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Assessment of Moringa Seed Oil: Fatty Acid Profile, Oxidative Stability and Antioxidant Activity

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ABSTRACT

Moringa oleiferais called the "miracle tree" and it has more vitamins than even some fruits and vegetables like oranges, carrots etc. **Objective:** To assess functional groups and compare oxidative stability, fatty acid profile, free fatty acid concentration, and antioxidant activity to commercial vegetable oil. **Methods:** The moringa seed oil was extracted by cold press extraction and solvent extraction by n-hexane and petroleum ether. Functional group, lipid peroxidation, fatty acid profile, antioxidant activity, and FFAs % were analyzed by FTIR, TBARS, GC-MS, DPPH, and titration respectively. **Results:** The FTIR spectra of prepared samples showed common functional groups of triglycerides, including a sharp peak at 2984 cm-1 for aliphatic C-H stretching. The maximum TBARS value was 0.234 ± 0.03% in T0 at 30 days of storage, whereas the lowest was 0.167 ± 0.04% in T1 at 0 day. The GC-MS analysis of screw press moringa oil showed a high percentage of monounsaturated fatty acids, with 71.38 ± 0.01% oleic acid, 7.01 ± 0.01% palmitic acid, and 1.92 ± 0.01% linoleic acid. At 15 days of storage, FFAs were 2.28 ± 0.06%, showing low hydrolytic rancidity. The Antioxidant Activity in DPPH analysis was 44.46 ± 0.02%, showing high antioxidant properties. **Conclusions:** The characteristics of moringa seed oil indicate that it could be an effective edible oil and suitable for the production of food items and other edible products in the food and nutraceutical sectors.

INTRODUCTION

The Moringaceae family includes the plant known as *Moringa oleifera* (*M. oleifera*). It is a perennial that is commonly found in Southern and Eastern Asia, and it is likely the most cultivated species in the northwest of the Indian subcontinent. Tropical and subtropical regions are suitable for *M. oleifera* cultivation [1]. It is considered as the "miracle tree" because of its many beneficial features, such ascontaining seven times more vitamin C than oranges and ten times more vitamins than carrots. It is extensively grown for the many uses of its tender seed pods and verdant leaves as food and medicine.Its high protein content makes it an excellent supplement as well [2]. Moringa seed oil have high amount of oleic acid (78%) and low amount of essential fatty acids such as linolenic acid 2.2% and linoleic acid 0.77% similar to olive oil [3]. It is also a good source of behenic acid wich is use to stabilize and solidify the semi-solid and solid fat foods without hydrogenation of oil [4]. According to, unsaturated fatty

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acids have potent antioxidant, anticancer, and antihyperlipidemic properties that are beneficial to human health [5]. Furthermore, α-and δ-tocopherol are abundant in moringa seed oil, with levels of 45–80 mg/100 g and 0.21–0.53 mg/100 g, respectively [6]. Tocopherols are important nutrients as well as antioxidants that protect cells from damage [7]. Moringa oil has been found to have excellent antioxidant property, as it has significant quantity of tocopherols. Moreover, low concentration of polyunsaturated fatty acids increases stability and oxidative resistance [8]. Commercial vegetable oil is an important constituent in different food composition and vital element of our daily diet. [9]. According to vegetable oil contains triesters (a byproduct formed by the interaction of fatty acids and glycerol), triglycerides (98g/100g) and other substances are also present in trace amount [10]. Vitamins, polyphenols, tocopherols, phytosterols and diglycerides shouldn't be removed during processing because these elements provide health benefits for people [11]. Although processing and refining of vegetable oil increases its shelf life but still there are some disadvantages. One of the disadvantages is the loss of compounds such as phytosterols, squalene, tocopherols, phospholipids and polyphenols that provide medicinal and technologicalbenefits. The formation of 3-MCPD-esters, trans fatty acids, glycidyl ester and polymeric triacylglycerolsare unwanted compounds during processing which is another drawback of processing [12]. These compounds affect the safety of oil at different levels [11]. There is a substantial correlation between high use of commercial edible oil and a high risk of coronary heart disease. The onset of Coronary Heart Disease (CHD) was caused by the buildup of atheromatous plaque in the arteries, which deprived the functioning heart of blood and oxygen. Numerous risk factors, such as elevated BMI, elevated triglyceride levels, diabetes, physical activity, and infection, contribute to the development of plaque in the arteries [13]. Due to their significant impact on cholesterol metabolism, dietary fatty acids may be linked to Cardiovascular Diseases (CVD). Non-Communicable Diseases (NCDs) will be responsible for over 75% of all deaths globally by 2030, according to health statistics published in the World Health Organization 2015 Gazette [14]. Even though Pakistan is an agricultural nation, the country nevertheless spends a lot of money importing edible oil [15]. Pakistan is only producing conventional oil-seed crops, which accounts for 18 % of the total need. To meet the needs, a significant sum of foreign currency is being used for the import of edible oil. A household's food expenses are significantly impacted by the import of edible oil [16]. Pakistan produces about 0.7 million tons of edible oil, compared to imports of between 1.8 and 8.0 million tons. Pakistan produces approximately 3 million tons of edible oil annually, of which 6.6 million tons are exported to meet the country's growing demands [17]. Oil extraction techniques have the potential to modify tiny components that have functional qualities and support stability during oxidation. Because solvent extraction is affordable and easy to use, it has gained a lot of popularity. It can result in high oil recovery efficiency and enable many extractions to be conducted simultaneously [18]. Petroleum ether and n-hexane were used to extract moringa oil, and the results showed a 29.98 and 33.47 weight percent oil yield, respectively [19]. The high tocopherol content of *M. oleifera* oil helps to preserve it from oxidative damage during processing and storage. Of all the tocopherols, α tocopherol stands out as being the most significant since it supports a number of bodily biochemical processes, models the expression of proteins involved in cholesterol metabolism, and inhibits and promotes cell growth [20]. Moringa oil has a higher tocopherol content than other oils. Moringa oil can be used as a vegetable oil source for human consumption in diets [21].

