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Chemical characteristics of Moringa oleifera oil as affected by harvest dates and extraction methods

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Abstract

Egypt has been suffering from a shortfall in the domestic production of vegetable oils for a long time. *Moringa* is a promising new alternate source for edible oil. The current research is aiming to evaluate chemical characteristics of *Moringa oleifera* oil as affected by different harvest-dates and extraction methods. Our study was carried out on three-year-old *Moringa oleifera* trees to add more information concerning the effect of harvest-date stages, where collecting fruit pods containing seeds after 60, 75, and 90 days from blooming in combination with different methods of extraction: warm press, cold press, and chemical solvent. Seed-oil yield of *Moringa oleifera* and its physical as well as chemical characteristics were evaluated. The main components of *Moringa oleifera* seed oil were analyzed and identified by GC-MS. The obtained results presented that on the 3rd harvest date (after 90 days from blooming) seed oil was markedly increased and seed oil was characterized by lower acid number and lower free-fatty acids. The chemical method (petroleum ether solvent) recorded the highest oil yield followed by the warm press and cold press methods, respectively. On the other hand, the cold press recorded rather high-quality features (very low acid number and free-fatty acids) followed by the warm press and petroleum ether solvent methods, respectively, and still acceptable in the case of warm-press for human consumption and suitable in the case of petroleum ether solvent for use in many industrial purposes. It could be concluded that harvest pods after 90 days from blooming in combination with oil extraction by cold press resulted in lower oil yield with rather high-quality features and excellent for human consumption.

Keywords: Moringa oleifera, Harvest stages, Extraction methods, Cold press, Warm press, Oil yield, Oil characteristics
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1. Introduction

Globally, vegetable oils constitute a very necessary part of human life. Vegetable oils contain very important polyunsaturated acids, such as oleic acid, linoleic acid, and palmitoleic acid. Polyunsaturated acids have antioxidant properties and could increase antioxidants, subsequently could improve many of the metabolic processes in humans [1]. For example oleic acid has been shown to help prevent coronary artery disease. Also, it is using a diet rich in monounsaturated fatty acids could prevent cardiovascular diseases [2].
Vegetable oil consumption is mainly dependent on traditional sources such as soybean, palm, canola, and sunflower oil with 31.6, 30.5, 15.5, and 8.6 million tons annually consumed, respectively. The traditional sources of vegetable oils can't meet the global growing up of oil demand for both industrial and domestic purposes [3]. Global demand for oils/fats is anticipated to expand significantly. For example, expand by 33 Million tons by 2030 [4], with a consequential increase in prices.

Moringa - the miracle tree carries important aspects to poor people as a food supplement for both humans and animals and for medical purposes according to its wide use in folk medicine as well as promising new alternate source for edible oil [5, 6, 7, 8 and 9]. The oil of Moringa seeds contains a brilliant yellow oil approximately 28:42 %, with high oleic acid. The crude oil of Moringa seeds has a pleasant

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peanut flavor, containing 39.3% crude fat, 0.60% moisture, 1.5% ash, 2.19% protein, and 56.4% total carbohydrates. The chemical characteristics of Moringa oil include pH 5.96 - saponification value of 164.09 mg/g - iodine value of 68.23g/mol - free fatty acid of 8.27 mg KOH/g and specific gravity of 0.86. Also, Moringa seed oil contains several fatty acids such as Palmitic acid (6.8%), Oleic acid (70%), Stearic acid (6.5%), Behenic acid (5.8%) Palmitoleic acid (2.9%), Arachidic acid (4.2%) and Linoleic acid (0.9%) [10].

Seed maturity at the harvest stage is the most important factor that can affect seed quality and oil yield. In addition, harvesting time is one of the critical steps in oil crop production. Where there is a period of very intense oil formation, which occurs about midway between blooming and the final maturity of the seed [8]. Unavoidable delays or those resulting from careless farmers' attention cause the exposition of the seeds to unfavorable environmental conditions and acceleration of the deterioration process. Hence, it is necessary to harvest at a time as close as possible to the physiological maturity, i.e., after the stabilization of dry matter translocation to the seeds, when they reach the maximum germination and vigor potential. The early harvest of seeds may result in low yield and poor seed quality. On the other hand, the late harvest of seeds may result in shattering seed yield [11, 12].

