

Comparative Study of the Physicochemical Properties of Oil from Underutilized Seeds of *Citrus Paradisi*, *Citrus Lemon* and *Citrus Sinensis*

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Article History:

Received: 25.02.2025 Revised: 12.03.2025 Accepted: 14.03.2025 Published: 14.03.2025

Abstract

Citrus paradisi, *Citrus lemon*, and *Citrus sinensis* seeds were cold extracted in this research using n-hexane after air drying and grinding, the percentage yield of *Citrus paradisi*, *Citrus lemon* and *Citrus sinensis* oil extract was 26.16 %, 24.79 % and 34.54 % respectively. Using standard methods, the physicochemical properties of the oils from *Citrus paradisi*, *Citrus lemon*, and *Citrus sinensis* were ascertained yielding the following results respectively; Acid value: 0.62 mg KOH/g, 2.26 mg KOH/g and 7.56 mg KOH/g; Saponification value: 195.86 mg KOH/g, 188.00 mg KOH/g and 193.65 mg KOH/g; Iodine value: 126.53 gI₂/100g, 110.66 gI₂/100g and 83.50 gI₂/100g; Peroxide value: 6.51 meq/kg, 0.62 meq/kg and 5.72 meq/kg; Specific gravity: 0.85 g, 0.85 g and 0.87 g; Density: 0.87 g/cm³, 0.95 g/cm³ and 0.88 g/cm³; pH value: 4.92, 4.26 and 4.21; Ester value: 195.24 mg KOH/g, 185.74 mg KOH/g and 186.09 mg KOH/g. Instead of allowing them to become a worrying threat as pollutants and waste in the environment, the oils from *Citrus paradisi*, *Citrus lemon*, and *Citrus sinensis* seeds could be used domestically in homes as edible oils and in the cosmetic and pharmaceutical industries to make soap, lubricant, creams, and drugs.

Keywords: *Citrus paradisi*, *Citrus lemon*, *Citrus sinensis*, Extraction, Rotary evaporator, Physicochemical properties.

Suggested citation: Babatolu, A.O., Akinsuli, V.O., Adekunbi, E.A., Oloyede, H.O., Oyebade, A., Babatolu, O.I., & Dada, S.F. (2025). Comparative Study of the Physicochemical Properties of Oil from Underutilized Seeds of *Citrus Paradisi*, *Citrus Lemon* and *Citrus Sinensis*. *European Journal of Theoretical and Applied Sciences*, 3(2), 294-301. [https://doi.org/10.59324/ejtas.2025.3\(2\).25](https://doi.org/10.59324/ejtas.2025.3(2).25)



Introduction

Citrus paradisi, an evergreen tree native to the Asiatic South West (West India, Tropical Asia), belongs to the Rutaceae family. The juicy, sour-bitter pulp of the grapefruit is yellow or yellow-red in colour. This fruit's flesh is utilized to treat poisoning and to cool down during a break (Sharamon and Baginski, 1998). Citrus paradisi seed extracts have been used to treat urinary tract infections, ulcers, cataracts, and gastrointestinal tract infections (Krzysztof *et al.*, 2003; Oyelami *et al.*, 2005).

Polyphenols and other reducing agents, such as vitamin C and E, known as antioxidants, found in citrus fruits, vegetables, and other fruits have been shown to protect the body against diseases related to oxidative stress (Rice-Evans and Miller, 1995; Drewnowski and Gomez-Carneros, 2000; Scalbert and Williamson, 2000). The oil from grapefruit peels has been used as an insecticide and antifeedant (Tirillini, 2000).

A significant medicinal plant in the Rutaceae family is the Lemon, commonly referred to as the *Citrus lemon*. The primary reason citrus lemons are grown is for their alkaloids, which have antibacterial and anticancer properties in crude extracts of various lemon leaf, stem, root, and flower sections. These extracts are utilized to combat clinically significant bacterial strains (Kawai *et al.*, 2000).

Oval citrus fruits, lemons have smooth, porous skins. Although lemons and limes have a similar appearance, lemons are typically larger and turn yellow when ripe, whereas limes are green (Mohanapriya *et al.*, 2013).