METHODS

Seeds of moringa oleifera (50 kg) were bought from a local market of Multan in the processed form of dried seeds after removal of its shell, were stocked for further use. All the reagents of analytical grade were purchased from Hale Marketing International. The purchased seeds of Moringa oleifera were sun dried and reduce into small particle sizes through the use of a grinding machine and were stored at 25oC for further use [22]. Moringa seed oil was extracted using two different methods. These methods include solvent extraction using n-hexane (T1) and petroleum ether (T2) and mechanical extraction (T3). For solvent extraction, 10g of moringa seed powderwas placed in a thimble and then fit in a Soxhlet extractor and used n-hexane as a solvent. The extraction was performed for 2-3 h at 50-60°C till 2-3 back siphon. For extraction with petroleum ether 10 g of moringa seed powder were treated with 210ml solvent for about 6 hours. Then, solvent was evaporated using rota-vapor and hot air oven. The extracted oil was then recovered by removing the hexane using an oven. For mechanical extraction, whole moringa seeds were used, and the oil was extracted using a mechanical extractor. The yield was calculated by dividing grams of extract over original powder respectively. Each Treatment (group) was denoted by T0, T1, T2 and T3, and three replicates were taken for each treatment. Each group contained approx. 12.5 kg seed before extraction. After the extraction of Moringa oil different analytical methods were performed to determine the different valuable properties of moringa seeds oil. Moringa seed oil free fatty acid composition was analyzed using method No. Ca 5a-40(2). 5g oil was mixed

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with 50ml ethanol and phenolphthalein indicator 2-3 drops were added, then titrated against 0.1 KOH (reagent grade, 90%, flakes) until a pink color endpoint [23]. Moringa seed oil peroxide value was determined using the approved Method No. Cd 8b-90. 5g oil was mixed with 30ml POV solution glacial acetic acid (99% ACS Grade) /chloroform (≥99% ACS Reagent Grade) in 3:2 and 30ml distilled water, then titrated with 0.01N sodium thiosulphate (Na2S2O4) (reagent grade 98, pellets anhydrous 1310-73-2) until a colorless endpoint was reached, using starch solution as indicator [23]. Moringa seed oil saponification value was determined according to, method no. Cd 3-25. 2g oil was refluxed with 0.5N ethanolic potassium hydroxide (0.1N Reagent Grade, c (KOH) = 0.1 mol/l 0.1N) for 45 min on reflux condenser, then cooled and titrated with 0.5N H2SO4 (96% ACS Reagent Grade) using indicator phenolphthalein [23]. A blank sample was run simultaneously, and the saponification value was calculated using formula. Moringa seed oil acidity value was determined according to method no. Cd 3d-63 [23]. A 10g oil sample was titrated with 0.1N NaOH (reagent grade, ≥98%, pellets anhydrous) until a light pink endpoint was reached, using 2-3 drops of phenolphthalein as indicator. The acidity value was then calculated using formula. Moringa seed oil fatty acid profile was analyzed using GC-MS. Methyl esters were prepared using methanol. The sample was injected through Agilent 7693A Auto sampler into the Agilent 5977B GC/MSD and Agilent 8890 GC system with helium as the carrier gas, and a temperature program was fixed between 70-280°C. The injection and detector temperatures were 240-250°C. Peaks were identified by comparison to standards, and fatty acid content were calculated as percentages (%) [24]. Moringa seed oil lipid peroxidation was assessed using thiobarbituric acid (reagent grade, ≥98%). 10ml oil was mixed with 50ml deionized water and 2.5ml of 4M HCl (ACS reagent, 37 7647-01-0), then heated to obtain total volume of 50ml. After boiling, 5ml was combined with 5ml TBA reagent and heated to 100°C for 35min. Absorbance was measured at 538nm using a Agilent Cary 60 UV-visible spectrophotometer that has a wavelength range from 190-1100 nm that can scanned in under 3 seconds, with deionized water as a blank [25].

$$\text{TBARS} = \frac{\text{Absorbance} \times \text{Volume Factor}}{\text{Slope of curve}} \times \text{Dilution Factor}$$

The free radical scavenging ability of Moringa seed oil was determined using DPPH. A 0.5 mL DPPH solution (0.15 mM) was added to 1g of the extract oil (in methanol). After mixing and 30 minutes at room temperature, the absorbance was measured at 517 nm using an Epoch Eliza reader (Bio-Tek Instruments, Inc., Winooski, VT, USA [26].