Oil extraction is one of the maximum crucial steps in seed oil processing as it determines the amount and quality of oil extracted. Optimization of the extraction situations for every extraction methodology complements oil yield and its quality. Meanwhile, a cautiously selected optimization system similarly has the ability to save time and heat requirements with an associated consequence on cost reduction of the entire process [13]. The most widely used two methods of extraction are mechanical press extraction and chemical solvent [14, 15]. Specifically, there are three major extraction methods used worldwide. Firstly, the mechanical cold-press method is often defined as a solid-liquid phase separation system used for oil extraction from seed with oil content below 20%. The advantages of mechanical oil extraction include simple use, a rapid realization of the process which leads to the short duration of the process, use of small quantities of raw materials, application of different seeds oil, and low cost. Also, as a by-product protein, rich press cake is obtained. While the disadvantage is that the yield is not as high as in chemical solvent extraction. The residue contains approximately 4-8% of oil, whereas it is only 1-2% when the material is extracted by chemical solvent [16]. Secondly, the mechanical warm-press method is carried out by applying heat to the seeds at a high temperature reaching 100˚C before or during pressing. The advantage of the warm-press method includes an increase in oil yield by up to 4-6% more than in the cold-press method. On the other hand, many temperature-sensitive phenolic compounds are lost and there are oxidations reactions occur along with the heat treatment. Also, the warm-press method produces oil with rather dark colors. Finally, the chemical solvent extraction method which in it a large number of different solvents can be used such as N-hexane, Petroleum Ether, Benzene, Chloroform, Ethyl Acetate, Diethyl Ether, Isopropyl Alcohol, Carbon Tetrachloride, and Ethanol. The chemical solvent should be able to solubilize the oil for efficient extraction and don't interact with oil components and be cheap. The advantages of chemical solvent extraction are low cost, simple equipment use, no need for filtrate of the oil obtained, and high efficiency. Also, the solvent extraction method in low and medium-fat seeds provides a higher oil yield by 11.5% compared to the cold-press method. But the disadvantages include the difficulty of removing the solvent from the oil, besides the fact that it is ecologically harmful and highly flammable [17].

The objective of current research is to study the effect of different harvest-dates and extraction methods on chemical characteristics of *Moringa oleifera* oil.

2. Materials and Methods:

Two field trials were conducted at a private farm located in Belbeis desert, Sharkia governorate (Egypt) as a model of newly reclaimed land on three-year-old *Moringa oleifera* trees cultivated by direct seeding in sandy soil in the open field initially at distances of 50 cm apart and then cultivated to the final destination in the field at 2m * 3m during the two successive seasons of 2019 and 2020.

Fruit pods were collected at three different harvest times were chosen as follows:

1. Seeds are collected after 60 days of blooming.
2. Seeds are collected after 75 days of blooming.
3. Seeds are collected after 90 days of blooming

Methods of oil extraction

Samples (100 g) from dry seeds of *Moringa oleifera* were collected and taken from untreated control plants of Moringa for the sake of studying the effects of extraction methods. Seed samples were subjected to three extraction methods, namely cold press, warm press, and organic solvent extraction.

1. Cold-press extraction (at 30-40˚C): It was executed using integrated screw oil press model 6YL-80A (made in China).

2. Warm-press extraction (at 100˚C): It was carried out using the same above-mentioned integrated screw oil press model 6YL-80A.
3. Chemical solvent extraction: It was performed using petroleum ether (40-60˚C) with a Soxhlet apparatus for six hours as stated in [18]. A rotary evaporator was used to evaporate the petroleum ether solvent.

Methods related to seed-oil quality features

1- Specific gravity: It was evaluated according to the procedure detailed by [19].

2- Refractive index: It was evaluated in oil samples with the help of an ATAGO hand refractometer model N-1E Brix 0- 32%. Two drops of oil were placed on the prism with the help of a syringe and the prism was firmly closed by tightening the screw head. The apparatus was allowed to stand for 5 min, and after that reading was recorded from the display screen according to the guidelines pointed out by [19].

3- Acid number: It was determined according to the procedure stated by [19].

