The hot excess alkali was then titrated with 0.5 M Hydrochloric acid, and a blank determination was also carried out under the same conditions according to ASTM D464.

The saponification value was estimated using the following Equation:

$$\text{Saponification Value, mgKOH/g} = \frac{(A - B) \times N \times 56.1}{W}$$

Where:

A: Volume of KOH required for the titration of the sample.

B: Volume of KOH required for the titration of the blank.

M: Molarity of the HCl.

W: Weight of the sample.

### Iodine Value

According to ASTM D5554 Iodine value was determined as following: 0.2 g of watermelon seeds oil was mixed with 20 ml cyclohexane and glacial acetic acid (1:1) to dissolve the fat content in 500 ml flask, 25 ml of Wijs' reagent (iodine monochloride solution) was added, and the mixture was then stored in dark place at room temperature for 30 minutes., and 20 ml of 10% potassium iodide (KI) solution was added, followed by 100 ml of distilled water. The mixture was titrated

immediately with 0.1 N sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) until the yellow color disappeared. At this point, 2 ml of the starch indicator was added, and the titration was continued until the blue color completely disappeared. The same was repeated with the blank sample.

The Iodine Value was estimated to be using below Equation:

$$\text{Iodine Value,} = \frac{(S - B) \times 0.1 \times 12.69 \times 100}{W}$$

Where:

B: Titration volume of blank.

S: Titration volume of sample.

N: Normality of  $\text{Na}_2\text{S}_2\text{O}_3$ .

W: Weight of the sample.

### Peroxide Value

Peroxide value analysis conducted according to AOCS CD 8-53 as follows; 2 g of watermelon seeds oil sample was added to conical flask containing a mixture of glacial acetic acid and chloroform in a ratio of 3:2 v/v, and the solution swirled gently to dissolve the oil. 0.5 ml of 0.1 N Potassium Iodide was added to the flask, and the solution was left in the dark cupboard for 5 minutes, 30 ml of Distilled water was added and titrated against 0.1 N Sodium thiosulfate, until the yellow color almost disappeared. 0.5 ml of 1% starch solution was added, and the titration continued with vigorous shaking until the blue color completely disappeared. The same titration was repeated for blank.

The peroxide value was calculated using the following equation:

$$\text{Peroxide Value,} = \frac{(S - B) \times N \times 1000}{W}$$

Where:

S: Volume of 0.01N sodium thiosulphate used for the sample.

B: Volume of 0.01N sodium thiosulphate used for the blank.

N: Normality of  $\text{Na}_2\text{S}_2\text{O}_3$ .

W: Weight of the sample.

### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted using a Nicolet iS5 FT-IR spectrophotometer (Thermo Scientific, Germany) equipped with an attenuated total reflectance (ATR) accessory using a ZnSe crystal. The infrared spectra were recorded to identify the functional groups present in the oil and confirm the presence of key components (Mahamuni and Adewuyi, 2009).

## RESULTS AND DISCUSSIONS

### Oil Extraction

The details of oil extraction from watermelon seeds using different methods. Presented in Table 1. Soxhlet extraction resulted in a higher oil yield (37.3%) compared to mechanical pressing (29.0%). Although mechanical pressing is more environmentally friendly

and suitable for significant production, it tends to leave a higher amount of residual oil. In contrast, the use of n-hexane as a solvent in the Soxhlet method enhances oil

recovery due to its strong ability to dissolve and extract lipids effectively (Handa *et al.*, 2008).

**Table 1: Extracted Oil yield from watermelon seed**

No.	Extraction method	Oil yield %
1	Soxhlet solvent Extraction	37.3
2	Mechanical Pressing	29.0

#### Fatty Acid Profile of Watermelon Seed Oil

The majority of fatty acids in watermelon seed oil were found unsaturated, accounting for approximately 70.5%, compared to about 29.5%

saturated fatty acids. Table 2 provides a detailed analysis of the fatty acid composition in watermelon seed oil. The main unsaturated fatty acids are linoleic acid (50.31%) and oleic acid (19.62%).

**Table 2: Fatty Acid Composition of Watermelon Seed Oil**

Fatty Acids	Formula	Area %	Structure
Linoleic Acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	50.31%	C18:2
Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	19.62%	C18:1
Palmitic Acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	15.25%	C16:0
Stearic Acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	12.38%	C18:0
Myristic Acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	0.06%	C14:0
Cis-11-Eicosenoic Acid	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	0.09%	C20:1
Hydroquinone	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	0.12%	C6:3
Margaric Acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	0.07%	C17:0
Docosanoic Acid (Behenic Acid)	C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>	0.07%	C22:0
Palmitoleic Acid	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	0.07%	C16:1
Stearolic Acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	0.19%	C18:1

#### Physicochemical Characteristics of Watermelon Seeds Oil

The Physicochemical properties of watermelon oil were determined according to ASTM and AOCS standard methods as presented in Table 3.

