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SPECTROSCOPIC AND CHROMATOGRAPHIC CHARACTERIZATION OF ALMOND OIL USING SOLVENT EXTRACTION.

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ABSTRACT

Almond oil, a nutrient-rich extract, is valued for its high content of essential fatty acids, vitamins, and natural antioxidants, which influence its stability and potential health benefits. This study investigates the composition of almond oil extracted via conventional solvent methods using Fourier Transform Infrared (FTIR) spectroscopy, Ultraviolet-Visible (UV-Vis) spectrophotometry, and Gas Chromatography-Mass Spectrometry (GC-MS). FTIR analysis revealed characteristic absorption peaks at 2919 cm^{-1} (C–H stretch), 1740 cm^{-1} (C=O stretch), and 1230 cm^{-1} (C–O stretch), confirming the presence of triglycerides and ester functional groups. UV-Vis spectroscopy showed significant absorbance at 400 nm and in the UV-B region (300–380 nm), indicating the presence of tocopherols and phenolic compounds, which contribute to antioxidant activity and UV protection. GC-MS analysis identified over 30 components, including key fatty acids such as oleic acid (retention time 19.249 min), linoleic acid (19.809 min), palmitic acid (14.941 min), and stearic acid (27.576 min), as well as antioxidants like vitamin E (32.065 min), phytosterols (32.437 min), and squalene (35.936 min). These compounds play critical roles in inhibiting lipid peroxidation and enhancing oil stability. The balance between oxidants (oleic and linoleic acids) and antioxidants (tocopherols and phytosterols) confirms the therapeutic and preservative potential of almond oil. The study demonstrates that almond oil extracted via n-hexane retains valuable bioactive compounds, making it suitable for applications in food, cosmetics, and pharmaceuticals.

KEYWORDS: Almond oil, Antioxidants. FTIR, Fatty acids, GC-MS, Solvent extraction,

INTRODUCTION

Almond oil is a lipid-rich substance that contains a wide array of bioactive compounds, notably antioxidants such as vitamin E, flavonoids, and phenolic compounds (Shahidi & Zhong, 2021). It is highly regarded for its nutritional, therapeutic, and cosmetic benefits but remains underutilized in certain regions, including Nigeria, where commercial production is limited. Almond oil is nutritionally rich in monounsaturated fatty acids, particularly oleic acid, and polyunsaturated fatty acids like linoleic acid (Bolaji *et al.,* 2018; Nwosu *et al.,* 2018). These essential fatty acids are linked to cardiovascular health, anti-inflammatory properties, and enhanced skin hydration.

Furthermore, the presence of natural antioxidants contributes to its potential in combating oxidative stress, thereby offering protection against chronic diseases. These attributes position almond oil as a valuable commodity for the food, pharmaceutical, and cosmetic industries (Ibrahim *et al.,* 2020).

The chemical composition of almond oil reveals significant bioactive and nutritional constituents. Ellis *et al.,* (2015) reported high concentrations of oleic and linoleic acids in the oil. A gas chromatography-mass spectrometry (GC-MS) analysis identified 21 principal components, with benzaldehyde (62.52%) and benzoic acid (14.80%) being the most prevalent (Akpabio, 2017; Okwuego, 2023). The proximate composition of almond seeds includes moisture (25.23%), ash (5.00%), lipid (33.73%), crude protein (33.69%), crude fiber (3.11%), and carbohydrate (25.47%), yielding a caloric value of 534.20 kcal (Akpabio, 2017). Mineral analysis of Indian almond nuts revealed notable quantities of calcium (845.45 ppm), sodium (245.65 ppm), iron (70.62 ppm), zinc (92.12 ppm), and other essential elements (Agunbiade & Olanlokun, 2019).

The method of extraction plays a crucial role in determining the yield, quality, and antioxidant content of almond oil (Aremu *et al.,* 2020). Extraction methods are generally classified into traditional and improved techniques. Conventional methods include manual pressing, grinding, roasting, sun drying, boiling, and heating. Although these approaches are cost-effective and accessible, they are time-consuming and produce lower-quality oil. Improved methods, on the other hand, include solvent extraction, supercritical fluid extraction (SC-CO_2), and mechanical pressing. Solvent extraction utilizes organic solvents, such as hexane, to dissolve and extract oil, and is known for its high efficiency and recovery rate (Martins *et al.,* 2018; Nwosu *et al.,* 2018). Despite potential drawbacks like solvent residue and thermal degradation, solvent extraction was selected for this study due to its balance of affordability, scalability, and high oil yield with acceptable quality retention.

