Comparative Analysis of Physico-chemical Properties and Fatty Acid Profiles Derived from *Moringa*, watermelon and sunflower seeds oil

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Abstract

A comparative study was conducted on *Moringa*, Watermelon, and Sunflower seed oils using conventional (n-hexane) and supercritical CO_2 (SC-CO_2) extraction to evaluate their potential for edible and industrial applications. Physico-chemical analysis revealed that SC-CO_2-extracted oils had slightly lower refractive indices and specific gravities, aligning with previously reported trends. *Moringa* oil extracted via SC-CO_2 demonstrated superior thermal stability, with a flash point of 288.50 °C and fire point of 296.40 °C, and lower peroxide (1.47–1.95 meq O_2/kg) and acid values (0.74–0.92%) compared to hexane-extracted oil (1.89 meq O_2/kg, 0.85%). Watermelon oil also exhibited improved thermal properties, with flash and fire points reaching 292.4 °C and 305.3 °C, respectively, and slightly reduced peroxide (1.8–2.9 meq O_2/kg) and acid values (2.24–2.93 mg KOH/g) than hexane-extracted oil (2.9 meq O_2/kg, 2.97 mg KOH/g). Similarly, Sunflower oil showed fire points of 339–342 °C, flash points of 314–316 °C, and peroxide and acid values of 6.15–6.93 meq O_2/kg and 3.13–3.86 mg KOH/g, slightly outperforming hexane oil (6.72 meq O_2/kg, 3.53 mg KOH/g). Fatty acid analysis confirmed high unsaturation: 66.04–79.98% (*Moringa*), 74.23% (Watermelon), and 88.13–91.14% (Sunflower), mainly cis-oleic and linoleic acids. SC-CO_2 extraction offers thermally stable, oxidation-resistant, and solvent-free oils.

Keywords; *Seed oils, Physicochemical properties, Supercritical CO_2 extraction, Gas-Chromatography, Oil quality.*

1 Introduction

Vegetable seeds and fruit seeds are major sources of vegetable oils, which find extensive uses beyond food. Some of these include use as precursor for the production of lubricants, bio-oil and biofuel production, drugs, food additives, drinks, cosmetics, and even as solvents [1,2]. Their high calorific value, low stickiness, and ability to carry flavors make them suitable for

food industry. Additionally, most vegetable oils are rich in vitamins such as A, D, and E, further supporting their use in pharmaceutical formulations and nutritional supplements [3]. The vegetable oils have fatty acids as main constituents, which are mostly triglycerides (95% - 98%) and remaining (2 - 5%) are less useful compounds like free fatty acids, tocopherols, sterols, pigmented compounds, oleoresin, etc. [4]. In the current scenario, the production and use of vegetable oils have increased rapidly which leads to demands for exhaustive research in this category of oils. Many oils fall under the category of vegetable oils such as Sesame, Soyabean, Peanut, Mustard, *Moringa Oleifera*, Sunflower, Pumpkin, Corn, Coconut, Flax etc [5,6]. India is one of the fastest-growing edible oil markets, with consumption rising at 6.5% annually, while supply grows only 2.17%, leading to a heavy reliance on imports about 56% of total consumption [7]. In 2023–2024 alone, India imported 17 .7 million tons of edible oil, placing a significant strain on foreign reserves. To reduce this dependency, there is a need to explore non-traditional domestic sources like Watermelon and *Moringa* seeds, which are rich in edible oil. At the same time, concerns over the health and environmental risks of chemical solvents have driven interest in clean technologies.

Extraction methods significantly influences the yield, purity, and bioactive composition of oils. Traditional solvent extraction, such as n-hexane, is widely used for its high efficiency but often leaves behind solvent residues that require further refinement [2]. The conventional n-hexane extraction method, although widely employed due to its high extraction efficiency and oil yield, presents several critical limitations from a scientific and industrial perspective. A major concern is the potential presence of residual solvent in the final product, which poses toxicological risks, particularly in food-grade and cosmetic formulations. Hexane, a petroleum-derived volatile organic compound, contributes to environmental degradation and raises significant sustainability concerns due to its non-renewable origin and atmospheric reactivity. Furthermore, its high flammability presents occupational hazards, increasing the likelihood of fire and explosion during large-scale processing [8]. The thermal conditions often employed during hexane extraction can cause the breakdown of compounds that are sensitive to heat, including tocopherols and polyunsaturated fatty acids, thereby reducing the nutritional and functional quality of the extracted oils. Additionally, hexane exhibits low selectivity, co-extracting non-lipid constituents such as waxes, pigments, and other impurities, necessitating further refining steps that increase energy consumption, operational complexity, and processing costs. These drawbacks collectively highlight the necessity for alternative extraction technologies such as SC-CO_2 extraction that offer improved safety,

selectivity, and environmental compatibility. In contrast, SC-CO_2 extraction offers a cleaner, solvent-free alternative that minimizes oxidation and preserves the oil's quality. To minimize or eliminate the harmful effects associated with conventional solvents in oil extraction, the present study explores low carbon technology using SC-CO_2 for the efficient recovery of oil. This method is gaining increasing popularity due to its environmental compatibility, high extraction efficiency, and the production of superior oil quality, making it a healthier option for culinary applications. Although solvent extraction is economical for large-scale oil production, SC-CO_2 extraction is increasingly favored for high-value applications in drugs extraction, cosmetics, and nutraceuticals due to its superior purity and minimal environmental impact. Optimizing extraction parameter, such as temperature and pressure is essential to achieving the best balance between yield, quality, and sustainability.

SC-CO_2 extraction is an advanced high-pressure technique used to extract solutes from solid materials. It utilizes CO_2, user-safe and non-flammable, and fully recyclable solvent with minimal environmental impact. Unlike conventional methods, it operates without the use of hazardous organic solvents, aligning with global initiatives for green and sustainable processing. In SC-CO_2 extraction, CO_2 is brought to its supercritical state a phase achieved when both temperature and pressure exceed its critical point where it exhibits enhanced solvating power and superior mass transfer properties. In this state, CO_2 exhibits a combination of gas-like and liquid-like properties. It has a density similar to liquids, which enhances the solubility of target compounds, and a viscosity similar to gases, allowing it to flow easily through porous structures. This leads to intermediate diffusivity, greater than that of liquids but lower than gases, enabling more efficient penetration into solid matrices. These properties improve mass transfer, increase extraction efficiency, and enhance the purity of the final extract. Compared to traditional solvent-based methods, SC-CO_2 extraction is cleaner, faster, and more selective [9]. SC-CO_2 methods are particularly effective in retaining essential fatty acids, antioxidants, and other bioactive compounds [10].

Numerous studies have shown the application of SC-CO_2 in extracting essential oils and valuable compounds from various plants sources [11–13]. For instance, Shalfoh et al. SC-CO_2 extract oil from fish waste for biofuel production [14]. Tzima et al. applied this technique to extract pigments, fats, and antioxidant from microalgae [15]. Similarly, some researcher also employed supercritical CO_2 for the preparation of nanoparticles [16–19]. In addition to extraction, SC-CO_2 has also been explored in polymer synthesis and processing, offering a clean and efficient alternative for producing advanced polymeric materials [20,21].

This study investigates oil extraction from *Moringa*, watermelon, and sunflower seeds using the conventional solvent method and SC-CO_2 extraction under varying operational conditions. Physicochemical characterization was carried out to evaluate the impact of different extraction approaches on the composition, stability, and overall quality of the extracted oils. In addition, optimization of SC-CO_2 extraction parameters was performed to enhance oil yield and process efficiency. A comparative analysis was conducted to measure variations in yield, purity, and extraction efficiency across techniques and seed types, providing insights into the most effective strategies for high-quality oil recovery. The selection of *Moringa*, Watermelon, and Sunflower seeds is based on their diverse biochemical composition, nutritional importance, and sustainability potential. *Moringa* seeds contains high amount of oleic acid and natural antioxidants, making them a valuable source of functional lipids. Watermelon seeds, often treated as agro-waste, are underutilized yet rich in essential fatty acids and nutrients, supporting waste valorization. Sunflower seeds offer high oil yield with linoleic acid and vitamin E, widely used in the edible oil industry. Their combined selection provides a varied matrix for evaluating SC-CO_2 extraction across different seed types and oil profiles. Limited studies have conducted the comparative evaluation of SC-CO_2 and hexane extraction for these specific seed types.