The radical scavenging activity of DPPH was measured by using the formula:

$$\text{Total antioxidant (\% Inhibition)} = \frac{\text{A Blank} - \text{A sample}}{\text{A Blank}} \times 100$$

According to the method of with some modifications, total phenolic content of moringa seed oil were determined by using Folin-Ciocalteu (FC) reagent [27]. A 300µLof oil sample were mixed with 600µL of 10% FC reagent and mixed with vortex. Then 2400µL of 700mM Na2CO3 (Powder, ≥99.5%, ACS reagent) solutionwere added. After this, mixed solution was placed in the dark at room temperature for 30 minutes. Epoch Eliza reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) were used to obtain the absorbance at 765 nm. These results were expressed as mg GAE (gallic acid equivalent) per 100g-1. Commercial oil and moringa seed oil samples were analyzed by using FTIR spectroscopy. Protocol explained by was followed with slight modifications [29]. A drop of each sample was placed on the IR crystal port and compared with conventional sample analysis. The spectra were obtained using an Agilent Cary 360 FTIR spectrometer, operating at a resolution of 4 cm-1 and scanning 10 times over a range of 4000-650 cm-1. The resulting spectra were analyzed using ORIGINPRO 8.5 software, measuring peak height and area. 100ml packaging of moringa seed oil was stored for 30 days at room temperature (25° C) in the dark place. Triplicate analyses were performed to ensure data reliability. Significant differences were assessed using ANOVA, following procedures [28]. The Completely Randomized Design (CRD) was computed using STATISTIX 8.1 software.

RESULTS

Moringa seed oil was extracted with solvent extraction (n-hexane and petroleum ether) and mechanical extraction. The yield of oil by solvent extraction (n-hexane and petroleum ether) and mechanical extraction were 24.77 ± 0.16%, 23.44 ± 0.20% and 22.61 ± 0.08% respectively as shown in (Figure 1). There was a significant difference ($p<0.05$) between the treatments for the analysis of oil extraction yield.

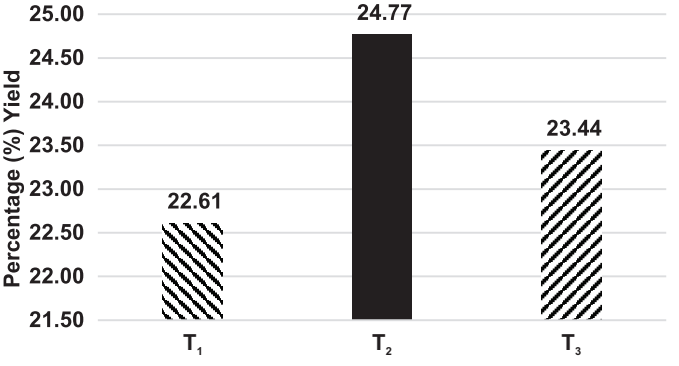

Figure 1: Percentage Oil Yield

Free fatty acid value was used to assess the hydrolytic rancidity and overall quality of an oil, indicating the quantity of free fatty acids present. The graph presents the values

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of free fatty acids in commercial edible oil and moringa seed oil extracted using solvent extraction (petroleum ether and n-hexane) and the cold press method at 0 day, 15 days, and 30 days of storage. The results showed that at 0 day, T0 had the highest content 4.47 ± 0.01% of FFA at day 15 and T1 had lowest content of 2.28 ± 0.06% FFA at day 1 as shown in (Table 1). Triglycerides' hydrolysis was the primary cause of an increase as it produces free fatty acids and other oxidation harms. Significant difference ($p<0.05$) was shown in between the treatments when analyzing free fatty acids in moringa seed oil.