Oxidative stability is an essential factor in evaluating almond oil's shelf life and nutritional integrity. Oxidants, such as peroxides and free radicals, cause oxidative rancidity, leading to a reduction in sensory and nutritional quality (Bolling *et al.,* 2020). Antioxidants act to inhibit or delay these oxidative reactions. Primary antioxidants neutralize free radicals and terminate chain reactions, while secondary antioxidants function through mechanisms like metal ion chelation and singlet oxygen quenching (Carocho & Ferreira, 2018). Natural antioxidants, particularly phenolic compounds and vitamin E in almond oil, significantly enhance its oxidative stability.

$$L^{\bullet} + AH \rightarrow LH + A^{\bullet}$$
$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$$
$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet}$$

Free radical antioxidants can interfere with chain propagation by forming peroxy-antioxidant compounds.

$$A^{\bullet} + LOO^{\bullet} \rightarrow LOOA$$
$$A^{\bullet} + LO^{\bullet} \rightarrow LOA$$

The effectiveness of antioxidants is influenced by the bond dissociation energy (BDE) of A–H and L–H bonds; antioxidants with lower A–H BDE are generally more efficient (Dreher & Junod, 2019). Analytical techniques such as the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) assay are commonly used to evaluate lipid oxidation. PV measures lipid hydroperoxides (ROOH), while TBARS quantifies malondialdehyde (MDA), a secondary oxidation product.

$$ROOH + 2H^+ + 2I^- \rightarrow I_2 + ROH + H_2O$$
$$ROOR + 2H^+ + 2I^- \rightarrow I_2 + 2ROH$$
$$I_2 + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I^-$$

These techniques are crucial for evaluating the antioxidant efficacy and overall quality of almond oil. The choice of extraction method and antioxidant evaluation technique directly influences the quality and applicability of almond oil. Enhancing extraction processes and accurately assessing oxidative stability are vital steps toward optimizing almond oil for broader applications in the food, health, and industrial sectors.

MATERIALS AND METHODS

Study Area and Sample Collections

Mature almond (***Prunus dulcis***) seeds were obtained from a local agricultural market in Zaria, Kaduna State, Nigeria (11.0635°N, 7.7050°E). A purposive sampling approach was employed to select healthy, undamaged mature seeds. The seeds were manually deshelled, air-dried under ambient conditions, and ground into a fine powder using a mechanical grinder. The powdered seed sample was stored in airtight, food-grade plastic containers in a calm, dry environment until further use for oil extraction.

EQUIPMENT AND REAGENTS

Equipment

Mechanical grinder**,** Analytical balance (±0.001 g sensitivity)**,** Conical flasks (500 mL and 1000 mL)**,** Magnetic stirrer with stir bars**,** Whatman No. 1 filter paper**,** Rotary evaporator (Buchi R-300 or equivalent)**,** Vacuum pump**,** Thermometer**,** Amber glass storage bottles (100 mL)**,** Refrigerator (maintained at 4°C)**.** PerkinElmer FTIR spectrometer with ATR accessory**,** Gas Chromatography-Mass Spectrometry (GC-MS) system (e.g., Agilent 7890B GC with 5977A MSD), equipped with DB-5MS capillary column**,** UV-Vis spectrophotometer (e.g., Shimadzu UV-1800)**.** Volumetric flasks, measuring cylinders, and pipettes

Reagents

N-Hexane (analytical grade, ≥99.0% purity), Anhydrous sodium sulfate (Na_2SO_4), Distilled water, Reference standards for GC-MS identification, High-purity helium gas (≥99.999%), Ethanol (used for glassware cleaning as needed). All reagents used were of analytical grade and sourced from BDH Chemicals, Bridge Head, Onitsha.

Oil Extraction Procedure

A total of 100 g of the ground almond seed powder was macerated in 300 mL of *n*-hexane in a 500 mL conical flask. The mixture was left to stand for 72 hours at room temperature, with intermittent stirring using a magnetic stirrer to enhance solubility. Post-maceration, the mixture was filtered through Whatman No. 1 filter paper, and the solid residue was discarded. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C to remove the solvent, yielding crude almond oil. The resulting oil was weighed using an analytical balance and stored in amber glass bottles at 4°C until further analysis was conducted. The crude oil was dried over anhydrous sodium sulfate to remove any residual moisture (Mmuo *et al.,* 2024). No additional purification steps were undertaken unless specified during instrumental analyses.