2 Material and Methods

2.1 Reagent and Standard

The Gas Chromatography (GC) grade standard of fatty acid methyl ester (FAME 37 mix) (CAS number 71076-49-8) was purchase from Sigma-Aldrich. The analytical grade ethanol (CAS number 64-17-5) was bought from Merck, Germany. Additionally, Sodium thiosulfate (CAS number 7772-98-7), n-hexane (CAS number 110-54-3), 2-propanol (CAS number, Methanol (CAS number 67-56-1), KOH (CAS number 1310-58-3), Potassium bisulfate (CAS number 7646-93-7), Toluene (CAS number 108-88-3), Anhydrous powder Sodium Sulfate (CAS number 7757-82-6) were procured from Merck, India. All necessary solutions were made using ultrapure water. High-purity (99.9%) liquid CO_2 was supplied local gas supplier in 30 kg deep-tube cylinders. Each cylinder provided approximately 17–18 hours of continuous operation.

2.2 Proximate composition

The proximate composition of flax seeds moisture, crude fat, protein, fiber, and ash was determined using (AOAC) methods [22]. Moisture content was estimated using hot air oven

at 105°C. Crude fat was obtained through Soxhlet extraction using hexane as the solvent. Protein content was examined through the Kjeldahl method [23], based on total nitrogen estimation. Crude fiber was determined through sequential acid and alkali digestion of the defatted sample. Ash content was obtained by incineration in a muffle furnace set at 550 °C. All measurements were performed in triplicate, with results was caluclated as percentages of total sample weight.

2.3 Extraction of Oil from *Moringa,* Sunflower and Watermelon seed

Solvent Extraction

Moringa, watermelon and sunflower seed total oil content was analyzed via the Soxhlet extraction technique. 20 ± 0.001 gm of dry seeds was loaded into a thimble made of glass and inserted into a Soxhlet equipment linked with round-bottom flask holding 200 mL of solvent. The solvent was brought to reflux, continually vaporizing and condensing *via* the sample to oil for 8–10 hours, with an average of 10–12 cycles per hour. Later solvent was evacuated by a rotary evaporator to leave the extracted oil behind.

SC-CO_2 Extraction

Liquid CO_2 is provided from a dip-tube cylinder to a high-pressure pump during the SC-CO_2 extraction process. The liquid CO_2 undergoes filtration through a 0.22 µm stainless-steel filter prior to pressurization to eliminate foreign particles and provide a clean, unpolluted supply of CO_2. The CO_2 is chilled to keep it in liquid form and avoid early vaporization. The liquefied CO_2 is subsequently pressurized to the desired supercritical pressure using a high-pressure pump (Figure 1). A heat exchanger is then used to elevate the CO_2 temperature to reach supercritical conditions. Having reached the desired temperature and pressure, the supercritical CO_2 enters the extraction vessel filled with seeds sample, which are loaded into a stainless-steel basket. Since the SC-CO_2 moves over the seed bed, it dissolves the oil and transports it outside the vessel. The mixture of CO_2 and oil goes through a metering valve where it is pressurized and depressurizes the CO_2 back to a gaseous state and from which it becomes separated from the extracted oil. The oil will then be obtained at the separator's bottom while the CO_2 may either be vented or recycled. The optimization of SC-CO_2 extraction conditions was performed based on a Design of Experiments approach. Specifically, small face-centered central composite design under response surface methodology was used. The extraction was conducted under varying conditions based on experimental trials and existing literature [24,25], with temperature range is 60 °C to 100 °C,

pressure range 200 to 400 bar, ethanol co-solvent concentration from 0% to 10%, particle 0.50 to 0.75 mm, and CO_2 flow rate ranging from 5 to 15 g/min.

2.4 Physical Properties of Oils

Refractive Index

Refractive index measurement of *Moringa*, Sunflower and Watermelon seed oils (Hexane as well as SC-CO_2 extracted) was done by means of a refractometer as suggested by Sangeetha et al.,[26] The instrument is calibrated with distilled water with refractive index of 1.33 at 20 °C. Clean and moisture free melted oil samples of 1-2 drops, were placed on clean and dry prisms. Now, instrument and lighting to obtain the different reading were adjusted to determined refractive index.

The refractive index at given temperature is calculated by the given formula:

$$R_0 = R_1 + K(To - T1)$$

R_0 = reading of the refractometer at particular temperature T_1 in °C.

R_1 = reading at temperature T_0 °C.

K = constant value (0.000385 for oils).

T_0 indicates the temperature at which the reading R1 is recorded.

T_1 = temperature, typically 40 °C.

Analysis of Specific Gravity

Specific gravity of fatty acid at 25ºC is estimated by method given is literature[27]. only filtered and moisture-free oil samples were utilized. Initially, a clean, dry pycnometer fitted with a thermometer is filled with pre-cooled distilled water at 25 °C and placed in a temperature-controlled water bath for 30 minutes. Once the water level stabilizes, the stopper is inserted, and the pycnometer is weighed. The specific gravity of each oil sample is measuring by given equation:

$$Specific\ Gravity\ = \frac{A - D}{Z - D}$$

A = weight (gm) of pycnometer with oil

D = weight (gm) of pycnometer without oil

Z= weight (g) of pycnometer with water

Flash and Fire Point

The flash and fire points of the fatty acid rich oil were measured by using Pensky Martin apparatus following the method outlined in literature [28]. Initially, the clean and dry cup of the Pensky apparatus was filled with the oil sample up to the specified level. The sample was heated at a rate of roughly 5 to 6 ºC per minute while being stirred. Every time the temperature rose by 5 ºC, stirring was stopped and the cup shutter was opened to introduce the test flame. Flash point was determined as the initial temperature at which the test flame ignited the vapours above the liquid. The sample was heated further to determine the fire point, or until the oil ignited and burned continuously for at least five seconds when exposed to the test flame.

2.5 Chemical Characteristics of Oils

Chemical properties like iodine number, unsaponifiable materials, saponification number, acid value and peroxide value were analyzed in the SC-CO_2 and hexane extracted oil.

Peroxide Value

The peroxide value is calculated using method discussed by Janporn et al., [27]. The peroxide value indicates the amount of active oxygen, expressed in milliequivalents, present in 1000 grams of the sample, based on the following procedure. A specific quantity of oil is kept in a conical flask sealed by stopper. To dissolve the oil, 30 mL of a glacial CH_3COOH and $CHCl_3$ mixture in a 2:3 ratio is added. 0.5 mL KI solution is added after dissolution, and the mixture is shaken for one minute. After that, 30 milliliters of purified water are added. 0.01 N sodium thiosulfate is added to the mixture until the yellow hue almost completely goes away. 0.5 mL of starch solution is then added, shaken, and the titration process is continued until the color completely goes away. The same blank test is administered in the same manner. The peroxide value is determined by the formula below.

$$Peroxide\ value\ = \frac{T \times N \times 1000}{gm\ of\ oil\ sample}$$

T = Volume of solution in mL (blank)

N = normality of solution

Acid Value

The acid value of the oil is ascertained using method previously used [27]. This shows how much KOH (in mg) is required to neutralize the free fatty acids present in one gram of the oil sampl. During this process, a conical flask is filled with around 1.00 ± 0.001gm of the oil sample. The oil sample is mixed with freshly made neutralized C_2H_5OH (10–20 mL) and 2

mL of phenolphthalein as indicator. After five minutes of boiling, the liquid is titrated with a 0.5 N KOH solution. The solution is vigorously shaken the entire while the titration is being done. After the oil sample is titrated, the acid value is determined by measuring the milligrams of KOH needed to neutralize the free fatty acid.

$$Acid\ value\ = \frac{56.1 \times V \times N}{G}$$

V = Volume in mL of KI utilized

N = Normality (KI)

G = Sample weight in grams.

$$\text{Free fatty acids (wt\%)} = \frac{28.2 \times V \times N}{G}$$

Saponification Number

A measurement called the saponification number tells us how many mg of KOH is needed to saponify 1 gm of oil. This value is calculated by using method suggested by Kabutey et al., [29]. Put 1.0–2.0 gm of dry, clean oil in Erlenmeyer flask with 25 mL of alcoholic KOH solution. Make the solution by refluxing 1.2 ml of alcohol with 10 gm KOH and 6 gm aluminum foil, distilling 1 mL, discarding the first 50 mL, cooling below 15°C, and dissolving 40 gm KOH in it. Let it stand overnight in the dark before putting it in a sealed bottle. Set up a blank titration with 25 mL of the same solution. After an hour, connect both flasks to air condensers and gently boil the solution until it is clear and leaves no oily residue. Rinse flasks and condensers with neutralized ethanol, and titrate the excess KOH with 0.5 N HCl using 1.0 mL phenolphthalein as indicator to estimate the saponification number.

$$Saponification\ number\ = \frac{56.1 \times (V - P) \times N}{G}$$

V = volume (in mL) HCl needed for the blank solution.

