

## Fatty Acid Composition and Spectroscopic Analysis of Oil from *Citrus Sinensis* Seed

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**Abstract:** Oil from *Citrus sinensis* seeds was extracted via cold maceration using *n*-hexane after air-drying and pulverization, yielding 36.21% oil. The physicochemical properties of the oil were determined, giving an acid value of 7.55 mg KOH/g, saponification value of 193.58 mg KOH/g, and iodine value of 83.48 g I<sub>2</sub>/100 g, indicating moderate unsaturation and suitability for industrial applications such as soap and cosmetic production. Gas Chromatography–Mass Spectrometry (GC–MS) analysis revealed that the oil is rich in unsaturated fatty acids (71.01%), with linoleic acid (35.43%) as the predominant component, followed by oleic acid (26.44%), while palmitic acid (20.90%) and stearic acid (5.10%) were the major saturated fatty acids. Fourier Transform Infrared (FT-IR) spectroscopy identified characteristic functional groups, including carbonyl (C=O), hydroxyl (O–H), and unsaturated (C=C) bonds, while UV–Visible spectroscopy showed absorption peaks at 290 nm and 370 nm corresponding to  $\pi$ – $\pi^*$  and  $n$ – $\pi^*$  transitions, respectively.

**Keywords:** *Citrus sinensis*; Fatty acid composition; Physicochemical properties; Spectroscopic analysis.

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### 1.0 Introduction

Vegetable oils are important natural resources widely utilized in food, pharmaceutical, and cosmetic industries due to their nutritional and functional properties. They are primarily obtained from plant sources such as seeds, fruits, or seedlings (Zielińska & Nowak, 2014). These plant-based oils are made up of triglycerides, which consist of both saturated and unsaturated higher fatty acids. In other words, these compounds are esters of glycerol and higher fatty acids, containing in their structure long aliphatic carbon chains (Lamer-Zarawska *et al.*, 2012). In recent years, increasing attention has been directed toward underutilized plant seeds as alternative sources of valuable oils with potential industrial and health applications. Fatty acids can be classified into two categories based on the presence and number of double bonds: saturated fatty acids, which

have no double bonds, and unsaturated fatty acids, which contain one or more double bonds (Zielińska & Nowak, 2014). Animal sources like sheep, cows, and pigs as well as plant sources like barley, oats, seeds, and fruits like avocados and coconuts, are the two main sources of fats and oils (Robinson, 1999). *Among such underutilized plant resources, Citrus sinensis (sweet orange), a member of the Rutaceae family, has attracted considerable interest due to its wide availability and rich phytochemical composition.*

(Bovili, 1996; Piccinelli *et al.*, 2008; Atolani *et al.*, 2012; Jorge *et al.*, 2016). As one of the natural staple foods for humans, *Citrus sinensis* provides an abundance of essential nutrients. Its juice has long played an important role in the human diet due to its nutritional value and health-promoting properties (Okwu & Emenike, 2006; Ezejiofor *et al.*, 2011). Citrus fruits, in general, are rich sources of vitamins, minerals, and enzymes. Studies indicate that while they are free from fat and cholesterol, they are rich in essential minerals such as silicon, calcium, phosphorus, magnesium, and potassium (Assa *et al.*, 2013). Citrus flavonoids are known for their high nutritional value and various applications, and have been reported to exhibit biological activity and antioxidant health benefits (Etebu & Nwauzoma, 2014; Tripoli *et al.*, 2007). Orange fruits possess anti-scurvy properties (Rapisararda, 1999), while orange waste/trash and other by-products have been utilized to produce value-added substances such as ethanol, hesperidin, and nanocellulose (Cypriano *et al.*, 2018). Previous studies have largely focused on the nutritional composition, antioxidant properties, and industrial applications of citrus fruits and their peels, with comparatively less attention given to the detailed chemical characterization of their seed oils.

Research has also shown that several underutilized seeds possess antimicrobial activity (Atolani *et al.*, 2019a; Atolani *et al.*, 2019b; Babatolu *et al.*, 2025b). *Citrus*

*sinensis* is a rich source of vitamin C and dietary fiber, a strong natural antioxidant. It also contains folate (vitamin B-9), which is crucial for red blood cell formation and the growth and function of healthy cells, and other bioactive compounds such as flavonoids and carotenoids, known for their roles in preventing cancer and degenerative diseases (Rapisararda, 1999; Cushnie & Lamb, 2005; Ejaz *et al.*, 2006; Pultrini *et al.*, 2006).

Despite these studies, there is limited information on the comprehensive characterization of *Citrus sinensis* seed oil, particularly with respect to its fatty acid composition and spectroscopic properties using combined analytical techniques such as GC-MS, FT-IR, and UV-Visible spectroscopy. Furthermore, the potential of these underutilized seeds as a sustainable source of industrial oil remains insufficiently explored.