Characterization of Almond Oil

FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was performed using a PerkinElmer FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) accessory. A small drop of crude almond oil was applied directly to the ATR crystal, and spectra were acquired in the range of 4000–450 cm^{-1}. The resulting spectra were analyzed to identify characteristic functional groups present in the oil (Okwuego *et al.,* 2025, Nkachukwu *et al.,* 2025; Okwuego *et al.,* 2021).

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) was used to determine the phytochemical constituents of the almond oil. A 1 µL aliquot of the oil, diluted with *n*-hexane (1:10 v/v), was injected into an Agilent 7890B GC system coupled with a 5977A mass selective detector. The separation was carried out on a DB-5MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness). The GC oven was initially set at 70°C, followed by a ramp of 10°C/min up to 280°C. The injector temperature was maintained at 250°C. High-purity helium was used as the carrier gas at a constant flow rate of 1 mL/min. Phytoconstituents were identified by comparing their mass spectra to entries in the National Institute of Standards and Technology (NIST) library database (Okwuego, 2023).

Ultra-Visible Spectrophotometry

The UV-Visible absorption spectrum of the almond oil was recorded using a Shimadzu UV-1800 spectrophotometer over a wavelength range of 200–800 nm. A 1:10 (v/v) dilution of the oil in *n*-hexane was prepared, and absorbance measurements were performed using quartz cuvettes with a 1 cm path length. This analysis aimed to elucidate the optical and electronic transition properties of the oil (Farhoosh et al., 2018).

RESULTS AND DISCUSSION

FTIR Analysis of Almond Oil

Fourier-transform infrared (FTIR) spectroscopy was employed to characterize the functional groups present in almond oil and to confirm its molecular composition. The spectrum exhibited several characteristic absorption bands consistent with the chemical structure of triglyceride-based vegetable oils. The major peaks and their assignments are presented in Table 1.0, along with their corresponding spectrum in Figure 1.0. below.

Table 1.0: FTIR Absorption Bands, Intensities, and Functional Group Assignments in Almond Oil

Wavenumber (cm^{-1})	Peak Type	Intensity	Assigned Bond	Functional Group / Component
3675	Sharp	Weak	O–H stretch	Trace moisture or free fatty acids
3458	Broad	Medium	O–H stretch	Hydrogen-bonded hydroxyl groups (water, free acids)

Wavenumber (cm^{-1})	Peak Type	Intensity	Assigned Bond	Functional Group / Component
3377	Sharp	Medium	N–H stretch	Amines or amides (nitrogenous compounds)
3248	Broad	Medium	O–H / N–H stretch	Water, free fatty acids, nitrogenous impurities
2919, 2850	–	Strong	C–H stretch	Aliphatic hydrocarbons (fatty acids)
1740	Sharp	Strong	C=O stretch	Ester carbonyl groups (triglycerides)
1650	–	Medium	C=C stretch	Unsaturated fatty acids (oleic, linoleic acids)
1460, 1375	–	Medium	C–H bend	CH_2 and CH_3 bending from fatty acid chains
1230, 1160	–	Strong	C–O stretch	Ester groups in triglycerides
1090	–	Medium	C–C stretch	Triglyceride backbone
800	–	Medium	C–H out-of-plane bend	Cis-alkene groups in unsaturated fatty acids