Table 1: Influence of Storage and Treatment on FFAs

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	4.28 ± 0.01^{B}	2.28 ± 0.06^{E}	3.61 ± 0.05^{D}	3.78 ± 0.01^{C}	3.48 ± 0.03^{B}
15th Day	4.47 ± 0.01^{A}	2.31 ± 0.05^{E}	3.57 ± 0.03^{D}	3.80 ± 0.06^{C}	3.53 ± 0.03^{A}
30th Day	4.37 ± 0.02^{A}	2.41 ± 0.09^{E}	3.58 ± 0.04^{D}	3.82 ± 0.03^{C}	B3.54 ± 0.04^{A}
Mean ± SD	B4.37 ± 0.01^{A}	2.33 ± 0.06^{D}	3.58 ± 0.04^{C}	3.80 ± 0.03^{B}	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

Peroxide value was a measurement of the amount of lipid oxidation in an oil, which provides a measure of its stability and freshness. According to graph, highest peroxide value was 2.86 ± 0.01meq O_2/kg found in T0 at day 30 and lowest peroxide value was 0.85 ± 0.04meq O_2/kg found in T1 at 0 day of storages shown in (Table 2). Which means it can be rancid and off flavor rapidly than the moringa seed oil extracted by cold press method. Significant differences ($p<0.05$) were assessed in between the treatments when analyzing free fatty acids in moringa seed oil.

Table 2: Influence of Storage and Treatment on Peroxide Value

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	2.80 ± 0.08^{A}	0.85 ± 0.04^{E}	1.35 ± 0.01^{D}	1.86 ± 0.02^{B}	1.71 ± 0.03^{B}
15th Day	2.85 ± 0.01^{A}	0.88 ± 0.012^{E}	1.44 ± 0.01^{C}	1.93 ± 0.01^{B}	1.77 ± 0.01^{A}
30th Day	2.86 ± 0.01^{A}	0.89 ± 0.02^{E}	1.46 ± 0.01^{C}	1.94 ± 0.01^{B}	1.78 ± 0.01^{A}
Mean ± SD	2.83 ± 0.03^{A}	0.87 ± 0.02^{D}	1.41 ± 0.01^{C}	1.91 ± 0.01^{B}	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

Saponification value can be obtained by determining the quantity of alkali required to saponify a specific volume of oil, thereby indicating the average molecular weight (or chain length) of the fatty acids present. The highest saponification value 189.37 ± 0.06mgKOH/g was observed in T0 and T3 had the lowest saponification value of 181.3 ± 0.05mgKOH/gas shown in (Figure 2). Saponification value between the treatments showed significant results ($p<0.05$) in moringa seed oil.

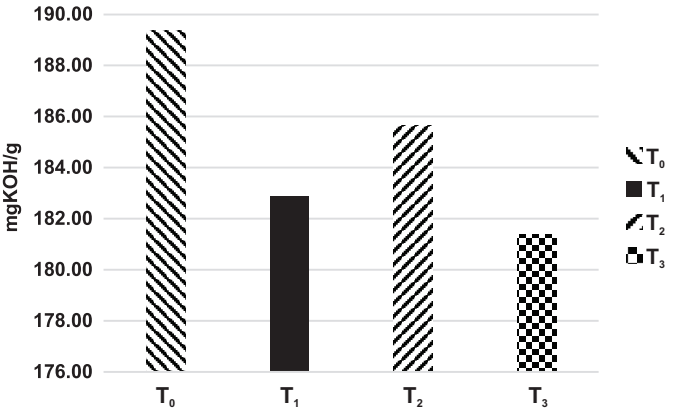

Figure 2: Influence of Treatment on Saponification Value

Overall, Moringa seed oil has relatively low acidity, which was reflected in its low free fatty acid level. It was also chemically stable and meets cosmetic and food industry standards. This indicates that the oil does not have high acidity, allowing the oil's benefits to be retained and increasing the storage time. The highest Acidity value was 0.577 ± 0.001% which observed in T2, and lowest value was 0.507 ± 0.002% observed in T1as shown in (Figure 3). Acidity between the treatments showed significant results ($p<0.05$) in moringa seed oil.

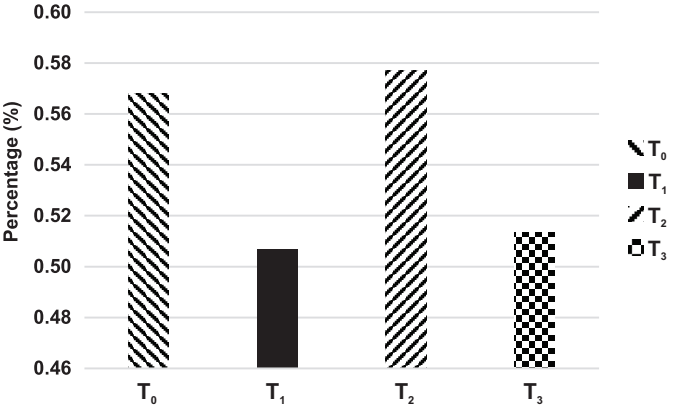

Figure 3: Influence of Treatment on Acidity (%)

Total phenolic contents were based on the F.C (Folin-Ciocalteu) reagent's response with the test sample. Due to the reduction of phosphotungstic and phospomolybdic acid in an alkaline media in the presence of phenolic substances, a blue chromophore was produced. According to Table 3, highest TPC value 46.35 ± 0.02mg GAE/g in T1 was found at 15 and 30 days and the lowest TPC value 38.34 ± 0.36 was observed in T0 at 0 day. Significantly differentiated ($p<0.05$) results were indicated in treatments when total phenolic content was analyzed.