This study, therefore, aims to extract and characterize oil from *Citrus sinensis* seeds using Gas Chromatography–Mass Spectrometry (GC-MS), Fourier Transform Infrared (FT-IR), and Ultraviolet–Visible (UV–Vis) spectroscopy to determine its fatty acid composition and evaluate its potential applications in food, cosmetic, and pharmaceutical industries. The outcomes of this study will contribute to the valorization of citrus seed waste, provide scientific data for industrial utilization, and promote sustainable resource management. Studies have also demonstrated that several underutilized plant seeds possess significant antimicrobial activity...

## 2.0 Materials and Methods

### 2.1 Plant Collection and Identification

Fresh *Citrus sinensis* fruits were collected from Ondo town, Ondo State, Nigeria, during the fruiting season. The plant material was authenticated by a botanist at the Department of Biological Sciences, Adeyemi Federal University of Education, Ondo, and a voucher specimen was deposited in the herbarium for future reference.

### 2.2 Solvents and Chemical Reagents



The solvents and chemical reagents used in the study included n-hexane, ethyl acetate, potassium hydroxide, methanol, sodium thiosulphate, ethanol, acetic acid, glacial acetic acid, chloroform, diethyl ether, potassium iodide, hydrochloric acid, among others. All chemicals were of analytical grade and were obtained from standard commercial suppliers.

### 2.3 Preparation of Seed Sample

The fresh fruits were sliced open with a knife and manually squeezed to extract the seeds from the pulp. The seeds were then air-dried at room temperature for six weeks. Once dried, they were manually de-shelled, pulverized using an electric blender, weighed, and stored in a container for subsequent analysis.

The powdered seed sample was subjected to cold maceration using n-hexane for 96 hours in a sealed container with intermittent agitation, and the filtrate/extract was concentrated using a rotary evaporator. The extracted oil was air-dried at room temperature (25–28 °C) for six weeks until constant weight was achieved. The percentage yield of the extracted oil was calculated using standard methods.

### 2.4 Physicochemical Parameters of the Oil

The physicochemical properties (acid value, saponification value, iodine value, and colour) were determined using standard methods as described by Gerpen (2005), Ibeto et al. (2012), Abdulhamid et al. (2014), Kayode (2015), and Atolani et al. (2016), with slight modifications.

### 2.5 Trans-esterification Determination

A 2 g sample of the oil was transferred into a beaker and mixed with 10 mL of 0.2 M methanolic HCl. The mixture was refluxed for 1 hour and subsequently transferred into a separating funnel. A mixture of n-hexane (20 mL) and distilled water (10 mL) was added and shaken vigorously to facilitate phase separation. The aqueous layer was discarded, and the organic layer containing fatty acid methyl esters (FAMES) was collected and

further purified with n-hexane. The resulting FAMES were stored for GC-MS analysis (Atolani et al., 2016).

### 2.6 Gas Chromatography–Mass Spectrometry (GC–MS)

The fatty acid composition of the trans-esterified oil was determined using an Agilent Technologies Network 6890N gas chromatograph, connected to an Agilent Technologies 5975B inert mass selective detector (MSD). The GC was equipped with a capillary column (specify type and dimensions if available), and helium was used as the carrier gas. The injector and detector temperatures, as well as the oven temperature program, were set according to standard analytical conditions.

Identification of the fatty acid methyl esters (FAMES) was carried out by comparing their mass spectra with the National Institute of Standards and Technology (NIST, 2008) database. The relative abundance of each component was expressed as a percentage, based on the peak areas obtained from the gas chromatography (GC) analysis using the total ion chromatogram (TIC).

### 2.7 Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR analysis was carried out using a Nicolet iS5 FT-IR spectrometer with KBr pellet technique to identify the functional groups present in the oil sample.

### 2.8 Ultraviolet–Visible Spectrophotometry Analysis

An ultraviolet–visible spectrophotometer (Beckman, UK) was used to record the absorption spectrum of the oil sample over a specified wavelength range.

## 3.0 Results and Discussion

### 3.1 Physicochemical Properties of the Seed Oil

The physicochemical characteristics of the oil were determined using standard procedures and are presented in Table 1. The oil extracted from *Citrus sinensis* seeds has an acid value of 7.55 mg KOH/g, as shown in Table 1, which aligns with the values previously reported for *Citrus sinensis* seed



oil (Babatolu *et al.*, 2025a). Lower acid values indicate reduced free fatty acid content and greater resistance to rancidity. The lower the acid values, the lower the free fatty acids it contains, which makes it less exposed to rancidity. Acid value is an important indicator of oil quality and reflects the extent of hydrolytic and oxidative degradation. Additionally, a low acid value may be attributed to the presence of antioxidants and phenolic compounds (Aremu *et al.*, 2015; Atolani *et al.*, 2016). As a result, the observed acid value supports the oil's potential use in cosmetic formulations (Afolabi, 2008).