P = volume (in mL) of standard HCl needed for the actual sample solution.

N = normality of the standard HCl.

G = gram of oil utilized for the test.

Unsaponifiable Matter

In a 250 mL conical flask, 1.0 gm of clean, dry oil was mixed with 10 ml alcoholic KOH and refluxed for 1 hour using an air condenser. The apparatus was rinsed with ethyl alcohol and cold distilled water, and saponified mixture was transferred into a separating funnel, followed by the adding petroleum ether. The contents were shaken thoroughly, and the resulting layers were allowed to separate. The lower soap layer was collected, and three additional ether

extractions (10 mL each) were performed. The combined ether extracts were cleaned with alcohol and distilled water, then evaporated in a beaker. Residual solvent was removed with acetone, followed by further evaporation in a 50 mL Erlenmeyer flask. The sample was dried at 100 °C for 30 minutes to remove ether traces. The residue was mixed in neutralized C_2H_5OH and titrated with 0.02 N sodium hydroxide. Free fatty acid content was calculated using: 0.282 × S × N, where S is the NaOH volume (mL) and N is its normality.

$$Unsaponifiable\ matter\ = \frac{C - D}{G} \times 100$$

D = weight (in gm) of the free fatty acid

G = weight (in gm) of the sample

C = weight (in gm) of the residue

Iodine Number

The iodine value measures the level of unsaturation in oils; a higher value signifies a greater number of double bonds. To determine it, 1 gm of clean, dry oil is dissolved in 10 mL carbon tetrachloride in a 500 mL conical flask. A blank (without oil) is prepared in parallel. Add 20 mL Wij's solution, swirl to mix, and keep both flasks in the dark for 2 hours. Subsequently, 10 mL of KI solution is introduced, followed by 100 mL of pre-boiled and cooled distilled water. Iodine is then titrated with $Na_2S_2O_3$ using starch as an indicator, continuing the titration until the blue coloration fades [30]. The procedure for a blank titration is the same as that for the test sample.

$$Iodine\ number\ = \frac{12.69 \times (V - P) \times N}{G}$$

V = volume (mL) of $Na_2S_2O_3$ blank solution

P = sample volume (in mL)

N = normality of the $Na_2S_2O_3$

G = required sample in grams.

2.6 Fatty Acid Analysis

Free fatty acid to low boiling point fatty acid methyl esters (FAME), one could determine the composition of high boiling point free fatty acid (FFA). For FAME analysis, gas chromatography has been employed.

Transesterification

FAME were produced from the free fatty acid of oil sample by applying Christie's process [31]. A test tube containing 50±0.001 mg of oil filled with 1 mL of methylbenzene to dissolve it. After attaching a condenser to the test tube, 2 mL of 1% H_2SO_4 in methanol was given. Over the course of two hours, the mixture was heated to 70 °C in complete reflux. Subsequently, a new 5% NaCl solution was added, and the generated FAME was successively extracted twice with 5 mL of n-hexane each. Using a Pasteur pipette, the top layer of n-hexane was isolated. After using 4 mL of recently made 2% $KHCO_3$ solution to rinse it, this layer was dried with Na_2SO_4. A rotary vacuum evaporator was used to evaporate the solvent at a lower pressure.

Gas-Chromatography for FAME Analysis

FAMEs were examined using a Thermo Trace Ultra gas chromatograph. Nitrogen served as the carrier gas at a flow rate of 30 mL/min, while air and hydrogen were supplied at 350 mL/min and 35 mL/min, respectively. The injector temperature was maintained at 250 °C with a split ratio of 50:1. The oven temperature program lasted 44.5 minutes, beginning at 120 °C (held for 1 minute), increasing to 145 °C at a rate of 5 °C/min, and then rising to 220 °C at 2 °C/min (held for 2 minutes). A 1 µL aliquot of the sample, diluted in 1 mL of n-hexane, was injected using autosampler. Chromeleon software (version A.09.01) was used for data processing and peak integration. Fatty acid identification was carried out by comparison with a Supelco 37-component FAME standard.

2.7 Statistical Analysis

Descriptive statistical analysis was conducted for all experimental data, and the data are given as mean values ± standard deviation (SD) to show the central tendency and variability of the measurements. All experimental tests were run in triplicate to provide reproducibility and reliability of the data. Statistical tests were conducted with IBM SPSS Statistics, Version 27.0 (IBM Corp., Armonk, NY, USA).

3 Results and Discussion

3.1 Proximate analysis and Oil Extraction

The proximate analysis of *Moringa*, Watermelon, and Sunflower seeds (**Table 1**) demonstrates distinct nutritional profiles, each with specific strengths. Watermelon seeds exhibited the highest fat content at 47.45%, followed closely by Sunflower seeds (44.46%), whereas *Moringa* seeds contained less fat (35.57%), indicating that Watermelon and

Sunflower are superior for oil extraction. In terms of protein, both *Moringa* (29.37%) and Watermelon (29.56%) seeds were almost identical and significantly richer than Sunflower seeds (20.19%), highlighting their potential as plant-based protein sources. Carbohydrate content was highest in Sunflower seeds (23.77%), suggesting a better energy yield, while Watermelon seeds had the lowest (7.34%), and *Moringa* showed a moderate level (17.74%). When analyzing crude fiber, *Moringa* again led with 7.28%, making it more favorable for dietary fiber intake, followed by Watermelon (5.41%) and Sunflower (3.91%). Ash content, which reflects total mineral content, was also highest in *Moringa* (4.28%), suggesting superior micronutrient density compared to Watermelon (3.12%) and Sunflower (3.19%). Lastly, moisture content was relatively low across all three, supporting good shelf life, with Sunflower seeds having the lowest moisture (4.58%), followed by *Moringa* (5.60%) and Watermelon (6.54%).

SC-CO_2 extraction was used to isolate fats from the seeds of *Moringa*, Watermelon, and Sunflower. The extraction process yielded 33% oil from *Moringa* seeds, 44% from Watermelon, and 41% from Sunflower seed. These findings demonstrate the efficacy of SC-CO_2 as a non-toxic, solvent-free extraction method for isolating plant-derived lipids, particularly from high-oil-content seeds. The observed differences in extraction yield reflect the inherent lipid profiles of the respective seeds, as well as the capacity of supercritical CO_2 to disrupt cellular structures and solubilize hydrophobic compounds with minimal thermal degradation, thereby preserving the biochemical integrity of the oils.

This result is in close arrangement with literature values reported by some researchers [32,33]. who found oil yields ranging between 33–38% using SC-CO_2 extraction. The highest oil yield, 78.10%, was obtained from *Pistacia khinjuk* using SC-CO_2, as reported by Shalfoh et al. [34].

3.2 Physical and Chemical Characteristics of *Moringa* (MO), Watermelon (WM) and Sunflower (SF) seed Oil

3.2.1 Physical and Chemical Characteristics of *Moringa* seed oil

The Supplementary material, **Table S-1** illustrate the Physico-chemical Characteristics such as acid value, iodine number, saponification number, un-saponifiable matter, flash and fire point, refractive index and specific gravity of *Moringa* seed oil extracted in run no. 1-26 and solvent extraction performed with n-hexane. Refractive index of oil extracts by SC-CO_2 varies from 1.422 to 1.487 whereas it is 1.487 for oil extracts by n-hexane. The most of the

RI values for oil being extracted s by SC-CO_2 and hexane are in agreement with those values given in past studies [35]. The high refractive index represents the greater number of carbon atoms in the fatty acids. The normal value of specific gravity of oils-extracted by SC-CO_2 and by hexane is 0.899 and 0.9203 respectively. The higher flash and fire points of MO seed oil make it ideal for cooking at high temperatures. However, oils extracted by hexane typically have lower flash and fire points compared to those extracted by SC-CO_2, likely due to traces of hexane present in the oil samples. Lower values indicate stronger resistance to oxidation. The peroxide value of an oil indicates its oxidative deterioration. In the case of MO oil extracts, peroxide value fall within 1.47-1.95 mequiv O_2/kg oil for extractions using SC-CO_2, and it is 1.89 mequiv O_2/kg oil for extractions using hexane. These peroxide values largely align with the values reported by Lalas and Tsaknis [35] for extraction by hexane (1.87) and a mixture of organic solvents (1.47). The acid values of *Moringa* oil (0.81-0.91% as oleic acid) are lower than or equal to those reported by Lalas and Tsaknis [35]. The iodine values for oils extracted by SC-CO_2 and hexane show no significant difference, aligning well with findings from previous studies on hexane-extracted oils [36]. For oils extracted by SC-CO_2, saponification values range from 176.11 to 188.36 mg of KOH/g of oil, while hexane-extracted oil has a value of 188.72 mg of KOH/g of oil. These findings align closely with those reported previous studies [35] for hexane-extracted oil (188.36) and a blend of organic solvents (186.32).