Table 3: Influence of Storage and Treatment on Total Phenolic Content (TPC)

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	38.34 ± 0.36^{G}	45.67 ± 0.26^{B}	43.78 ± 0.30^{D}	42.56 ± 0.25^{E}	42.58 ± 0.29^{C}
15th Day	39.15 ± 0.02^{F}	46.35 ± 0.02^{A}	44.36 ± 0.01^{C}	44.37 ± 0.03^{C}	43.55 ± 0.02^{B}

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30th Day	39.25 ± 0.02^{F}	46.35 ± 0.02^{A}	45.25 ± 0.02^{B}	45.23 ± 0.02^{B}	44.02 ± 0.02^{A}
Mean ± SD	38.91 ± 0.13^{D}	46.12 ± 0.1^{A}	44.46 ± 0.11^{B}	44.05 ± 0.1^{C}	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

DPPH has a dark red color and was a very stable radical. It has a 1, 1-diphenyl-2-picrylhydrazyl chemical structure. Food's color changes from dark red to yellow when antioxidants present in it absorb free radicals. According to Table 4, highest DPPH value 46.44 ± 0.02% was found at 0-day storage in T2 and the lowest DPPH value 31.22 ± 0.02% was observed at 30 days' storage in T0. Significant differences ($p<0.05$) were assessed in between the treatments when analyzing DPPH value of moringa seed oil.

Table 4: Influence of Storage and Treatment on DPPH Value

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	31.46 ± 0.02^{J}	35.45 ± 0.02^{G}	46.44 ± 0.02^{A}	45.65 ± 0.02^{D}	39.75 ± 0.02^{A}
15th Day	31.34 ± 0.02^{K}	35.35 ± 0.02^{H}	46.36 ± 0.01^{B}	45.37 ± 0.03^{E}	39.60 ± 0.02^{B}
30th Day	31.22 ± 0.02^{L}	35.25 ± 0.04^{I}	46.25 ± 0.02^{C}	45.23 ± 0.02^{F}	39.60 ± 0.02^{C}
Mean ± SD	31.34 ± 0.02^{D}	35.35 ± 0.02^{C}	46.35 ± 0.01^{A}	45.41 ± 0.02^{B}	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

FTIR spectroscopy was an analytical technique used to determine the chemical composition and functional groups of substances by analyzing their infrared absorption spectra. It was widely used for quality control and characterization in several industries, including pharmaceuticals and food. FTIR analysis of commercial edible oil and moringa seed oil obtained from solvent extraction (n-hexane and petroleum ether) and cold press technique, showed a peak at 2905 cm-1 for O-H stretching and 2984 cm-1 for C-H stretching. The wavelength of 1691 and 2290 cm-1 for the stretching vibration of the -C=O and O-C=O functional groups respectively. The peak at 1390 cm-1 representing the bending vibration of –C-C- bond. The absorption peak at 1155 cm-1 was related to the bending motion of C-O-C chemical bond. Moreover, the absorption peak showed at 1028-1302 cm-1 and 959-971 cm-1 were related to the stretching of C-O bonds and bending of C-H bonds. All the oils showed similar characteristics bands that can further be used for qualitative measurementas shown in (Figure 4-7). Theoretically, oils need conversion to react with alkali. However, FTIR spectra of moringa seed oil showed no significant alteration in the absorption band of molecules. Significantly differentiated ($p<0.05$) results were observed of FTIR analysis between treatments of moringa seed oil (Figure 4).

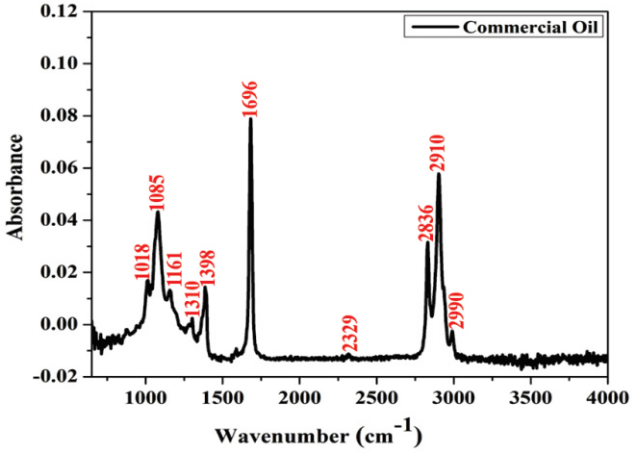

Figure 4: FTIR Analysis of Commercial Edible Oil

Figure 5 displayed the FTIR analysis of cold-pressed moringa seed oil, revealing prominent peaks for functional groups such as triglycerides, with a characteristic aliphatic C-H stretching observed at 2984 cm^{-1}.