The acid value of watermelon seeds oil was found to be 6.47 mg KOH/g, which is lower than that of melon oil at 7.85 mg KOH/g and 9.76 mg KOH/g for moringa oil reported by (Afolayan *et al.*, 2014). The acid value of an oil is a key indicator of its quality, typically reflecting the amount of free fatty acids (FFAs) formed as a result of enzymatic hydrolysis (Ogbeide *et al.*, 2022). The acid value and free fatty acids are very important in determining the use of oil for industrial or edibility purposes. The acid value of oil which is suitable for edible purposes should not exceed 0.4 mgKOH/g, which indicates that the acid values of the study samples fall outside the nutritional limit. (Japir *et al.*, 2017).

Free fatty acid (FFA) content in watermelon seeds oil, measured as oleic acid, was found to be 3.2%. The level of free fatty acids (FFAs) in oil is a key measure of its quality and degree of deterioration.

purposes; the lower the free fatty acid the more stable the oil. (Japir *et al.*, 2017)

The saponification value was found to be 191.1 mg KOH/g, which is close to 191.89 mg KOH/g that for watermelon oil in another study reported by (Ogunwale, 2015). The higher saponification value of WSO indicated that this oil is more suitable for soap-making (Keke, *et al.*, 2023).

The Iodine value of watermelon seed oil was found to be 104.22 gI<sub>2</sub>/100g, less than the 157.15 gI<sub>2</sub>/100g reported by (Ogunwale, 2015). This high iodine value indicates a high content of unsaturated fats in watermelon seed oil. The iodine value is a measure of the degree of unsaturated fatty acid present in the oil, reflecting the oil's susceptibility to oxidation (Ogbeide *et al.*, 2022).

Peroxide value of Watermelon oil was found to be 6.82 meq/kg. It is lower than the values recommended by AbuBakr *et al.*, (2020), which were found to be 8.0 meq/kg. The peroxide value indicates the degree of primary oxidation products and the oxidative stability of oil production (Dermiş *et al.*, 2012).

**Table 3: The Physicochemical characteristics of watermelon seed oil**

Test	Method	Watermelon seed oil
Total Acid Number, mg KOH/g	ASTM D 974	6.47
Free Fatty Acids (as Oleic acid), %FFA	AOCS Ca-5a-40	3.2
Iodine Value, gI <sub>2</sub> /100g	ASTM D 5554	104.22



Test	Method	Watermelon seed oil
Saponification Value, mg KOH/g	ASTM D94	191.1
Peroxide, meq/1000g	AOAC 965.33	6.82
Kinematic Viscosity at 40°C	ASTM D445	35.355
Density 15°C, g/ml	ASTM D4052	0.9236
Refractive Index	ASTM D1747	1.473
Water Content, wt%	AOCS Ca 2e-84	0.936
Cloud Point, °C	ASTM D2500	3.5
pour point, °C	ASTM D97	0

Kinematic Viscosity was found to be 35.35cSt at 40 °C. This result of kinematic viscosity is much higher than the Diesel fuel level, which is in the range between 2 – 5 cSt according to ASTM D975.

The density of watermelon seed oil at 15°C was found to be 0.9236 g/ml, which is similar to the reported value of 0.9157 g/ml for Karaya seeds oil (Galander *et al.*, 2017).

The refractive Index for watermelon seed oil was found to be 1.473. Which is closer to the Refractive index for crude and refined groundnut oil, varied from 1.463 to 1.466, which is reported by (Shumi and Getacho, 2022).

The refractive index of a medium is the ratio of the speed of light at a definite wavelength in vacuum to its speed in the medium. The value of the refractive factor depends on both the wavelength of the light used and the temperature of the material (Lakhe *et al.*, 2022).

The cloud point of the obtained watermelon seeds oil was found to be 3.5°C, and the pour point was

0°C. Water Content of watermelon seeds oil was found to be 0.936 wt.% which is less than the 5.8 wt.% which was reported by (Ogunwole, 2015).

#### Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy into a valuable technique for qualitatively analyzing organic compounds found in natural products (Rai *et al.*, 2015). The watermelon oil sample was subjected to FT-IR analysis to investigate the functional group. Figure 2 shows the plot of transmittance (%) versus wavenumber (cm<sup>-1</sup>) from the FT-IR study. The peak at 1743 cm<sup>-1</sup> showed the existence of the carbonyl functional group (C=O), and the carbon skeleton vibration peak at 723 cm<sup>-1</sup> Furthermore, the stretching vibration of the C-O group attached to -CH<sub>2</sub> corresponds to the triglyceride spectrum peak at 1163 cm<sup>-1</sup>, at 1453 cm<sup>-1</sup> absorption wavelength the -C-H bending of alkanes was observed. The frequency bands at 1652.88 are attributed to -C=C- symmetric stretching of alkenes, unsaturated C-H stretching vibration peak of carbon chain at 3008 cm<sup>-1</sup>, saturated carbon chain C-H stretching vibration peak at 2923 cm<sup>-1</sup> and 2857 cm<sup>-1</sup> stretching vibration peak of C-O in triglyceride at 1163 cm<sup>-1</sup> (Bichi *et al.*, 2022).

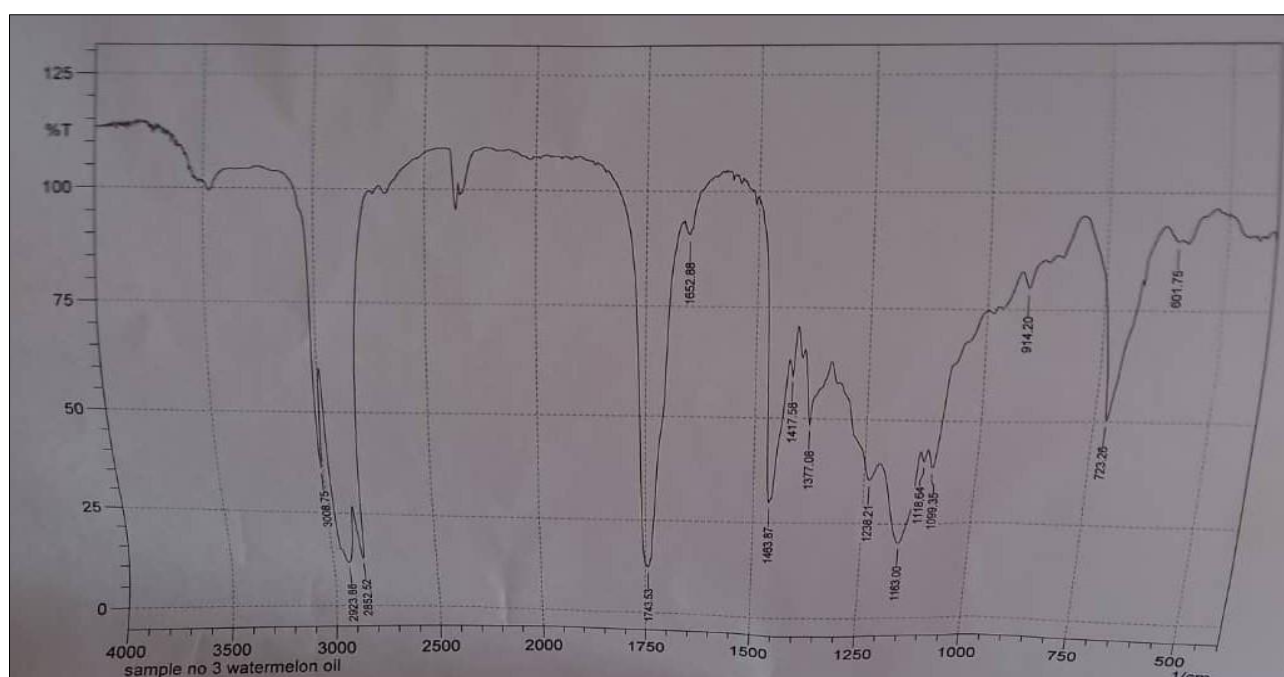


Figure 2: FT-IR spectra of watermelon seed oil

## CONCLUSIONS

The following results could be concluded from this study:

- Chemical extraction using the Soxhlet method yielded the highest yield of oil and was more useful than mechanical pressing.
- Watermelon seed oil contains 70.5% unsaturated fatty acids and 29.5% saturated fatty acids.
- The Physical and Chemical properties of watermelon oil emerge as a potential feedstock for biodiesel production.

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