3.2.2 Physical and Chemical Characteristics of Watermelon seed oil

The physico-chemical characteristics of WM seed oil, such as its acid value, saponification number, flash and fire points, refractive index, iodine number and specific gravity, are shown in the Supplementary material, **Table S-2**. Oil extracted using SC-CO_2 has a refractive index of 1.456 to 1.473; oil extracted using hexane has a refractive index of 1.487. These refractive index values align with previous studies [37]. A higher refractive index indicates that fatty acids contain long chain of hydrocarbon. Average specific gravity of oils extracted by SC-CO_2 and hexane is 0.899 and 0.9203, respectively. WM seed oil extracted with SC-CO_2 has an average flash point of 290 ºC and fire point of 304ºC, making it suitable for high-temperature cooking. On the other hand, oil extracted using hexane has lower flash and fire points, potentially due to residual hexane in the sample. Peroxide value is lower in the SC-CO_2 extracted oil at 2.8 mequiv O_2/kg oil, compared to 2.9 mequiv O_2/kg oil in hexane-extracted oil. Most peroxide values for both methods are lesser than those reported by Baboli et al. [37] for hexane extraction, which was 3.24. The acid value of oil extracted by SC-CO_2

varies from 2.27 to 2.92 mg KOH/gm oil whereas it is 0.97 for oil extracts by using hexane as a solvent. These values are in good agreement as reported in literature [38] for oil extracted by hexane and the acid value of WM oil shows its suitability for cooking and deep-frying purpose. The WM seed oil extracted with hexane has a little greater Iodine number than the oil extracted with SC-CO_2, but both values are less than the hexane-extracted oil values reported [37]. The saponification values for oil extracted via SC-CO_2 (range from 185 to 188 mg KOH/gm oil) are lower than those for hexane extraction (198 mg KOH/gm oil). The unsaponifiable matter value of the oil indicates the presence of several components such as alcohols, tocopherols, sterols, and waxes [37].

3.2.3 Physical and Chemical Characteristics of Sunflower Seed Oil

The Supplementary material, **Table S-3** shows the physico-chemical properties of Sunflower seed oil for oils extracted in run no.1-26 and solvent extraction performed using n-hexane. The values are arranged according to run numbers. Oil extracted using SC-CO_2 has a refractive index (RI) of 1.452 to 1.478, while oil extracted using hexane has a RI of 1.472. The majority of the RI values for oil extracts by hexane and SC-CO_2 are consistent with those found in earlier research [5,37,39]. Oils extracted by SC-CO_2 and hexane have average specific gravities of 0.905 and 0.916, respectively. These results fall within the range found in previous research which support the present study [5,40].

3.3.4 Comparison of Physicochemical properties of *Moring* seed, watermelon and sunflower seed oil

The physicochemical profiles of *Moringa* (MO), Watermelon (WM), and Sunflower (SF) seed oils varied significantly with extraction method SC-CO_2 or hexane. SC-CO_2-extracted oils exhibited higher refractive indices in WM (1.472) and SF (1.473) compared to MO (1.445), indicating greater unsaturation and molecular complexity (**Figure 2(a)**). Hexane-extracted WM and SF oils showed higher specific gravity (0.920) than MO (0.900), reflecting denser compositions (**Figure 2(b)**). Thermal stability was notably superior in SC-CO_2 SF oil, with a flash point of 339°C and fire point of 316 °C, while MO and hexane oils remained below 300 °C (**Figure 2(c,d)**).

Oxidative indicators revealed elevated peroxide values in SC-CO_2 SF (6.6 meq O_2/kg) and WM (2.8 meq O_2/kg), suggesting higher susceptibility to lipid peroxidation, whereas MO and hexane oils exhibited lower values (1.8–2.0 meq O_2/kg) (**Figure 2(e)**). Acid values followed a similar trend: SF (3.6 mg KOH/g) and WM (2.6 mg KOH/g) from SC-CO_2 had greater free

fatty acid content, while MO and hexane oils were more stable (0.8–0.9 mg KOH/g) (**Figure 2(f)**). The highest saponification value was observed in SC-CO_2 WM oil (198 mg KOH/g), indicating more short-chain fatty acids (**Figure 2(g)**). SC-CO_2 also enhanced unsaponifiable content, especially in WM (2.6%) and SF (1.2%), compared to MO (0.8%) (**Figure 2(h)**).

Iodine values, reflecting unsaturation, were highest in hexane WM (148 g I_2/100g), followed by SC-CO_2 WM (138) and SF (127), while MO remained significantly lower (65–67), (**Figure 2(i)**) confirming its higher oxidative stability but lower nutritional complexity. Overall, SC-CO_2 extraction yielded oils richer in unsaturated and bioactive compounds especially from WM and SF seeds—making it superior for nutraceutical and functional food applications, whereas hexane extraction offered slightly more oxidatively stable, but less nutritionally enriched oils.

3.3 Fatty Acid Profile of MO, WM and SF Seed Oil

GC was used to evaluate the fatty acid analysis of MO, WM, and SF seed oils after the high-boiling-point free fatty acids were converted into lower-boiling-point FAMEs. **Figure 3** displays the variations in the amounts of saturated and unsaturated fatty acids found in seeds from *moringa*, watermelon and sunflower.

3.3.1 Fatty Acid Profile of MO

The GC has been used for FAME identification. The Supplementary material, **Table S-4**, organized with Run No. provide details of fatty acid content of MO seed by using SC-CO_2 and hexane, with corresponding GC chromatogram. The analysis shows that MO seed oil contains unsaturated fatty acids between 66.043% and 79.978% and saturated fatty acids ranging from 20.03% to 33.963%. On average, the oil contains about 24.07% saturated and 77.14% unsaturated fatty acids. Cis-oleic acid is the predominant unsaturated fatty acid in both extraction methods, with high levels ranging from 70.52% to 76.2% these results are within the range that obtained by Rai et al., [41]. Additionally, minor amounts of trans-oleic acid, palmitoleic acid, linoleic acid, and eicosenoic acid contribute to the unsaturation of MO seed oil. The saturated fatty acids present, such as myristic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, and lignoceric acid, each are involved in the overall composition of the oil similar to previous observations by Zhao et al. [32,42]. While there is a slight difference in the composition of fatty acid between SC-CO_2 and hexane extraction, the use of SC-CO_2 without organic solvents like hexane enhances the quality of MO seed oil.

3.3.2 Fatty Acid Profile of WM

The Supplementary material, **Table S-5** details the fatty acid profile of WM seed by using SC-CO_2 and hexane. The oil consists of about 25.12% saturated fatty acids and 74.23% unsaturated fatty acids, with linoleic acid being the primary unsaturated component, constituting approximately 61% of the oil's composition parallel outcomes were reported by Rai et al., [41]. WM seed oil consists of high amount of cis-oleic acid, contributing to stability and health benefits, including heart disease prevention. Small amounts of palmitoleic acid and eicosenoic acid further enhance unsaturated profile of Watermelon oil. Different percentages of saturated fatty acids such as stearic acid, palmitic acid, myristic acid and arachidic acid are present in the overall oil composition. WM seed oil remains consistent between SC-CO_2 and hexane extraction methods, with the use of SC-CO_2 contributing to improved oil quality by eliminating the need for organic solvents like hexane.

3.3.3 Fatty Acid Profile of SF

The fat content of SF seed oil that was extracted by hexane and SC-CO_2 is shown in The Supplementary material, **Table S-6** Between 88.133% and 91.145% of the oil's total weight are unsaturated fatty acids, whereas between 9.458% and 12.041% are saturated fatty acids. The percentage of saturated and unsaturated fatty acids in the oil is 9.98% and 90.32%, respectively. It may be observed that cis-oleic acid makes up 51.826% to 56.426% of the unsaturated fatty acid present in sunflower seed oil. Additionally, overall oil comprises substantial levels of linoleic acid, ranging from 33.313% to 36.179% which is smaller than as reported by Vicentini-Polette et al., [43]. Together, cis-oleic acid and linoleic acid contribute to roughly 85% of the unsaturated fatty acids in Sunflower oil. Linoleic acid, an omega-6 fatty acid, supports skeleton system, hair growth, metabolic regulation, and reproductive health. Cis-oleic acid, a crucial monounsaturated oleic acid, promotes stability and offers health benefits, including heart disease prevention. SF oil also contains trace amounts of hexadecanoic acid and eicosenoic acid further enhancing its unsaturated fatty acids profile similar outcomes were observed in litrature [44].