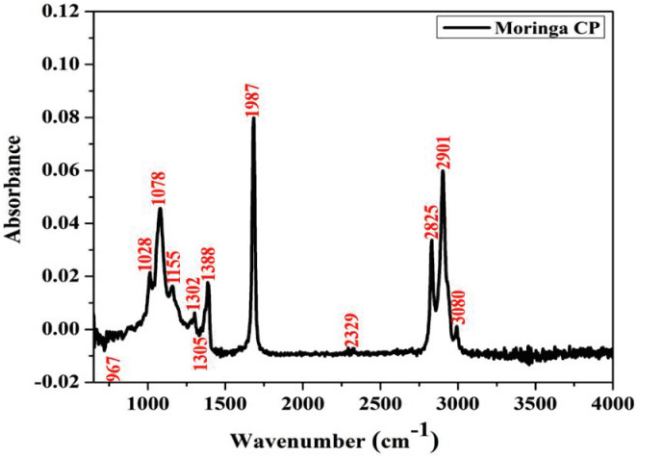

Figure 5: FTIR Analysis of Moringa Seed Oil Extracted with Cold Press

Figure 6 displayed the FTIR analysis of moringa seed oil extracted with n-hexane, revealing characteristic peaks for triglycerides, including a distinct aliphatic C-H stretching band at 2984 cm^{-1}.

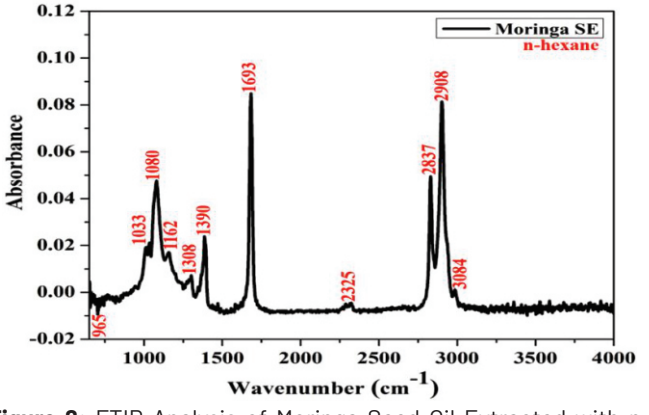

Figure 6: FTIR Analysis of Moringa Seed Oil Extracted with n-hexane

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Figure 7 displayed the FTIR analysis of moringa seed oil extracted with petroleum ether, revealing characteristic peaks for triglycerides, with a distinct aliphatic C-H stretching observed at 2984 cm^{-1}.

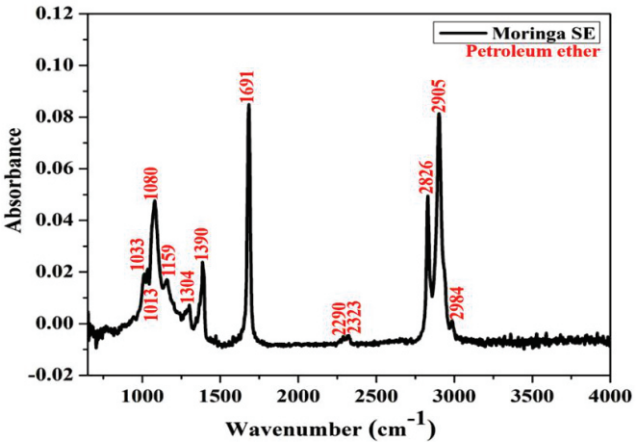

Figure 7: FTIR Analysis of Moringa Seed Oil Extracted with Petroleum Ether

The TBARS analysis Measures Malondialdehyde (MDA) and other secondary oxidation-related compounds that result from lipid peroxidation. As the result shows in Table 5, the highest TBARS content of 0.234 ± 0.03 mg MDA/kg was found in T0 at 30 days of storage and lowest content 0.167 ± 0.04 mg MDA/kg was observed in T1 at 0-day storage. Significant differences ($p<0.05$) were shown in between the treatments when analyzing TBARS value in moringa seed oil.