**Table 1: Physicochemical properties of *Citrus sinensis* seed oil.**

Parameter	<i>Citrus Sinensis</i>
Acid value	7.55 (mg KOH/g)
Saponification value	193.58 (mg KOH/g)
Iodine value	83.48 (gI <sub>2</sub> /100g)
% Oil trans-esterified	91.88
Colour	Golden-yellow
Physical state at ambient temperature (27 <sup>o</sup> C)	Liquid
% Oil Yield	36.21

The seed oil of *Citrus sinensis* recorded a saponification value of 193.58 mg KOH/g, as shown in Table 1, which aligns with previously reported values for *Citrus sinensis* seed oil (Babatolu *et al.*, 2025a). This high saponification value suggests the presence of fatty acids with lower molecular weights, and

therefore falls within the recommended range of edible oils, which indicates that the oil has potential applications in cosmetic and personal care products. (Emmanuel *et al.*, 2012; Aladekoyi *et al.*, 2016; Babatolu *et al.*, 2025c).

The iodine value serves as an indicator of the degree of unsaturation in oils, with higher values reflecting a greater level of unsaturated fatty acids. Based on iodine values, oils are classified as drying (>130), semi-drying (90–130), or non-drying (<90) based on their iodine values (Güner *et al.*, 2006). (Güner *et al.*, 2006). The *C. sinensis* seed oil exhibited an iodine value of 83.48 g I<sub>2</sub>/100 g (Table 1), categorizing it as a non-drying oil, which makes it more appropriate for use in cosmetic products like soaps and creams.

At ambient temperature, the oil remained liquid and had a pleasant fragrance. The percentage yield of trans-esterified oil from *Citrus sinensis* seed was 91.88%, which is consistent with previously reported values (Babatolu *et al.*, 2025a).

### 3.2 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of Trans-esterified Oil

The Gas Chromatography Mass Spectrometry (GC-MS) result of the oil is depicted in Table 2. The predominance of unsaturated fatty acids (71.01%) over saturated fatty acids (28.99%) suggests that the oil may possess favorable nutritional and functional properties, as unsaturated fats are associated with improved health benefits.

**Table 2: Fatty acid composition of *C. sinensis* seed oil**

Fatty Acids	Short name	% Composition
Palmitic acid	C16:0	20.90
Palmitoleic acid	C16:1	0.40
Heptadecenoic acid	C17:1	0.44
Stearic acid	C18:0	5.10
Elaidic acid	C18:1n9t	1.40
Oleic acid	C18:1n9c	26.44
Linoleic acid	C18:2n6c	35.43



<b><math>\alpha</math>-Linolenic acid</b>	C18:3n3	4.10
<b>Arachidic acid</b>	C20:0	0.62
<b>Eicosadienoic acid</b>	C20:2	0.36
<b>Heneicosanoic acid</b>	C21:0	0.38
<b>Arachidonic acid</b>	C20:4n6	0.38
<b>Cis-11,14,17-eicosatrienoic acid</b>	C20:3n3	0.37
<b>Behenic acid</b>	C22:0	0.30
<b>Cis-13,16-docosadienoic acid</b>	C22:2n6	0.74
<b>Tricosanoic acid</b>	C23:0	1.31
<b>Lignoceric acid</b>	C24:0	0.38
<b>Nervonic acid</b>	C24:1	0.40
<b>Docosahexaenoic acid</b>	C22:6n3	0.42
<b>Total Saturated</b>		28.99
<b>Monounsaturated</b>		29.21
<b>Polyunsaturated</b>		41.80
<b>Total Unsaturated</b>		71.01

The fatty acid profile of *Citrus sinensis* seed oil, as shown in **Table 2**, reveals the presence of key fatty acids such as palmitic acid (20.90%), oleic acid (26.44%), linoleic acid (35.43%), and stearic acid (5.10%). Of the total fatty acid content, 28.99% is saturated, while 71.01% is unsaturated.

From the table, the fatty acid composition of *Citrus sinensis* is similar to that of the fatty acid composition of *Citrus paradisi* and *Citrus lemon* seed oils previously reported by Babatolu *et al.* (2025d).