3.3.4 Comparison of Fatty Acid Profile of MO, WM and SF Seed Oil

The fatty acid analysis of *Moringa*, Watermelon, and Sunflower seed oils reveals significant differences in their nutritional profiles and functional properties. *Moringa* oil is predominantly rich in cis-oleic acid (74%), a monounsaturated fatty acid known for its oxidative stability and heart-health benefits. This high oleic content, combined with low levels of linoleic acid (0.5%), indicates that *Moringa* oil is highly resistant to oxidation,

making it suitable for high-heat applications and long-term storage. In contrast, Watermelon seed oil is characterized by a high concentration of linoleic acid (63%), a polyunsaturated fatty acid that supports skin health and immune function, but also makes the oil more prone to oxidative degradation. It also contains moderate levels of palmitic acid (14%), cis-oleic acid (13%), and stearic acid (11%), contributing to a balanced blend of saturated and unsaturated fats. Sunflower oil on the other hand displays a dual-rich profile, with cis-oleic acid (55%) and linoleic acid (35%) offering both oxidative stability and essential fatty acids. Its low saturated fat content (palmitic and stearic acids <5%) enhances its appeal as a heart-friendly oil. The fatty acid compositions of *Moringa*, Watermelon, and Sunflower oils highlight their distinct functional, nutritional, and industrial potentials. In contrast, Watermelon oil comprises a huge amount of linoleic acid (63%) and lower oleic acid (13%), making it more oxidation-prone but valuable for skin health and nutritional formulations due to its essential omega-6 content. Sunflower oil offers a balanced profile (56% oleic and 35% linoleic acid), combining moderate stability with essential fatty acid benefits, suitable for both culinary and cosmetic use. Oleic acid in *Moringa* and Sunflower oils supports heart health and reduces inflammation, while linoleic acid in Watermelon and Sunflower oils aids membrane integrity and skin function, though its oxidation sensitivity limits use in high-heat processing. Thus, the analysis underscores how varying fatty acid profiles define the specific applications and health benefits of each oil. Overall, *Moringa* oil excels in oxidative stability due to its high monounsaturated fat content, Watermelon oil offers high nutritional value with rich polyunsaturated fatty acid, and Sunflower oil provides a balanced fatty acid profile suitable for both health and culinary applications.

4. Conclusion:

Seed oils from *Moringa*, Watermelon, and Sunflower are rich in health-promoting monounsaturated and polyunsaturated fatty acids, also contain vitamin E, which contribute to cardiovascular support, immune modulation, and skin nourishment. In addition to their nutritional value, these oils have broad industrial relevance, particularly in cosmetics, pharmaceuticals, and biofuel production. Sunflower oil, with its high energy content and unsaturated fatty acid profile, is especially promising for biodiesel applications. This comparative study demonstrated that SC-CO_2 extraction offers notable advantages over conventional n-hexane extraction for *Moringa,* Watermelon, and Sunflower seed oils in both edible and industrial applications. SC-CO_2-extracted *Moringa* oil exhibited enhanced thermal stability and lower peroxide and acid values, indicating superior oxidative stability.

Watermelon oil also displayed improved flash and fire points along with reduced degradation markers. Similarly, Sunflower oil extracted via SC-CO_2 had higher thermal resistance and marginally lower peroxide and acid values than hexane-extracted counterparts. These findings highlight SC-CO_2 as a cleaner, safer, and more efficient extraction method, yielding higher-quality oils suitable for high-temperature use and longer shelf life. SC-CO_2-extracted oils demonstrated superior thermal stability, including higher flash and fire points, making them more appropriate for high-temperature culinary applications. Moreover, SC-CO_2 extraction offers significant sustainability benefits: it eliminates the use of toxic organic solvents, thereby preventing solvent residues in the extract and reducing environmental effect. Fatty acid methyl ester analysis confirmed high unsaturation across all oils up to 79. 98% in *Moringa* (mostly cis-oleic acid), 74.23% in Watermelon (rich in linoleic acid), and 91.14% in Sunflower oil. Overall, SC-CO_2 extraction not only enhances oil quality and safety but also supports environmentally sustainable and cleaner processing practices, aligning well with green technology and industrial innovation goals. SC-CO_2 extraction serves as a sustainable, high-quality alternate to traditional methods, aligning with industrial demands for safer and greener oil processing. Its implementation can promote innovation in environmentally friendly, value-added oil production systems.

Supplementary data:

The Supplementary data is available at:

file:///C:/Users/User/Downloads/supplimetary%20file-Rai%20(70-SCI-2505-10289).pdf

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Figures

Figure 1: Photograph of complete Supercritical Fluid Extraction unit

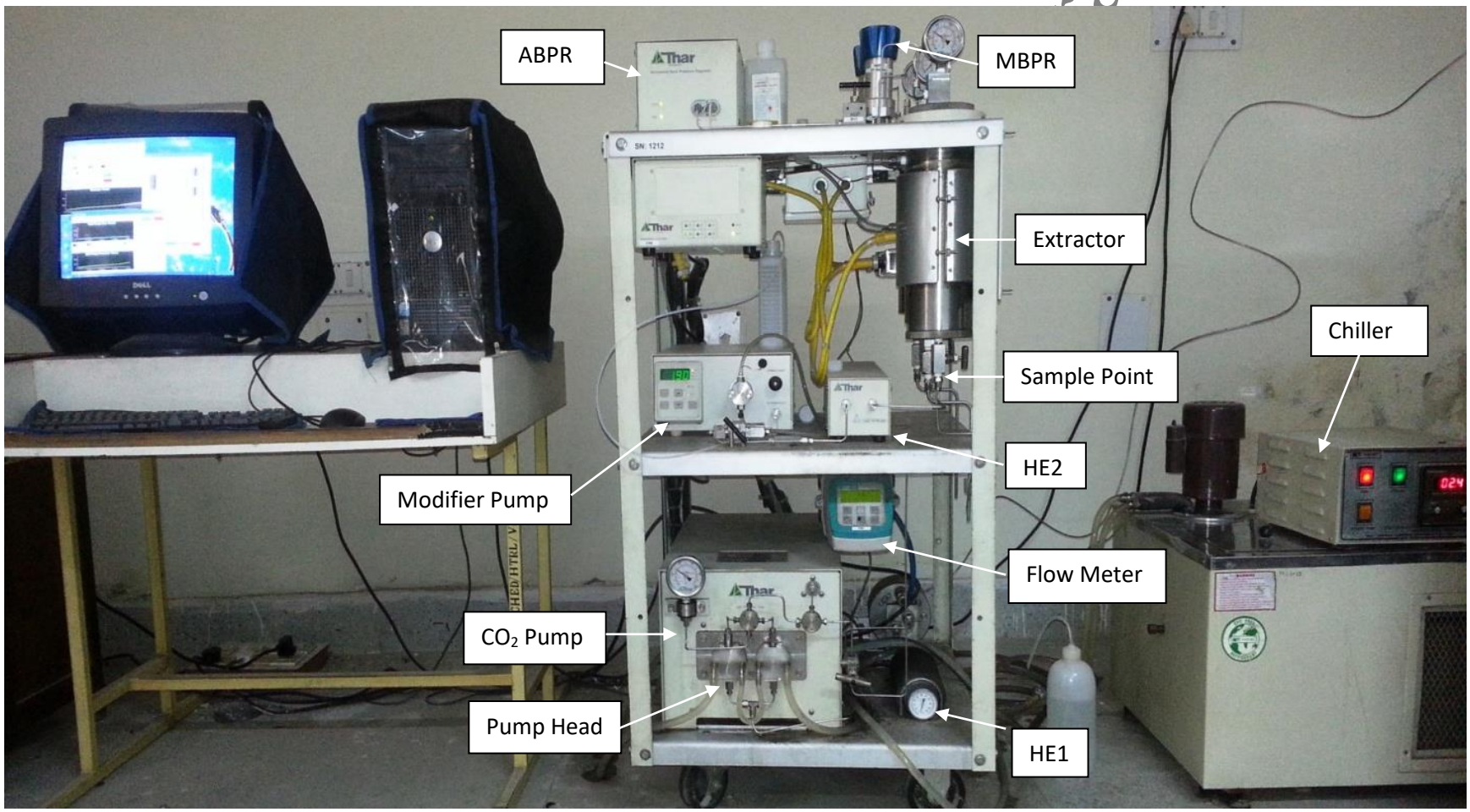
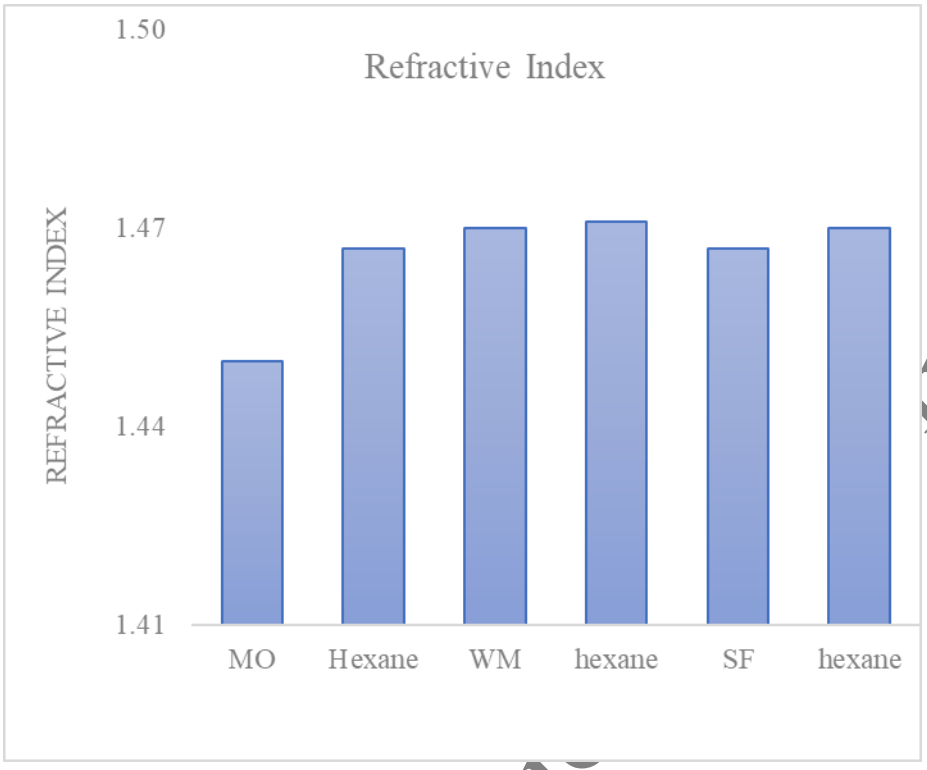