4- Saponification number. It was determined according to the procedure narrated by [19].

5- Ester number: It was calibrated from the formula:

Ester number = (Saponification number – Acid number)

6- Free-Fatty Acids: It was calibrated from the formula:

Free-Fatty Acids = Acid number × 0.503

7- Seed-oil composition: It was determined by Gas Chromatography/Mass Spectrometry (GC-MS) analysis.

Preparation of fatty acid methyl esters:

Lipids obtained after extraction of seed oil and fat samples were converted to corresponding fames by trans-esterification with Potassium hydroxide ISO 5509, 2000 to do it, put 5ml of hexane and 250µL of 2N KOH has added to 0.24g oil. The mixture was shaken for 2 minutes in a closed 20mL vial. After settling, the supernatant was injected.

The GC-MS analysis of the fatty acids methyl ester was carried out using a gas chromatography-mass spectrometry instrument located at the Medicinal and Aromatic Plants Research Dept., National Research Center with the subsequent specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a Thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.e., 0.25 µm film thickness). Analyses have been achieved by using helium as a carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 80 ºC for 1 min; rising at 4 ºC/min to 300 ºC and held for 5 min. The injector and detector were held at 240 ºC, 3 µL of the sample was the standard amount always injected. Most of the compounds have been recognized through the use of the analytical method: mass spectra (proper chemicals, Wiley spectral library collection, and NSIT library). Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 35-550.

Statistical Analysis of Result Data

The experiment was performed in a split-plot design, with three replicates. The effects of the two experimental factors were subjected to analysis of variance. LSD test was used to compare the mean values of examined treatments as reported by [20].

3. Results

The result data presented in Table (1) showed the effect of harvest date on seed oil yield and its physical and chemical characteristics. Seed-oil content (%) in the 3rd harvest date (after 90 days from blooming) was increased. The physical and chemical characteristics namely: specific gravity and refractive index were increased to their highest values. Meanwhile, four of them decreased to their lowest values, namely: free fatty acids, acid number, saponification number, and ester number.

Whilst, on the 1st harvest date (after 60 days from blooming) acid number and saponification number were increased to their highest values. In the meantime, oil yield, specific gravity, and refractive index were decreased to their lowest values.

Data obtained in Table (2) presented the effect of three different extraction methods on *Moringa oleifera* seed oil yield and seed-oil physical and chemical characteristics. Seed-oil content (%) tended to give the same values when extracted by either warm-press or petroleum ether solvent. Also, there were no recorded differences between the three methods of extraction methods (cold press, warm press, and petroleum ether solvent) in their effect on values of two physical/chemical characteristics, namely: specific gravity and refractive index.

In addition, warm press extraction resulted in rise to increase values of two other physical/chemical characteristics, namely: acid number and free-fatty acid number. On the other hand, warm-press extraction caused declined values of another two characteristics, namely: saponification number and ester number.

In contrast, both cold press and petroleum ether solvent extraction methods were equal concerning their positive increased effect on the two abovementioned characteristics, namely: saponification number and ester number.

Main Components of the *Moringa oleifera* seed-oil as analyzed and identified by GC-MS when affected by harvest-date stages of fruit pods and by three seed-oil extraction methods:

The main components of *Moringa oleifera* fixed-seed oil (as identified by GC-MS) when affected by harvest-date stages and the three extraction methods in the second season (2020) are presented in Table (3) and Figures (1, 2, 3, 4, 5 and 6). While admitting that the result data in Table (3) were not subjected to statistical analysis, still there were clear indicators in the general trends of the obtained results which will be highlighted and stressed in the paragraphs.

Exactly 16 compounds were identified and accounted for in the components of *Moringa oleifera* fixed-seed oil samples of the second season (2020) when analyzed by GC-MS.

The main compound identified in plenty was oleic acid (methyl ester) in almost all cases. Notably, oleic acid was increased slightly in seeds harvested at the green-pod stage. It decreased in seeds harvested at the semi-dry pod stage and increased dramatically again in seeds harvested in the dry stage. Also, oleic acid increased by petroleum ether solvent extraction method. It gradually decreased by the warm-press extraction method with a further decrease by the cold-press extraction method.