Citrus fruit contains flavonoids that have a wide range of biological activity, such as antiviral, antifungal, antidiabetic, antibacterial, and anticancer properties/activities (Burt, 2004; Ortuno *et al.*, 2006). According to Duthie and Crozier (2000), flavonoids can regulate enzymatic activity, restrict or halt cell proliferation, and act as direct antioxidants and free radical scavengers. Moreover, citrus fruits include fiber that contains bioactive substances/compounds like polyphenols, the most significant of which is vitamin C (also

known as ascorbic acid), which prevents and treats vitamin C deficiency, which results in scurvy (Aronson, 2001).

According to Assa *et al.* (2013), they are generally great providers/sources of minerals, vitamins, and enzymes. They are also free of fat and cholesterol and include essential mineral components including silicon, potassium, calcium, phosphorus, and magnesium.

According to Mohanapriya *et al.* (2013), *Citrus lemon* is used to treat a variety of conditions, including respiratory problems/disorders, eye care, gout, gums, piles, weight loss, peptic ulcers, urinary disorders, constipation, arthritis, rheumatism, diabetes, fatigue, and heart ailments/diseases.

Citrus sinensis is a member of the Rutaceae family and the Aurantioideae subfamily. It is grown extensively in Nigeria and many other tropical and subtropical regions (Piccinelli *et al.*, 2008). Orange, also known as the sweet orange (*Citrus sinensis* L. Osbeck), is a major source of vitamins, particularly vitamin C, as well as a sufficient amount of folacin, Calcium, Potassium, Thiamine, Niacin, and Magnesium (Angew, 2007). Due to its high nutritional value and other uses, citrus flavonoids have been reported to have biological action and health effects as antioxidants (Etebu and Nwauzoma, 2014; Tripoli *et al.*, 2007).

Citrus sinensis is rich in vitamin C, contains dietary fiber, a potent natural antioxidant, folate (vitamin B-9) which is essential for the production of red blood cells as well as for the growth and function of healthy cells, and other bioactive components like flavonoids and carotenoids that prevent cancer and degenerative diseases (Ejaz *et al.*, 2006). Consuming meals high in vitamin C strengthens the body's defence against infectious diseases and helps the body rid itself of harmful, pro-inflammatory free radicals. Hesperetin and naringenin are among the many phytochemicals found in sweet orange. As an antioxidant, scavenger of free radicals, anti-inflammatory, and immune system modulator, naringenin has a

bioactive impact on human health (Etebu and Nwauzoma, 2014).

In view of the medicinal significance of *Citrus paradisi*, *Citrus lemon* and *Citrus sinensis*, this work was carried out to ascertain and compare the physicochemical properties of the fixed oil from the seeds of *Citrus paradisi*, *Citrus lemon* and *Citrus sinensis* in order to establish other beneficial uses/applications beyond their use in nutrition.

Materials and Methods

Collection and Preparation of Sample

Fresh fruits of *Citrus paradisi*, *Citrus lemon* and *Citrus sinensis* were bought from the market in Ondo town, Ondo State. Using a knife, the fruits were cut open, and the seeds were taken out. Following a water rinse, the seeds were allowed to dry for five weeks at room temperature. The dried seeds were de-shelled and pulverized with a blender, weighed and packed in a container for further analysis.

A glass jar containing approximately 365.45 g of grape seeds, 315.42 g of lemon seeds, and 110.00 g of orange seeds each were subjected to a maximum of five days of cold extraction using n-hexane. It was filtered and concentrated using rotary evaporator.

After the oil was air dried to eliminate any leftover solvent, it was weighed. The oil was poured into a sample bottle for further analysis. The percentage yield of the oils was determined.

Physicochemical Properties of the Oil

The physicochemical parameters of the oil were determined using the standard procedures.

Determination of Saponification Value

A conical flask was filled with precisely 1 g of each oil, and 25 ml of ethanolic potassium hydroxide was then added. For half an hour (30 minutes), the mixture was refluxed in a bath of boiling water. After adding 3 drops of phenolphthalein, the pink colour was titrated against 0.5 M HCl until it vanished. A blank titration was also performed (Pearson, 1991; Abdulhamid *et al.*, 2014; Kayode, 2015).