The seed oil contains a high proportion of essential fatty acids, particularly linoleic acid (omega-6) and linolenic acid (omega-3), which are vital for human nutrition but cannot be synthesized by the body. Additionally, oleic acid (omega-9) and palmitic acid are present in substantial amounts, both of which are valuable in industrial applications such as paint production and cosmetic formulation. Linoleic acid is especially known for its skin-moisturizing and wound-healing properties, making it a common ingredient in cosmetic products. Oils rich in linoleic and linolenic acids are recognized as effective natural anti-inflammatory agents, useful in managing acne and related skin conditions (Lautenschläger, 2003; Vermaak *et al.*, 2011). Linoleic acid, a major component of the oil, is widely recognized for its skin-moisturising, anti-inflammatory, and wound-

healing properties, making it valuable in cosmetic formulations (Lautenschläger, 2003; Kanlayavattanukul & Lourith, 2011). Thus, the fatty acid composition of *C. sinensis* seed oil indicates its potential for use in the food and cosmetic industries, as well as in the formulation of drugs and dietary supplements, if properly harnessed and optimized for industrial applications.

### 3.3 Fourier Transform Infrared (FTIR) Analysis of the extracted oil

The FT-IR spectral characteristics of the oil are presented in Table 3.

**Table 3: FT-IR Value for *Citrus sinensis* seed oil**

Wave number (cm <sup>-1</sup> )	Assignment/Infrared bands
<b>1743</b>	C=O stretch
<b>1238</b>	C-O stretch
<b>2924</b>	Sp <sup>3</sup> C-H stretch
<b>3010</b>	Sp <sup>2</sup> C-H stretch
<b>1650</b>	C=C stretch
<b>3433</b>	O-H stretch
<b>1464</b>	C-H bending

The FTIR spectrum of *Citrus sinensis* (orange) seed oil exhibited an absorption band at 1743 cm<sup>-1</sup>, indicating the presence of a carbonyl group (C=O), while the band at 1238 cm<sup>-1</sup> corresponded to a C-O stretch,



confirming the presence of an ester functional group. An absorption band observed at  $3433\text{ cm}^{-1}$  revealed the presence of an alcohol (O–H) group. The stretch at  $3010\text{ cm}^{-1}$ , attributed to  $\text{sp}^2$  C–H, signified the presence of an alkene (C=C), further supported by a peak at  $1650\text{ cm}^{-1}$ . Additionally, absorption at  $2924\text{ cm}^{-1}$  was due to  $\text{sp}^3$  C–H stretching, indicating saturated hydrocarbon chains,

along with a C–H bending vibration observed at  $1464\text{ cm}^{-1}$ . These functional groups are characteristic of triglyceride structures commonly found in vegetable oils.

### 3.4 Ultraviolet-Visible Spectroscopic Data of the extracted oil

The UV–Visible spectroscopic data of the extracted oil are presented alongside the FT-IR results in Table 4.

**Table 4: Results of UV–Visible Spectroscopy and FT-IR Analysis of *Citrus sinensis***

Oil	UV Wave length (nm)	IR ( $\text{cm}^{-1}$ )	Transition/Assignment	Remark
<i>Citrus sinensis</i>	290	1650	C=C band ( $\pi$ - $\pi^*$ )	C=C band
	370	1743	C=O band ( $n$ - $\pi^*$ )	C=O band

The oil exhibited two absorption peaks in the UV spectrum, corresponding to  $\pi$ - $\pi^*$  transitions of C=C bonds and  $n$ - $\pi^*$  transitions of C=O functional groups at 290 and 370, respectively.

### 4.0 Conclusion

This study successfully extracted and characterized oil from *Citrus sinensis* seeds using physicochemical analysis, Gas Chromatography–Mass Spectrometry (GC–MS), Fourier Transform Infrared (FT-IR), and Ultraviolet–Visible (UV–Vis) spectroscopy. The oil yield was appreciable, and the physicochemical parameters indicated good stability and suitability for industrial applications. GC–MS analysis revealed that the oil is rich in essential fatty acids, particularly linoleic acid, oleic acid, and palmitic acid, with a high proportion of unsaturated fatty acids. FT-IR and UV–Vis analyses further confirmed the presence of key functional groups such as carbonyl and unsaturated bonds, characteristic of triglyceride-based oils.

Overall, the findings demonstrate that *Citrus sinensis* seed oil, often regarded as waste, possesses significant potential as a valuable raw material for food, cosmetic, pharmaceutical, and nutraceutical applications. Its utilization could contribute to waste valorization, sustainable resource

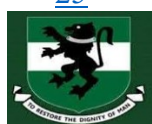
management, and the development of value-added industrial products.

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Not Applicable

**Availability of data and materials**

The publisher has the right to make the data public

**Conflict of Interest**

The authors declared no conflict of interest

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**Authors' Contributions**

Ayotunde O. Babatolu led the study, sourcing plant material, performing extraction, physicochemical and trans-esterification analyses, and GC-MS interpretation. Hammed O. Oloyede handled spectroscopic analyses and drafting. Ibrahim O. Oloruntele guided characterization and revisions. Justinah S. Amoko supported design and spectroscopy. Tunde S. Ogungbemi assisted preparation and data collection, while Abidemi I. Demehin supervised, validated results, and edited. All authors approved.