Noteworthy, there were four components in the fixed-seed oil which appeared in the green-pod stage and disappeared in both the semi-dry and dry-pod stages, viz. pathalic acid, cyclopropaneoctanoic acid, stearic acid (ethyle ester) and octadecanamide.

Interestingly, there were five components in the fixed-seed oil which appeared due to the petroleum ether solvent extraction method and disappeared in both warm and cold-press extraction methods, viz. pathlic acid, palmetoleic acid, stearic acid, octadecanamide, and arachidic acid.

Noticeably also, there were three components in seed oil which appeared in the dry-pod stage while disappearing on both the green and semi-dry pod stages, viz. stearic acid (methyl ester), hexadecanamide and 11-eicosenoic acid.

At the same time, palmitic acid – like oleic acid - increased in the green-pod stage while being decreased in the semi-dry pod stage and increased again in the dry-pod stage. It also seemed to increase due to the petroleum ether extraction method with a notable decline as a result of both warm-press and cold-press extraction methods.

Lastly, one component in seed oil, viz. linolelaidic acid appeared only in two cases, i.e. as a result of the cold-press extraction method and in the semi-dry pod stage of harvest. Also, another component, viz. hexadecanamide did appear due to the warm-press extraction method while disappearing as a result of both cold-press and petroleum ether solvent extraction methods. Although mentioned earlier, cyclopropaneoctanoic acid (as a component in the green-pod stage), this same component failed to materialize and show up in seed-oil component results when analyzed by GC-MS in any samples that were related to the three extraction methods (cold press, warm press, and petroleum ether solvent).

Table 1. Effect of three harvest-date stages on *Moringa oleifera* seed-oil yield and on seed-oil physical and chemical characteristics in 2019 and 2020.

First season (2019)

Oil yield/ oil characteristics	Oil yield (%)	Specific Gravity	Refractive Index	Acid Number	Saponification Number	Ester Number	Free-Fatty Acids
Harvest Dates							
1st harvest	17.3	0.57	1.27	2.14	223.2	221.1	1.06
2nd harvest	22.3	0.64	1.45	1.94	230.9	229.0	0.97
3rd harvest	28.4	0.66	1.65	0.50	146.7	146.2	0.26
LSD at 5%	*1.1*	*0.016*	*0.13*	*0.17*	*3.8*	*3.8*	*0.06*

Second season (2020)

Oil yield/ oil characteristics	Oil yield (%)	Specific Gravity	Refractive Index	Acid Number	Saponification Number	Ester Number	Free-Fatty Acids
Harvest Dates							
1st harvest	19.8	0.62	1.25	1.76	223.6	221.2	0.93
2nd harvest	23.0	0.60	1.20	2.87	226.7	223.8	1.54
3rd harvest	30.7	0.68	1.47	0.44	142.8	144.03	0.25
LSD at 5%	*1.3*	*0.015*	*0.11*	*0.25*	*4.6*	*4.7*	*0.09*

Table 2. Effect of three extraction methods (cold press, warm press, and solvent extraction with petroleum ether) on seed-oil yield and seed-oil characteristics of *Moringa oleifera* plants in the 2019 and 2020 seasons.

First season (2019)

Oil yield/ oil characteristics	Oil yield (%)	Specific Gravity	Refractive Index	Acid Number	Saponification Number	Ester Number	Free-Fatty Acids
Extraction methods							
Cold press	20.05	0.73	1.82	0.44	148.36	147.93	0.22
Warm press	34.40	0.69	1.9	1.09	107.86	106.77	0.54
Petroleum ether	40.09	0.62	1.87	0.56	146.79	146.23	0.28
LSD at 5%	*Ns*	*Ns*	*Ns*	*0.49*	*29.35*	*19.48*	*0.25*

Second season (2020)

Oil yield/ oil characteristics	Oil yield (%)	Specific Gravity	Refractive Index	Acid Number	Saponification Number	Ester Number	Free-Fatty Acids
Extraction methods							
Cold press	24.50	0.66	1.92	0.28	132.17	131.89	0.14
Warm press	36.65	0.73	1.7	0.96	112.84	111.88	0.48
Petroleum ether	43.00	0.62	1.92	0.56	137.81	137.25	0.28
LSD at 5%	*11.3*	*Ns*	*Ns*	*0.19*	*19.09*	*25.38*	*0.09*

Ns: not significant

Table 3. The main components of fixed-seed oil of *Moringa oleifera* plants harvested at three stages of harvest dates and extracted by three extraction methods (cold press, warm press, and petroleum ether solvent extraction) in the second (2020) season.