Determination of Iodine Value

A precise 1 g sample of each oil was weighed into a 250 ml conical flask, and 25 ml of carbon tetrachloride was used to dissolve the oil. The flask was sealed and shaken after 25 ml of wiji's solution was added. One hour was spent letting the mixture stand in the dark. After that, the released/liberated iodine was titrated using a starch indicator against 0.1M sodium thiosulphate. A blank titration was also performed (Pearson, 1991; Gerpen, 2005; Ibeto *et al.*, 2012; Kayode, 2015).

Determination of Acid Value

A flask containing precisely 1 g of each oil, 25 ml of diethyl ether, 25 ml of methanol, and 3 drops of phenolphthalein indicator was added to the mixture. According to Pearson (1991); Gerpen (2005); Ibeto *et al.* (2012); Abdulhamid *et al.* (2014); Kayode (2015); the mixture was warmed over a water bath for five minutes before being titrated against 0.1M KOH while being continuously shaken until the pink colour appeared, signifying the end point.

Determination of Specific Gravity and Density

A 50 ml measuring cylinder was washed, dried, and weighed was recorded as W_0 in order to establish the specific gravity. 1.4 ml and 1.8 ml of the oil was measured into the measuring cylinder and re-weighed, and was recorded as W_1 . The measuring cylinder was washed and dried. 1.4 ml and 1.8 ml of distilled water was measured into the measuring cylinder and weighed, and the value was recorded as W_2 (Pearson, 1991; Abdulhamid *et al.*, 2014; Kayode, 2015). The specific gravity was calculated using the expression;

$$\text{Specific gravity} = \frac{W_1 - W_0}{W_2 - W_0} \quad (1)$$

A 50 ml measuring cylinder was properly washed, dried, weighed, and marked as M_0 in order to calculate density. 1.4 ml and 1.8 ml of the oil was measured into the measuring cylinder and weighed, and was recorded as M_1 . The volume of the oil samples was recorded as V_1

(Pearson, 1991; Abdulhamid *et al.*, 2014; Kayode, 2015).

Density was calculated using the expression;

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}} \quad (2)$$

Determination of Peroxide Value

Each oil sample weighed precisely 0.5 g and was placed in a conical flask with 1 g of potassium iodide. It was mixed with a solvent mixture of 20 ml (13 ml glacial acetic and 7 ml chloroform). The conical flask was placed on a water bath for about one minute after which 20 ml of 5% potassium iodide and 25 ml of water were added to the mixture inside the conical flask. Then, using a starch indicator, this was titrated against a 0.002 M sodium thiosulphate solution until it was colourless. Blank titration was also performed (Pearson, 1991; Gerpen, 2005; Ibeto *et al.*, 2012; Abdulhamid *et al.*, 2014; Kayode, 2015).

Determination of Ester Value

The difference between the acid value and the saponification value was used to calculate the ester value (Pearson, 1991; Gerpen, 2005; Ibeto *et al.*, 2012; Abdulhamid *et al.*, 2014; Kayode, 2015).

Determination of Trans-Esterification

After weighing a precise 2 g sample of each oil, it was put into a beaker with 10 ml of 0.2 M methanolic HCl. After an hour of refluxing, the mixture was transferred into a separating funnel. After adding 20 ml of n-hexane and 10 ml of water to the separating funnel and shaking it vigorously to allow the two layers to separate, the aqueous layer (water) was decanted into a beaker and then poured back into the separating funnel to be cleaned again with 10 ml of n-hexane. The fatty acid methyl esters (FAMES), which make up the organic layer (oil), were also decanted and kept for GC-MS analysis. The percentage yield of the trans-esterified oil was determined using the expression (Atolani *et al.*, 2016);

$$\text{Percentage yield} = \frac{\text{weight of trans-esterified oil} \times 100}{\text{Weight of lipid}} \quad (3)$$

Determination of pH

The pH value of the oils was measured at room temperature and recorded after the pH electrode was calibrated/standardized using buffer solution (Pearson, 1991; Abdulhamid *et al.*, 2014; Kayode, 2015).