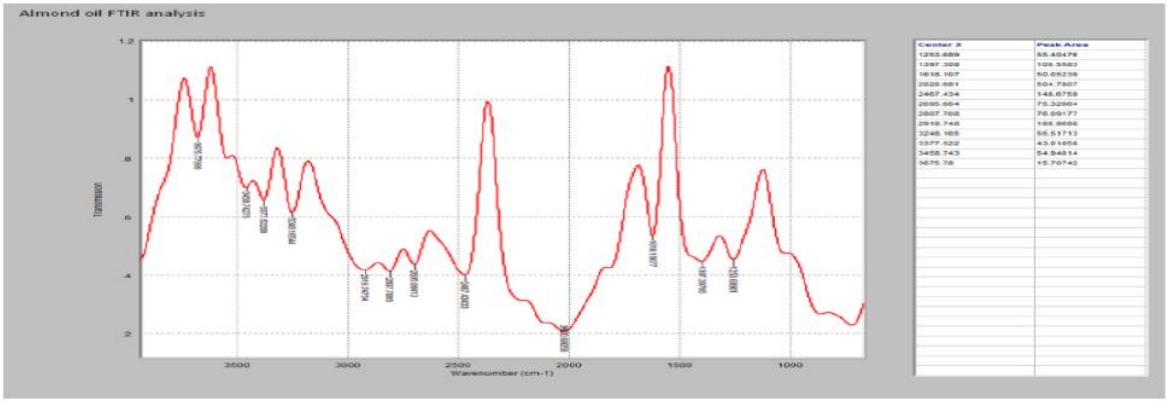

Figure 1.0: FTIR Spectrum of Almond Oil

The strong absorption bands at 2919 cm^{-1} and 2850 cm^{-1} are attributed to C–H stretching vibrations of methylene (–CH_2–) and methyl (–CH_3) groups, indicating the presence of long-chain aliphatic hydrocarbons typical of fatty acid chains in both saturated and unsaturated triglycerides. These peaks are characteristic of plant-derived oils and confirm the lipid nature of the sample (Okwuego, 2023). A prominent peak at 1740 cm^{-1} corresponds to C=O stretching vibrations of ester carbonyl groups, confirming the presence of triglycerides, the primary constituents of almond oil. This is consistent with the typical absorption ranges for ester functional groups in natural fats and oils, as reported in previous studies (Okwuego et al., 2021; Okwuego *et al.,* 2025).

The absorption band at 1650 cm^{-1} is assigned to C=C stretching vibrations, indicative of unsaturated fatty acids, such as oleic and linoleic acids, which are commonly found in almond oil. This suggests a significant degree of unsaturation, which contributes to the oil's

nutritional and oxidative properties. The spectrum also reveals broad O–H stretching bands at 3675 cm^{-1}, 3458 cm^{-1}, and 3248 cm^{-1}, which may be associated with free fatty acids, moisture, or trace hydrogen-bonded hydroxyl compounds. These signals are typical of minor polar components or impurities, including water or partially hydrolyzed lipids. A distinct peak at 3377 cm^{-1} is attributed to N–H stretching, which may suggest the presence of amines or amides, potentially arising from nitrogenous impurities or protein-derived residues. This indicates a small degree of contamination or naturally occurring bioactive compounds in the sample.

In the fingerprint region, several key bands further confirm the presence of triglycerides. These include: C–H bending vibrations at 1460 cm^{-1} and 1375 cm^{-1}, confirming the presence of aliphatic CH_2 and CH_3 groups. C–O stretching at 1230 cm^{-1} and 1160 cm^{-1}, characteristic of ester bonds in triglycerides. A C–C stretching vibration at 1090 cm^{-1}, indicating the structural integrity of the triglyceride backbone. C–H out-of-plane bending at 800 cm^{-1}, typically associated with cis-di substituted alkenes, supporting the presence of unsaturated fatty acids like oleic acid.

These results provide comprehensive molecular evidence for the complex lipid composition of almond oil, dominated by triglycerides with saturated and unsaturated fatty acids, along with minor constituents such as free fatty acids, moisture, and nitrogen-containing compounds. The FTIR profile aligns with previous spectral analyses of plant-based oils, corroborating the purity and chemical identity of the almond oil sample (Okwuego, 2023; Ochie *et al.,* 2025).

Uv-Vis Spectrophotometry

The UV-Vis absorption spectrum of almond oil was recorded over the wavelength range of 200–800 nm using a UV-Vis spectrophotometer. To minimize scattering and ensure accurate absorbance measurements, the oil was diluted with hexane in a 1:10 (v/v) ratio before analysis. The resulting spectral profile is shown in Figure 2.0, with key spectral features summarized in Table 2.0 below.