Compound	Rt (min)	Relative percent					
		Zn2B2 green pods	Zn2B2 semi dry pods	Zn2 B2 dry pods	Cold press extraction	Warm press extraction	Solvent extraction
Isobutyl phthalate	26.63	—	1.74	0.66	3.82	6.44	2.10
Phthalic acid, bis(7-methyloctyl) este	27.11	0.50	—	—	—	—	0.54
Palmitoleic acid, methyl ester	27.30	0.77	—	1.70	—	—	2.00
Palmitic acid, methyl ester	27.86	23.52	4.84	15.47	9.24	10.57	18.65
Dibutyl phthalate	29.06	62.35	76.21	—	5.44	5.27	10.65
Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	30.39	0.56	—	—	—	—	—
Linolelaidic acid, methyl ester	31.86	—	0.86	—	5.82	—	—
Oleic acid, methyl ester	32.17	8.25	5.37	71.32	33.95	39.69	48.79
9,12-Octadecadienoic acid, methyl ester	32.38	2.24	0.85	—	2.86	3.26	—
Stearic acid, methyl ester	32.65	—	—	5.47	3.42	4.32	7.93
Stearic acid, ethyl ester	34.09	0.52	—	—	—	—	0.54
Hexadecanamide	34.79	—	—	0.59	—	2.79	—
Octadecanamide	35.04	0.91	—	—	—	—	1.49
11-Eicosenoic acid, methyl ester	36.47	—	—	1.47	3.91	2.48	1.39
Arachidic acid methyl ester	37.04	0.68	—	1.00	—	—	1.46
9-Octadecenamide, (Z)	38.56	0.61	4.13	—	10.39	11.57	0.98

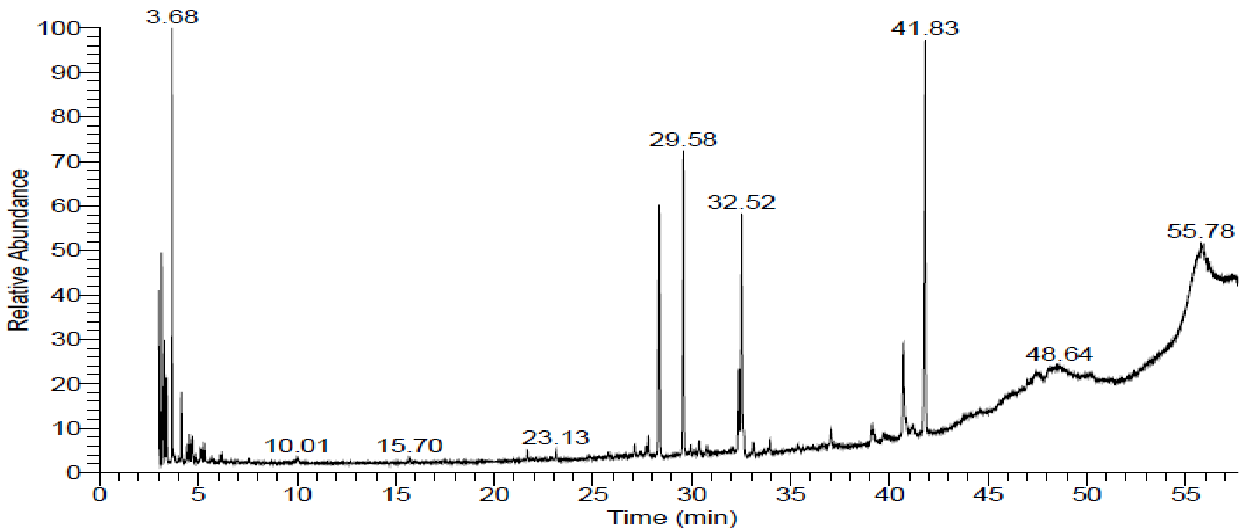

Fig 1. Chart of Gl chromatogram of *Moringa oleifera* fixed-seed oil from the green-pod stage of plants treated with Zinc at 0.4% and Boron at 0.4% during the 2020 season.