Results and Discussion

Table 1. Physicochemical Parameters of Oils from Seeds of *Citrus paradisi* (Grape), *Citrus lemon* (Lemon) and *Citrus sinensis* (Orange) Fruits

Parameter	<i>Citrus paradisi</i>	<i>Citrus lemon</i>	<i>Citrus sinensis</i>
Saponification value (mg KOH/g)	195.86	188.00	193.65
Iodine value (gI ₂ /100g)	126.53	110.66	83.50
Acid value (mg KOH/g)	0.62	2.26	7.56
Specific gravity (g)	0.85	0.85	0.87
Density (g/cm ³)	0.87	0.95	0.88
Peroxide value (meq/kg)	6.51	0.62	5.72
Ester value (mg KOH/g)	195.24	185.74	186.09
% oil trans-esterified	71.65	91.55	91.86
pH value	4.92	4.26	4.21
% yield of extract	26.16	24.79	34.54
Colour	Light yellow	Light yellow	Golden-yellow
Physical state at ambient temperature (27 ^o C)	Solid	Liquid	Liquid

At room temperature, the oil from the seed of *Citrus paradisi* (grape) was solid, whereas that of *Citrus lemon* (lemon) and *Citrus sinensis* (orange) was liquid and had a pleasant scent/smell.

The percentage yield of trans-esterified oil for *Citrus paradisi* (grape) gave 71.65 % while that of *Citrus lemon* (lemon) and *Citrus sinensis* (orange) gave 91.55 % and 91.86 % respectively.

Acid value represents the amount of free fatty acids presents in an oil and it is an excellent predictor of oil degradation induced/caused by hydrolysis (Nizam, 2009). Acid value of the *Citrus paradisi* (grape) gave 0.62 mg KOH/g which was within the range reported for oil from seed of Palm kernel 0.834 mg KOH/g suitable for soap production (Afolabi, 2008). In contrast, *Citrus lemon* (lemon) and *Citrus sinensis* (orange) had acid values of 2.26 mg KOH/g and 7.56 mg KOH/g, respectively, which were lower than the oil from *Dennettia tripetala* (pepper fruit) that had an acid value of 5.34 mg KOH/g (Nwinuka and Nwiloh, 2009) and Shea butter that had an acid value of 10.3 mg KOH/g (Warra *et al.*, 2009).

The saponification value of *Citrus paradisi* (grape) was found to be 195.86 mg KOH/g, while that of *Citrus lemon* (lemon) and *Citrus sinensis* (orange) yielded 188.00 mg KOH/g and 193.65 mg KOH/g, respectively. These values were within the range of oil reported for olive oil (192 mg KOH/g) and sunflower oil (188.7 mg KOH/g), but higher than that of beeswax (93 mg KOH/g), which are frequently used in soap production (Mabrouk, 2005). Additionally, the saponification values of *Citrus paradisi* (grape), *Citrus lemon* (lemon), and *Citrus sinensis* (orange) are lower than those of neem seed oil (213 mg KOH/g) and coconut oil (253 mg KOH/g) (Akpan, 1999; Oshinowo, 1987). This suggested that the oil could be used to make soap because its saponification value is within the range of these oils, which, due to the lower molecular weight of the fatty acids in the oils, fell within the approved range of edible oils. Saponification value represents the average molecular weight of triglycerides in the oil. The lower the saponification value, the larger the molecular weight of fatty acids in the glycerides and vice versa (Nizam, 2009).