Figure 2: Physicochemical properties of *Moringa* seed, watermelon and sunflower seed oil

a



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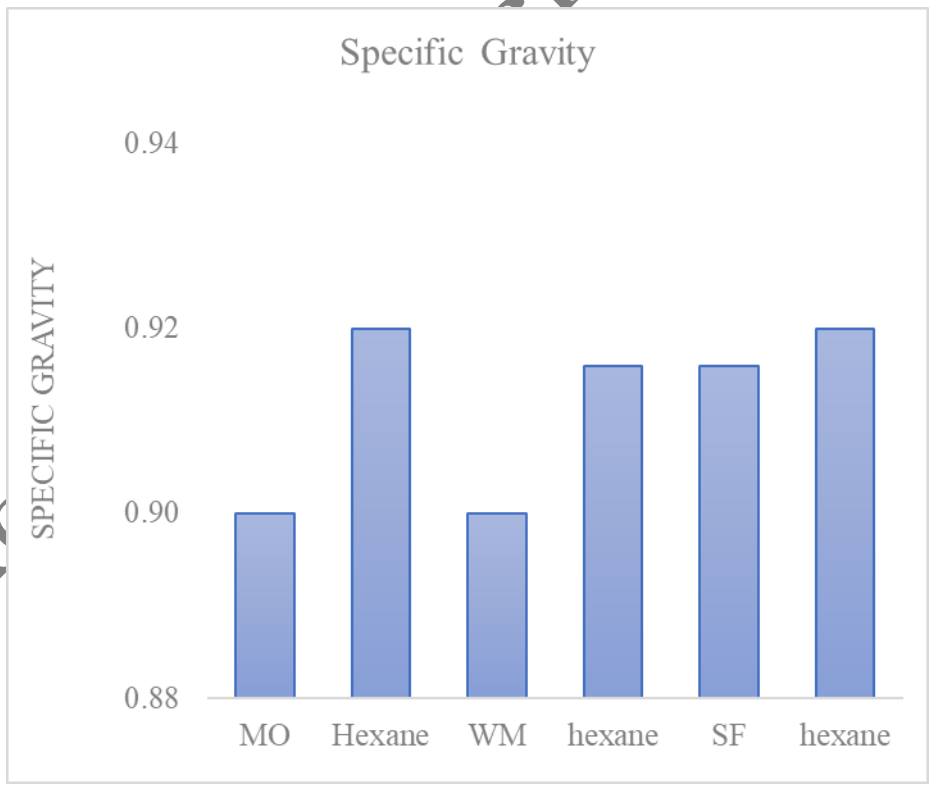