Table 2.0: Presentation of the Ultraviolet Spectrum of Almond Oil

Wavelength [nm]	Peak/Absorbance	Bond/Compound
800	Near-infrared (NIR)	Likely light scattering or baseline artifact, no specific compound
600–700	Visible range	Minor pigments or impurities
400	Absorbance of tocopherols	Tocopherols (Vitamin E), providing UV protection
300–380	UV-B region, multiple peaks	Tocopherols or minor phenolic compounds, with minimal UV-B absorbance
<280	UV-C region, no absorbance	Indicates a lack of UV-C protection

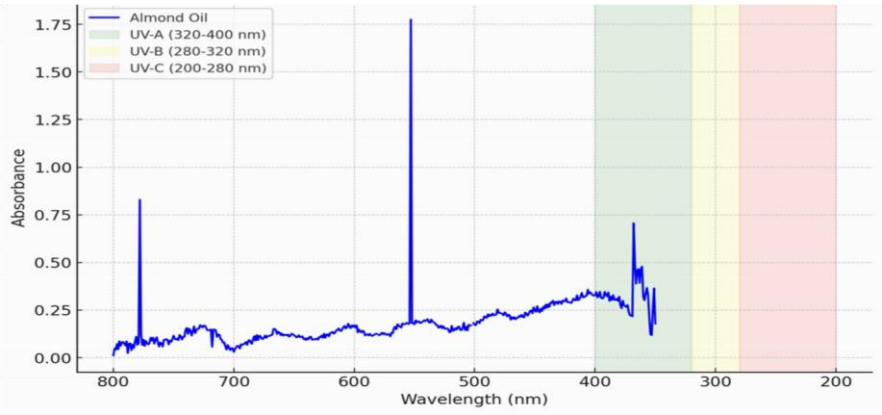

Figure 2: Ultraviolet Spectrum of Almond Oil

The absorption pattern revealed distinct features in both the UV and visible regions, which correlate with the presence of specific chemical constituents known to be found in almond oil. No appreciable absorbance was observed at 800 nm wavelength. As this region falls within the near-infrared (NIR) zone, any weak signal is likely the result of light scattering or instrumental baseline noise, rather than absorbance by chemical components. This is consistent with other plant-based oils, which typically exhibit negligible absorbance in the Near IR region (Net, 2015; Farhoosh *et al.,* 2018). A low-intensity absorbance signal was detected in the visible region, from 600 to 700 nm, potentially due to trace levels of pigments such as chlorophyll or carotenoids. These pigments are commonly found in vegetable oils and are known to absorb weakly in the visible spectrum (Net, 2015). Their presence may be the result of minimal processing or natural variation in the composition of almond oil.

A distinct absorption peak was recorded at approximately 400 nm, which is characteristic of tocopherols (Vitamin E)**.** Tocopherols are potent lipid-soluble antioxidants prevalent in almond oil and are known to absorb in the near-UV range (Mmuo *et al.,* 2024). Their presence at this wavelength supports previous studies identifying α-tocopherol as a major contributor to the oxidative stability and UV-protective properties of nut oils (Net, 2015). Multiple minor absorption bands were observed within the UV-B range from 300–380 nm, suggestive of phenolic compounds or tocopherol isomers. These compounds are commonly reported in almond and other plant oils, contributing to mild photoprotective effects (Net, 2015; Farhoosh *et al.,* 2018). However, the low absorbance intensity indicates that the oil offers limited protection against UV-B radiation.

Virtually no absorbance was recorded in this high-energy region below 280 nm. The absence of significant chromophores capable of absorbing UV-C radiation suggests that almond oil does not protect this range. Similar findings have been reported for other cold-pressed oils lacking synthetic UV filters (Popa, 2015; Mmuo *et al.,* 2024). The almond oil demonstrates moderate UV-absorbing activity, primarily due to the presence of tocopherols, which are most active near 400 nm. While

trace levels of other compounds (e.g., pigments, phenolic) contribute slightly to the absorbance in the UV-B and visible regions, the oil shows limited efficacy in the UV-C region. These findings align with existing literature and support the potential application of almond oil as a natural UV-protective agent, particularly for its antioxidant and mild photo-protective benefits in the UV-A and UV-B ranges.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of almond oil revealed a complex mixture of fatty acids, antioxidants, hydrocarbons, and other bioactive compounds. The major constituents identified, based on their retention times, include oleic acid**,** linoleic acid**,** palmitic acid**,** stearic acid**,** vitamin E (tocopherols)**,** phytosterols**,** and squalene as presented in Table 3.0 and Figure 3.0 below.

Table. 3.0: Gas Chromatography-Mass Spectrometry Result of Almond Oil.