Table 5: Influence of Storage and Treatment on TBARS

Days	T_0	T_1	T_2	T_3	Mean ± SD
0 Day	0.23 ± 0.03^{A}	0.16 ± 0.04^{F}	0.17 ± 0.03^{D}	0.18 ± 0.05^{B}	0.185 ± 0.03^{C}
15th Day	0.23 ± 0.03^{A}	0.17 ± 0.02^{E}	0.17 ± 0.03^{D}	0.18 ± 0.05^{B}	0.187 ± 0.03^{B}
30th Day	0.23 ± 0.03^{A}	0.17 ± 0.02^{E}	0.18 ± 0.02^{C}	0.18 ± 0.05^{B}	0.19 ± 0.03^{A}
Mean ± SD	0.23 ± 0.03^{A}	0.16 ± 0.02^{D}	0.17 ± 0.02^{C}	0.18 ± 0.05^{B}	-

T_0: (Commercial edible oil)
T_1: (Screw press extracted moringa seed oil)
T_2: (n-hexane extracted moringa seed oil)
T_3: (Petroleum ether extracted moringa seed oil)

Gas Chromatography-Mass Spectrometry (GC-MS) was a technique that combines gas chromatography with mass spectrometry to separate and identify compounds in a sample. Widely employed in environmental investigation, forensics, and food safety. GC–MS analyses were carried out for oil samples using an Agilent 5977B GC/MSD instrument. Results showed that unsaturated fatty acids in the and moringa seed oil obtained by mechanical extraction and solvent extraction (petroleum ether and n-hexane) were higher than the amount of saturated fatty acids which was best suited having healthful attributes. GC-MS analysis performed for the moringa oil indicated that fatty acids profile of commercial edible oil and moringa oil by solvent extraction contained 63.79 ± 0.01%, 67.77 ± 0.01% and 68.2 ± 0.1% oleic acid respectively. Fatty acids profile of moringa oil by mechanical extraction contained 71.38 ± 0.01% oleic acid. Other fatty acids like linolenic acid, linoleic acid and palmitic acid were present in amount 1.92 ± 0.01%, 0.976 ± 0.001%, and 7.01 ± 0.01% respectively as shown in (Figure 1). Significantly differentiated ($p<0.05$) were shown in fatty acid profiling between the treatments were analyzed in moringa seed oil (Figure 8).

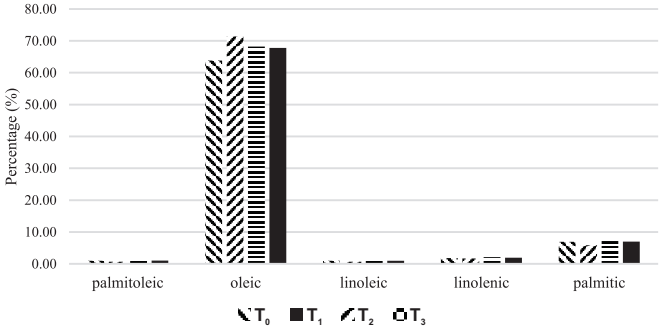

Figure 8: Influence of Treatment on Fatty Acid Composition (%)

DISCUSSION

Moringa seed oil was extracted with solvent extraction (n-hexane and petroleum ether) and mechanical extraction. Triglycerides' hydrolysis was the primary cause of an increase as it produces free fatty acids and other oxidation sources. The percentage of moringa oil indicated by was 40%, 33.6% and 36.7% respectively in accordance to the results of this study as shown in figure 1 [30, 31]. As compared to the investigating the free fatty acid concentration of moringa seed oil and table 1obtained different results [32, 33]. Found the same outcomes when analyzing the free fatty acids of moringa seed oil extracted through cold pressing. The results were identical as used a cold pressed oil to determine the free fatty acids of moringa seed oil [34]. The findings of the investigation showed that the concentration of the utilized extract influenced the levels of free fatty acids. When comparing results of v with table 2in which they compared the peroxide value of moringa seed oil, they give contrasting outcomes [35]. When examining the peroxide value of moringa seed oil by solvent extraction, determined the same conclusions [36]. They concluded that the essential oil derived from the moringa seeds has the lower value of peroxide value, of 1.5±meq O_2/kg compared to other sections. Similar outcomes were observed by who assessed the peroxide value of moringa seed oil by cold press as the results came in figure 3 [37]. The results of the analysis showed that the concentration of the extract employed affected peroxide value. In contrast, observed different outcomes when comparing the saponification values of sage and fennel essential oils [38, 39]. Investigated the same outcomes during analysis of the saponification value of moringa seed oil according to the results of figure 2. They also identified that among all the oils, the oil extracted from the seeds of moringa had the