Iodine value expresses the unsaturation level of the oil; higher iodine value indicates higher degree of fatty acids unsaturation. The iodine value of *Citrus paradisi* (grape) was found to be 126.53 gI₂/100g, which falls within the range reported for *Glycine max* 126 gI₂/100g (Okullo *et al.*, 2010). *Citrus lemon* (lemon) and *Citrus sinensis* (orange) yielded 110.66 gI₂/100g and 83.50 gI₂/100g, respectively, which were higher than that of cashew nut oil (44.4 gI₂/100g) (Aremu *et al.*, 2006a). According to their iodine values, triglyceride oils can be classified as drying, semi-drying, or non-drying. A drying oil's iodine value is greater than 130, whereas a semi-drying oil's is between 90 and 130. If the iodine value is less than 90, the oil is classified as non-drying (Guner *et al.*, 2006), which categorizes *Citrus paradisi* and *Citrus lemon* seed oil as semi-drying oil and *Citrus sinensis* seed oil as non-drying oil, which is more suitable for soap production.

Citrus paradisi (grape) had a peroxide value of 6.51 meq/kg, while *Citrus lemon* (lemon) and *Citrus sinensis* (orange) had peroxide values of 0.62 meq/kg and 5.72 meq/kg, respectively. *Citrus sinensis* (orange) had a value range similar to that of egusi (5.80 meq/kg) and higher than the peroxide values for pawpaw (3.12 meq/kg) (Dugo and Bonaccorsi, 2013; Abdulhamid *et al.*, 2014). *Citrus paradisi* (grape), *Citrus lemon* (lemon), and *Citrus sinensis* (orange) oil had low peroxide value, suggesting that they could be used for human consumption. According to FAO/WHO (2009), edible oils should have a peroxide value of less than 10 meq O₂/kg. Fresh oils have been shown to have peroxide values lower than 10 mg/g oil and oils become rancid when the peroxide value ranges from 20.0 to 40.0 mg/g oil (Pearson, 1976; Onyeike and Acheru, 2002).

Peroxide value is used as an indicator of oil rancidity. Rancidity is caused by autoxidation of double bonds/alcohols in the oil to aldehydes and ketones (Nizam, 2009). Also, low peroxide value serves as indicators of the presence of anti-oxidants in the oils (Bennion, 1995).

According to Bamgboye and Adejumo (2010), specific gravity is the ratio of a substance's density to that of water at 4 °C. *Citrus paradisi*

(grape) oil had a specific gravity of 0.85 g, while *Citrus lemon* (lemon) and *Citrus sinensis* (orange) oil had specific gravities of 0.85 g and 0.87 g, respectively. These values fell within the range of specific gravities reported for linseed oil (0.938 g) and beeswax oil (0.953 g) (Nwokonkwo, 2008), as well as the range of specific gravities reported for a few Nigerian oil seeds (Onyeike and Acheru, 2002).

Citrus paradisi (grape) oil had a density of 0.87 g/cm³, whereas *Citrus lemon* (lemon) and *Citrus sinensis* (orange) oil had densities of 0.95 g/cm³ and 0.88 g/cm³, respectively. These levels fell between 0.915 g/cm³ for oil derived from *Sesamum indicum* L. seeds (Mohammed and Hamza, 2008) and 0.89 g/cm³ for oil derived from African bean seeds (Odoemelam, 2005), suggesting that the oil could be used on a commercial scale.

Citrus paradisi (grape), *Citrus lemon* (lemon), and *Citrus sinensis* (orange) oil had pH values of 4.92, 4.26, and 4.21, respectively. These values were lower than those reported for oil derived from castor plant seeds (Omari *et al.*, 2015).

Citrus paradisi (grape) had an ester value of 195.24 mg KOH/g, whereas *Citrus lemon* (lemon) and *Citrus sinensis* (orange) oil had ester values of 185.74 mg KOH/g and 186.09 mg KOH/g, respectively.

Conclusion

The results from this study showed that oils from the seeds of *Citrus paradisi*, *Citrus lemon*, and *Citrus sinensis* could be a potential antioxidant agent to improve formulation of cosmetics products and prevent free radical-induced oxidative stress. *Citrus paradisi* (grape), *Citrus lemon* (lemon), and *Citrus sinensis* (orange) have great potential because they may be used productively in homes for domestic use as edible oils, as well as in the pharmaceutical and cosmetic industries serving as industrial raw materials and nutraceuticals in the production of soap, lubricants, creams, and medications.

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