Retention Time(min)	Possible Compound (s) Common in Almond Oil	
14.941	Palmitic Acid	Yes (common fatty acid)
19.249	Oleic Acid	Yes (primary fatty acid)
19.809	Linoleic Acid	Yes (essential fatty acid)
27.576	Stearic Acid	Yes (minor fatty acid)
32.065	Vitamin E (Tocopherols)	Yes (main Antioxidant)
32.437	Phytosterols, e.g, Betasitosterol	Yes (beneficial skin)
35.936	Squalene	Yes (natural antioxidant)
14.943	Tetradecane	Yes
17.404	2,4-Di-tert-butylphenol	Yes
19.249	Dodecanoic acid	Yes
19.565	Z-8-Hexadecene	Yes
19.692	4-tetradecene	Yes
19.809	hexadecane	Yes
21.542	Dodecyl acrylate	Yes
23.490	Tetradecanoic acid	Yes
24.002	3-Eicosene	Yes
27.576	n-Hexadecanoic acid	Yes
28.039	3-Eicosene	Yes
29.762	trans-13-Octadecenoic acid	Yes
30.243	1-Docosene	Yes
31.483	17-Pentatriacontene	Yes
31.525	Carbonic acid, eicosyl vinyl ester	Yes
31.623	Cyclotetracosane	Yes
31.822	1-Docosene	Yes
31.896	Erucic acid	Yes
31.935	2-Piperidinone N-[4-bromo-n-butyl]	Yes
31.977	cis-Vaccenic acid	Yes
32.065	Bis(2-ethylhexyl) phthalates	Yes
32.326	ethanol	Yes
35.907	hydroxypropyl ester	Yes
35.936	yethyl ester	Yes

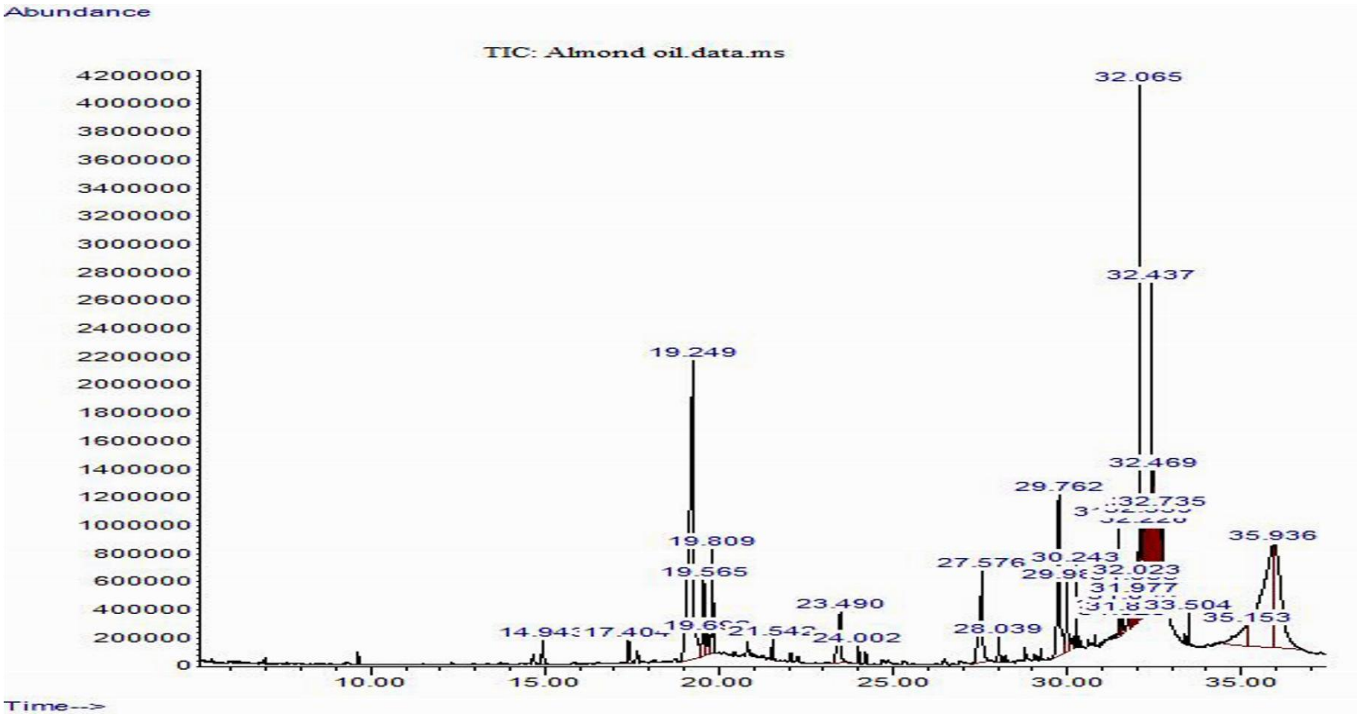

Figure. 3.0: Gas Chromatography-Mass Spectrometry Chromatogram of Almond Oil.