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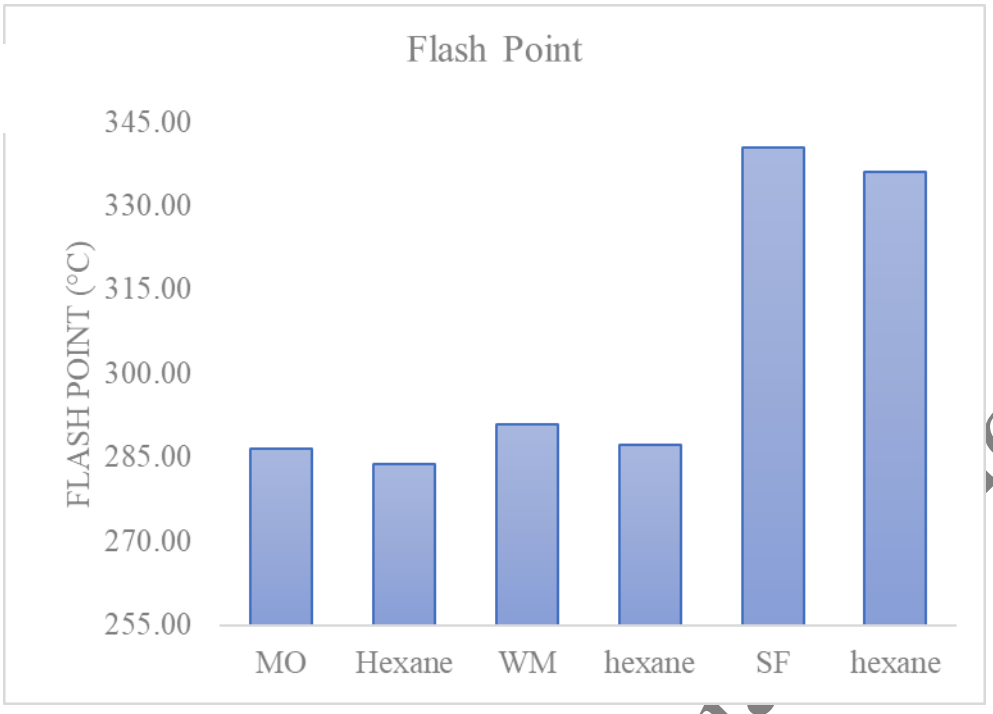
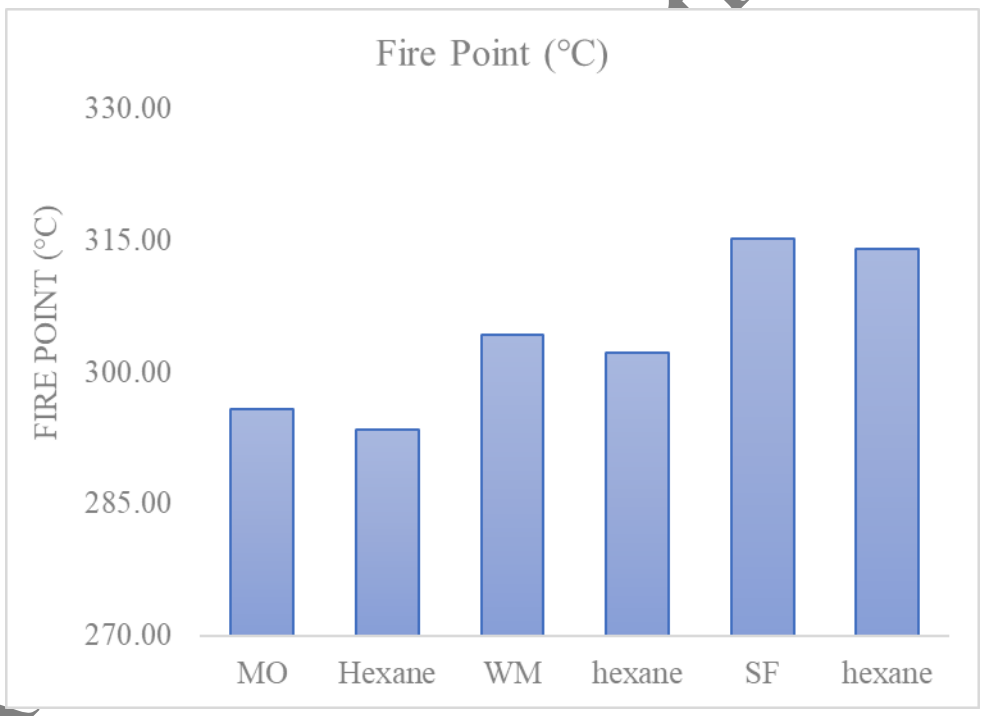
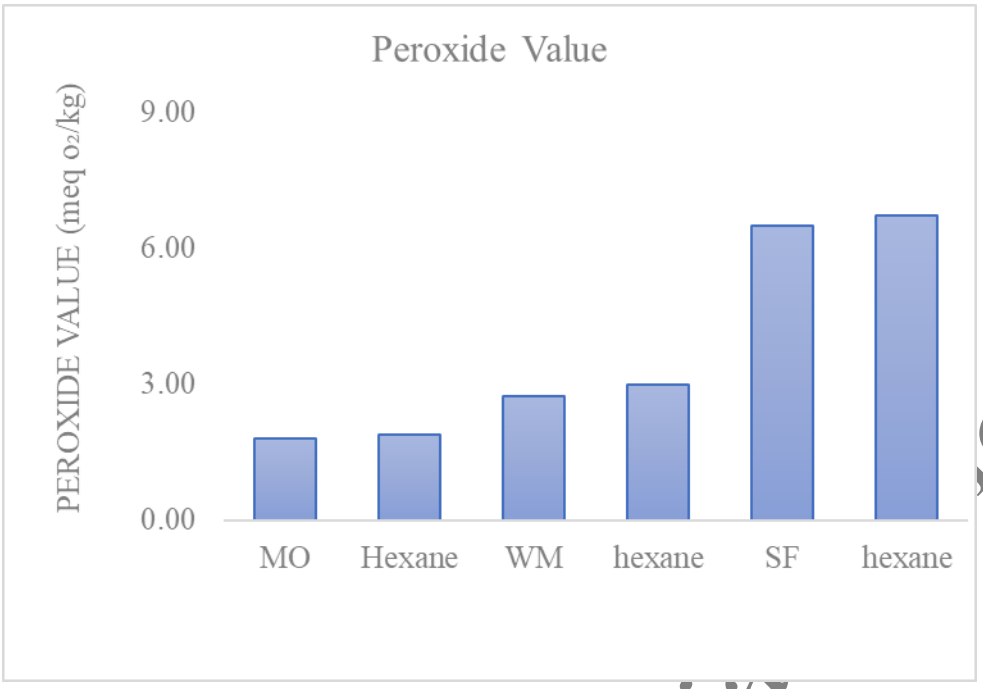

Flash Point
FLASH POINT (°C)
345.00
330.00
315.00
300.00
285.00
270.00
255.00
MO
Hexane
WM
hexane
SF
hexane

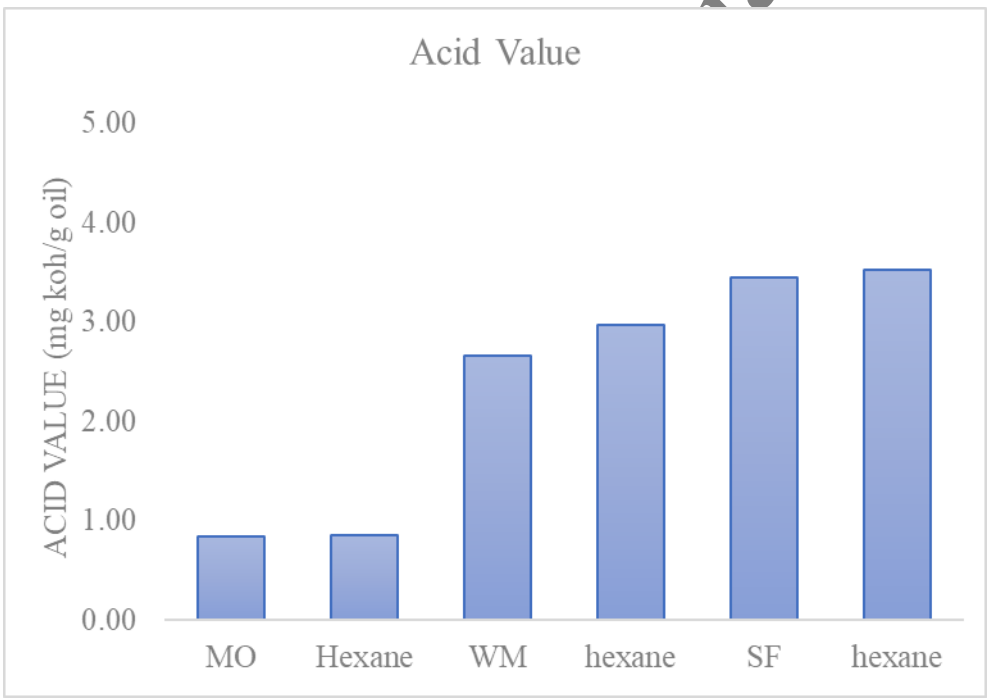
d


Fire Point (°C)
FIRE POINT (°C)
330.00
315.00
300.00
285.00
270.00
MO
Hexane
WM
hexane
SF
hexane

e


Peroxide Value
PEROXIDE VALUE (meq o2/kg)
9.00
6.00
3.00
0.00
MO
Hexane
WM
hexane
SF
hexane