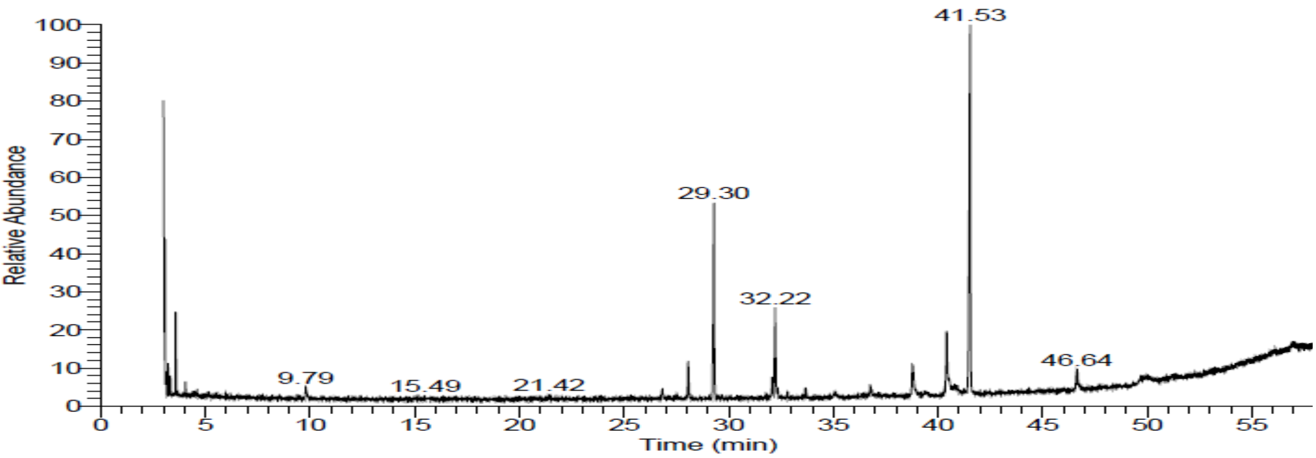

Fig 2. Chart of Gl chromatogram of *Moringa oleifera* fixed-seed oil from semi-dry pod stage of plants treated with Zinc at 0.4% and Boron at 0.4% during the 2020 season.

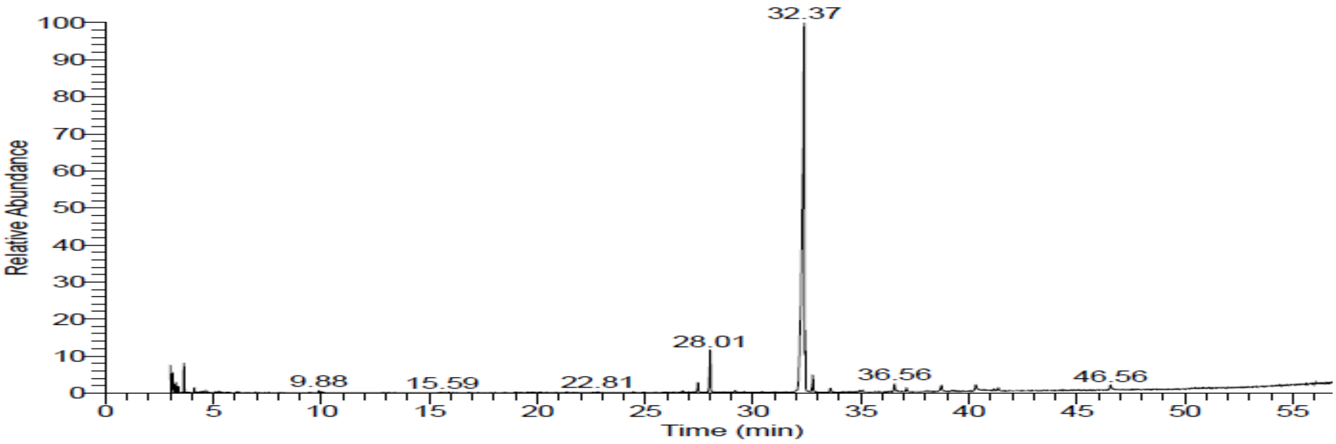

Fig 3. Chart of Gl chromatogram of *Moringa oleifera* fixed-seed oil from the dry-pod stage of plants treated with Zinc at 0.4% and Boron at 0.4% during the 2020 season.