These findings are consistent with previous studies, particularly Okwuego (2023), which reported a similar fatty acid profile in almond oil. Oleic acid (RT = 19.249 min) was identified as the predominant fatty acid, corroborating the well-known fact that almond oil is rich in monounsaturated fats, primarily oleic acid, which constitutes 60–70% of its composition (Okwuego, 2023). The presence of linoleic acid (RT = 19.809 min), an essential polyunsaturated fatty acid, further supports the nutritional value of almond oil.

Palmitic (RT = 14.941 min) and stearic acids (RT = 27.576 min), identified as saturated fatty acids, were also detected, albeit in lower concentrations. These components are typical of plant-derived oils and contribute to the physical stability and oxidative resistance of the oil (Brahmi *et al.,* 2021). The detection of vitamin E (RT = 32.065 min) is particularly significant due to its role in oxidative stability and skin protection. Tocopherols act as natural antioxidants, which prevent lipid peroxidation and prolong shelf-life (Sánchez-Machado *et al.,* 2004). Likewise, phytosterols such as β-sitosterol (RT = 32.437 min)**,** which are known for their cholesterol-lowering and anti-inflammatory properties, were identified and align with prior work on almond oil's skin-healing potential (Antolovich *et al.,* 2022; Okwuego, 2023).

Squalene (RT = 35.936 min)**,** another potent antioxidant, was also detected. Its presence is valuable not only for skin hydration and elasticity but also as a scavenger of free radicals, thereby enhancing the oil's pharmaceutical and cosmetic appeal (Popa, 2015). The GC-MS profile also showed the presence of hydrocarbons such as tetradecane (RT = 14.943 min) and hexadecane (RT = 19.809 min). While these may be naturally occurring in trace quantities in some plant oils,

their presence warrants further investigation to determine whether they result from contamination or are intrinsic to the oil. Interestingly, compounds such as bis(2-ethylhexyl) phthalates (RT = 32.065 min) and 2-piperidinone derivatives (RT = 31.935 min) were also detected. These are not typically associated with natural almond oil and may reflect environmental contamination or leaching of packaging materials, as previously noted in oil analysis studies (Net, 2015). This underscores the importance of monitoring post-extraction handling and storage conditions. Other esters and alkenes, such as dodecyl acrylate, 3-eicosene, trans-13-octadecenoic acid, and 1-docosene, further reflect the complex lipidic nature of the oil and may contribute to its unique functional characteristics.

The GC-MS profile of almond oil validates its well-known nutritional and therapeutic properties through the identification of key unsaturated fatty acids and potent antioxidants. The results are in strong agreement with existing literature (Popa, 2015; Okwuego, 2023), thereby supporting the oil's potential use in nutraceuticals, cosmetics, and functional food formulations. However, the detection of possible contaminants underscores the need for rigorous quality control during the processing and packaging stages.

CONCLUSION

This study successfully elucidated the chemical composition and potential functional applications of almond oil through a multi-analytical approach employing FTIR, UV-Vis spectrophotometry, and GC-MS. FTIR analysis confirmed the lipid-rich molecular profile characteristic of triglycerides and unsaturated fatty acids. At the same time, UV-Vis data highlighted the presence of tocopherols and minor phenolic compounds contributing to the oil's moderate UV-absorptive properties. GC-MS further validated the oil's nutraceutical value by identifying essential fatty acids, antioxidants such as tocopherols and squalene, and phytosterols. However, the detection of possible contaminants emphasizes the importance of stringent quality control in processing and packaging. While the findings reinforce the potential of almond oil for use in cosmetic, nutraceutical, and functional food applications, the study's limitations, such as reliance on a single sample, absence of quantitative profiling, and lack of biological validation, highlight the need for further research. Future investigations should adopt broader sample sets, incorporate rigorous quantification protocols, trace contamination pathways, and substantiate functional claims with biological assays. These steps are crucial for promoting the safe and effective use of almond oil in both commercial and therapeutic contexts.

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