f


Acid Value
ACID VALUE (mg koh/g oil)
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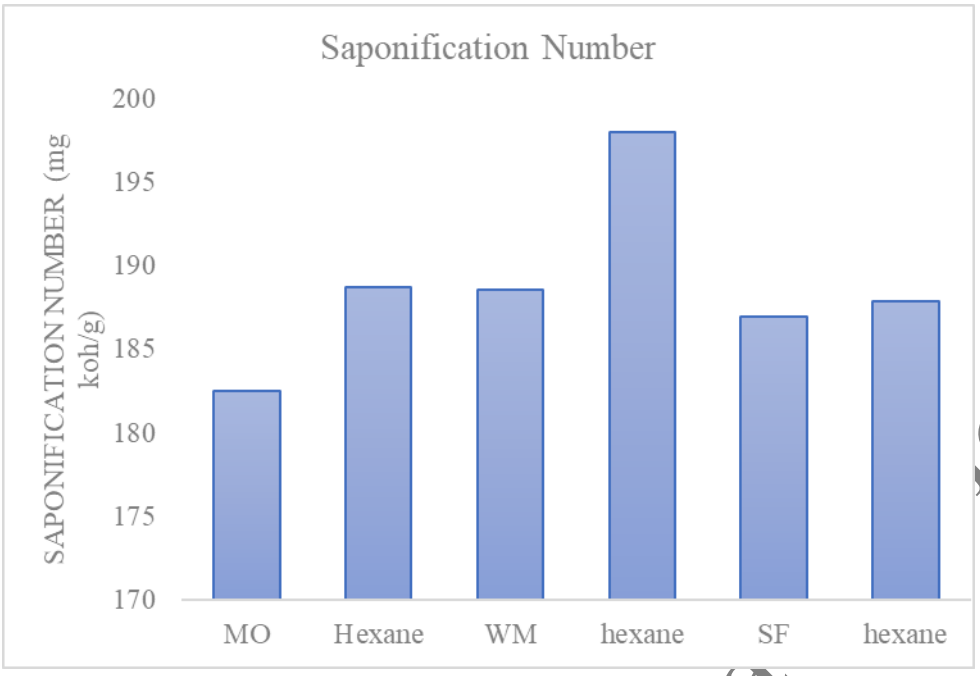
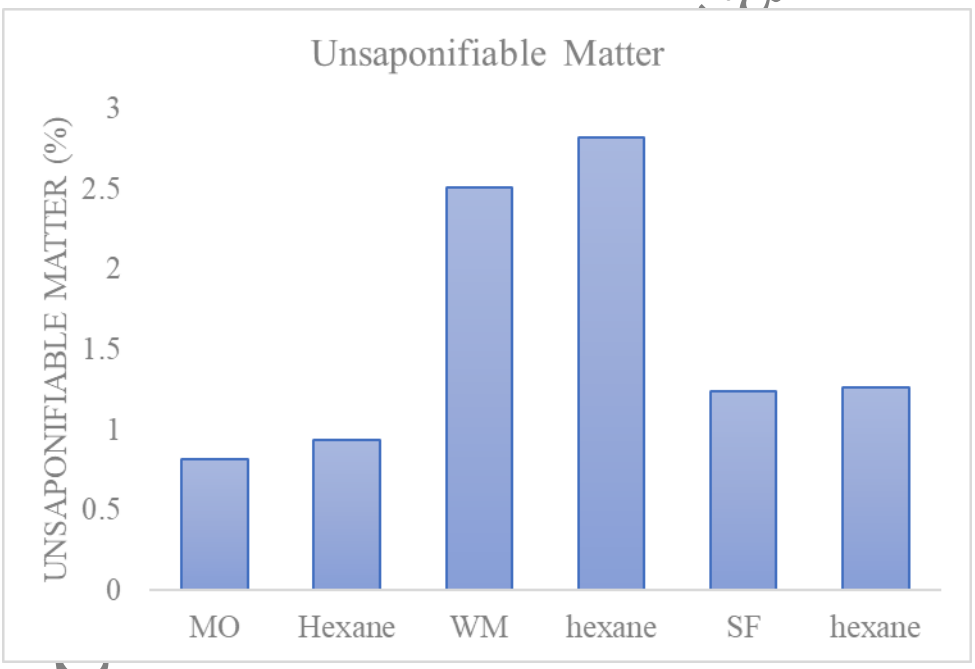
4.00
3.00
2.00
1.00
0.00
MO
Hexane
WM
hexane
SF
hexane

g


Saponification Number
SAPONIFICATION NUMBER (mg koh/g)
200
195
190
185
180
175
170
MO
Hexane
WM
hexane
SF
hexane

h


Unsaponifiable Matter
UNSAPONIFIABLE MATTER (%)
3
2.5
2
1.5
1
0.5
0
MO
Hexane
WM
hexane
SF
hexane

i

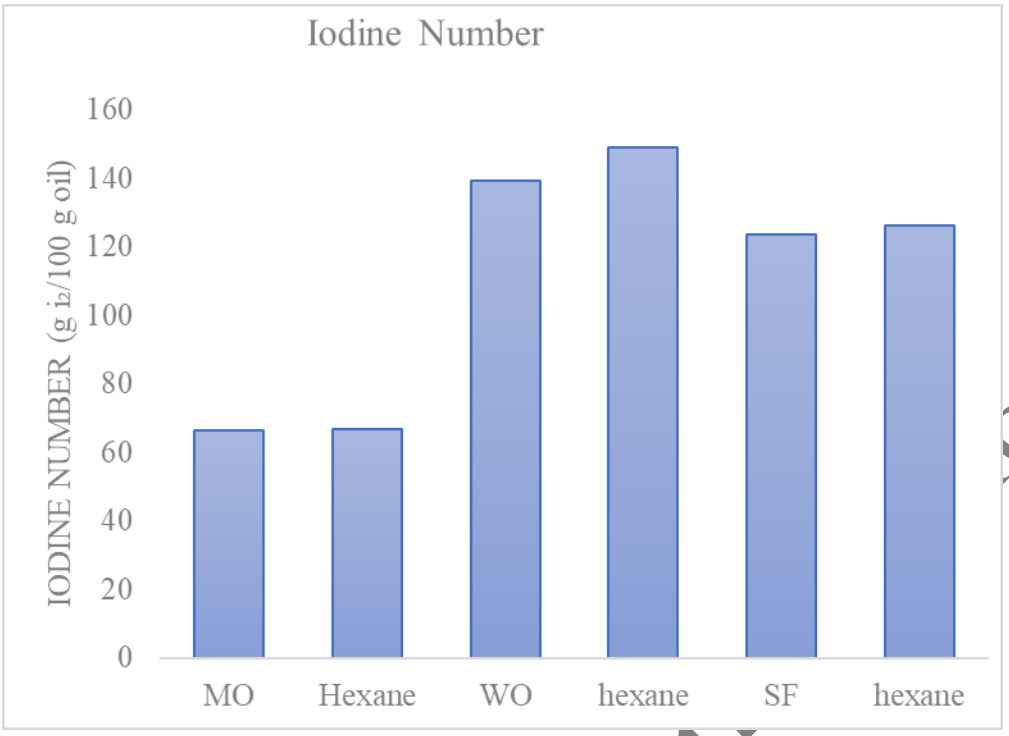


Figure 3: Differences in the content of saturated and unsaturated fatty acids derived from *Moringa Oleifera,* Watermelon and Sunflower seed

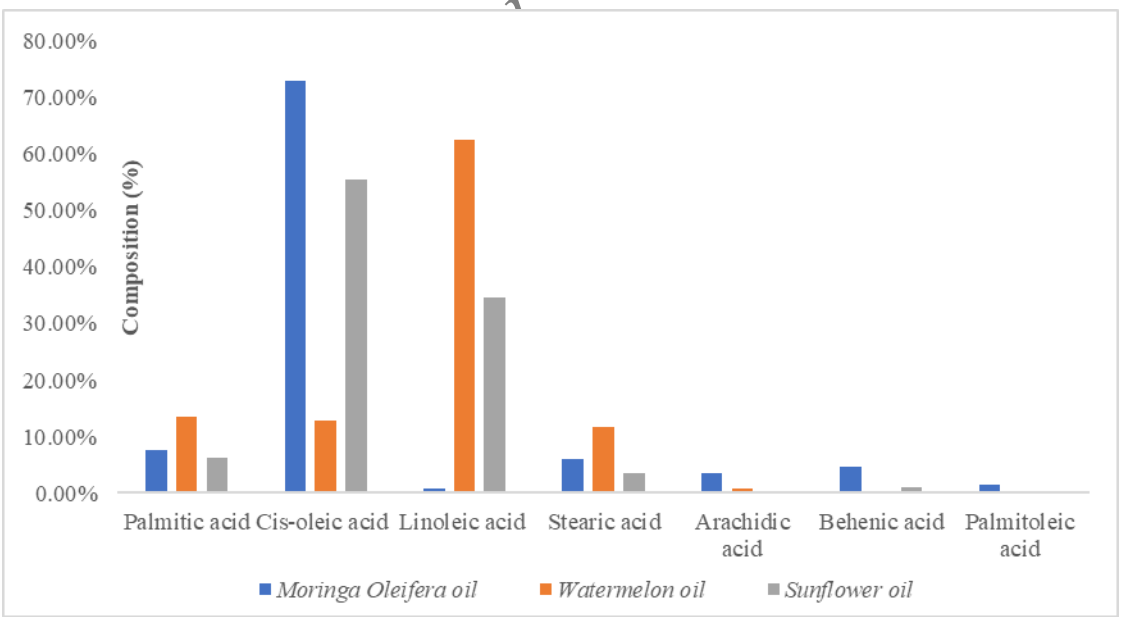


Table 1: Proximate composition of *Moringa,* watermelon and sunflower seed

Constituents (%)	Percent composition		
	Moringa seed	Watermelon	Sunflower Seed
Moisture	5.60 ± 0.12	6.54 ± 0.21	4.58 ± 0.13
Carbohydrate	17.74 ± 0.11	7.34 ± 0.15	23.77 ± 0.03
Protein	29.37 ± 0.14	29.56 ± 0.16	20.19 ± 0.05
Fat	35.57 ± 0.13	47.45 ± 0.62	44.46 ± 0.64
Crude fiber	7.28 ± 0.03	5.41 ± 0.05	3.91 ± 0.04
Ash	4.28 ± 0.07	3.12 ± 0.07	3.19 ± 0.03

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