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least value of saponification, at 179. 5±0. They were also comparatively lower at 25mgKOH/g compared to the other sections [40]. Established the same research results, using solvent extraction to determine the saponification value of moringa seed oil. Consequently, the analysis clearly showed that the extract's concentrate level impacted the saponification value [38]. Observed different outcomes in contrast to figure 3when comparing the acidity values of sage and fennel essential oils. The identical conclusions were examined by when they analyzed the acidity value of moringa seed oil [39]. In comparison to the other sections, they determined that the oil extracted from moringa seeds has the lowest acidity value of 0.494 ± 0.025%. Similar results with figure 5have been identified in the study conducted by in which the acidity value of moringa seed oil was evaluated extracted with solvent extraction [40]. As demonstrated by the results of the analysis, the acidity value was influenced by the concentration of the extract utilized. Antioxidant activity of olive oil in showed the contrasting results with figure 7 [41]. They used ethanolic extract of olive seeds to measure total phenolic contents and DPPH. Results revealed that total phenolic contents of olive oil were 42.87mg GAE/g at concentration of 25 microgram/ml. When measuring the total phenolic contents of two moringa seed species of Portugal according to the antioxidant activity of table 3 given above, shows the same results and findings [42]. They showed through their findings that both species were excellent sources of phenolic chemicals. In M. stenopetala, extract had a total phenolic concentration of 43.45 ± 0.023mg GAE/g, which was higher than the 42.18 ± 0.024 mg GAE/g found in methanolic extract. However, in the M. ovalifolia species, methanolic extracts (44.56 mg GAE/g) had higher phenolic levels than aqueous extracts (41.12 mg GAE/g). Comparable outcomes were found with the study of as shown in table 3 [43]. When they assessed the phenolic concentration of moringa seed oil by cold press. The phenolic fractions were extracted using cold press machine, and the TPC was then measured. The results of the TPC study showed that the cold press extract had a TPC value of 44.13mg GAE/g, which was similar to our findings. Contrasting results were also measured with this study mentioned in table 4by in which they compared the antioxidant activity of essential oils [44]. They compared three different species of essential oils at 60 degrees Celsius for a month, varying the concentrations from 600 to 1000 g/ml. They found that essential oil demonstrated 100% more radical scavenging activity. From their research, they concluded that essential oil has the greatest DPPH, PV, TBA, and BCB values. When examining the radical-scavenging capabilities of moringa seed oil, determined the same conclusions with table 4 [26]. They concluded that the essential oil derived from the moringa seeds has the maximum degree of free radical elimination activity, at 47.95%, compared to other sections. Similar outcomes of table 4were also observed by who assessed the antibacterial and antioxidant properties of moringa seed crude methanol extract [45]. They used 87.97 g of moringa seed powder to make 38.50 g of extract. After that, the concentrate was dried. They compared conventional ascorbic acid at the same concentrations to extract concentrations of 25, 50, 100, 200, and 400 g/ml in order to determine antioxidant activity. The results of the analysis showed that the concentration of the extract employed affected antioxidant activity. The antioxidant activity decrease with change in extraction method. Contrasting results were also observed by with figure 5 in which they studied the peaks for O–H, C–H, C=O, and O–C=O stretching [3]. Spectra showed distinctive peaks 3005 cm-1 peak for cis-olefinic bonds, indicative of unsaturated fatty acids, and the 1747 cm-1 peak for ester groups, representing total lipids. In contrast, observed different outcomes when comparing the FTIR analysis of moringa oil as observed in figure 6 [46]. Variations in environmental conditions during FTIR measurement, such as temperature or humidity, can introduce minor spectral differences. Similar findings were also determined by as they conducted the TBARS content of moringa seed oil as this study has mentioned in table 5 [47]. Variation is observed in the results due to differences in cultivars and variety. In the study to investigate the effects of lipid peroxidation on cold press moringa seed oil, stated that the cessation oil obtained from the moringa seeds contains a lesser value of TBARS content 0.192±0.09 mg MDA/kg [48]. Similar outcomes were observed by who assessed the lipid peroxidation 0.179±0.06 mg MDA/kg of moringa seed oil extracted by cold press [49]. The results of the analysis showed that the concentration of the extract employed affected TBARS value. The value change with the change in extraction method. Contrasting results of this study were shown with the study when comparing fatty acid profiles [50]. Similar results have been identified in the study conducted by in which fatty acid profile of moringa seed oil was evaluated, studies highlight oleic acid (C18:1) as the predominant fatty acid as with the analysis mentioned in figure 8 [51]. Variations with other studies results were due to Moringa plant sources, such as different species or varieties of Moringa oleifera, or even geographic origin, can lead to differences in the fatty acid composition of the extracted oil. The change in percentage of extracted oil yield was due to the change in environment, variety, difference in cultivar and soil condition or the chemical composition of moringa plant.

CONCLUSIONS

The nature of the oil extracted from *Moringa oleifera* seeds, variety PKM 1 showed that this oil can be used effectively as one of the edible oils for human consumption. The potential beneficial aspects of moringa oil were superior to other ordinary vegetable oils. Its composition, which was very different from most other oils in terms of the range and density of its components comprising essential fatty acids

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and antioxidants, means there was great potential for high nutritional and industrial value from the oil. According to the research, moringa oil contains many valuable nutrients and health-enhancing compounds. It was highly stable when exposed to oxygen, thus making it a valuable ingredient in food supplements and technical applications. It has a great effect in improving people's health and promoting sustainable approaches in different sectors.

Authors Contribution

Conceptualization: MHUH, SA, UF, UM,
Methodology: MHUH, SA, UM, MAK, MI
Formal analysis: MHUH, MS, SA, UF, MI
Writing, review and editing: SA, MAK, UF, HN, UM, AM, TBQ

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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