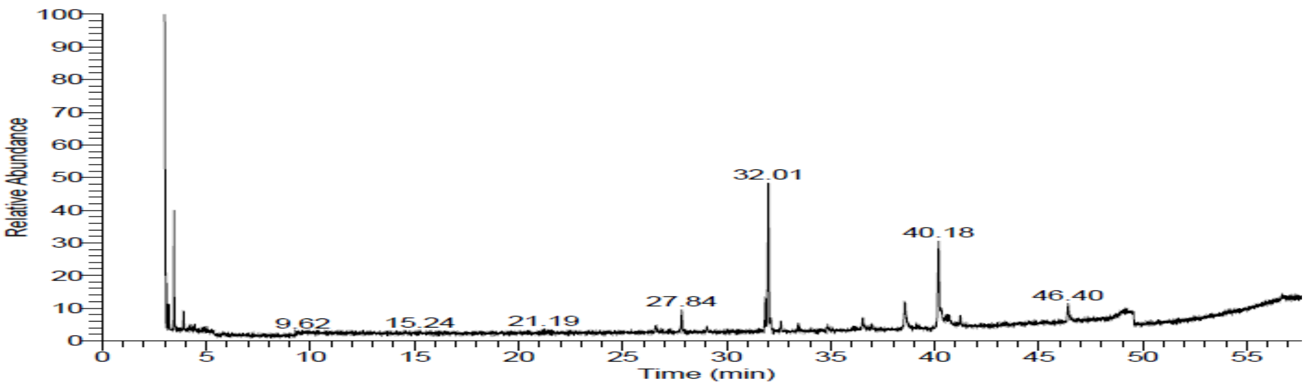

Fig. 4. Chart of Gl chromatogram of *Moringa oleifera* fixed-seed oil from the dry-pod stage of untreated control plant extracted with cold-press method during 2020 season.

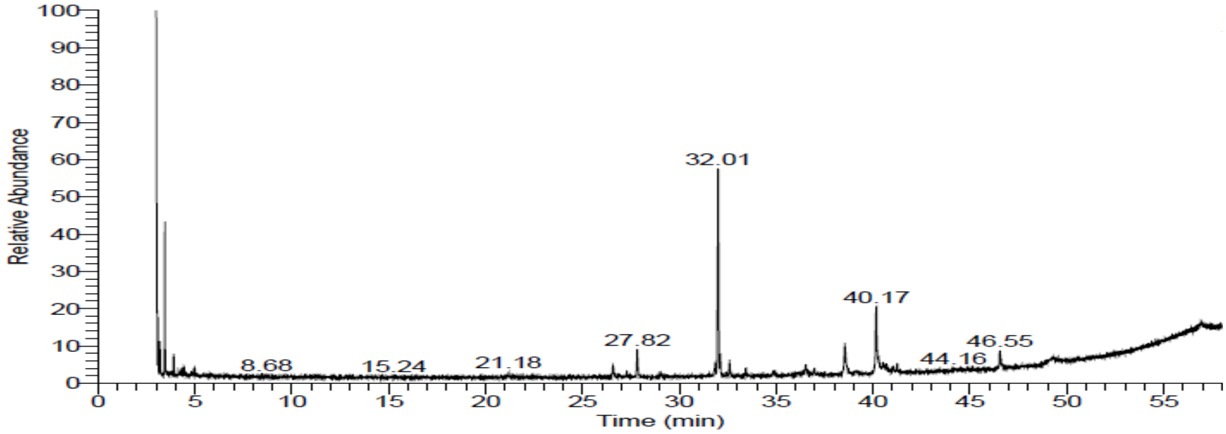

Fig 5. Chart of Gl chromatogram of *Moringa oleifera* fixed-seed oil from the dry-pod stage of untreated control plant extracted with warm-press method during 2020 season.

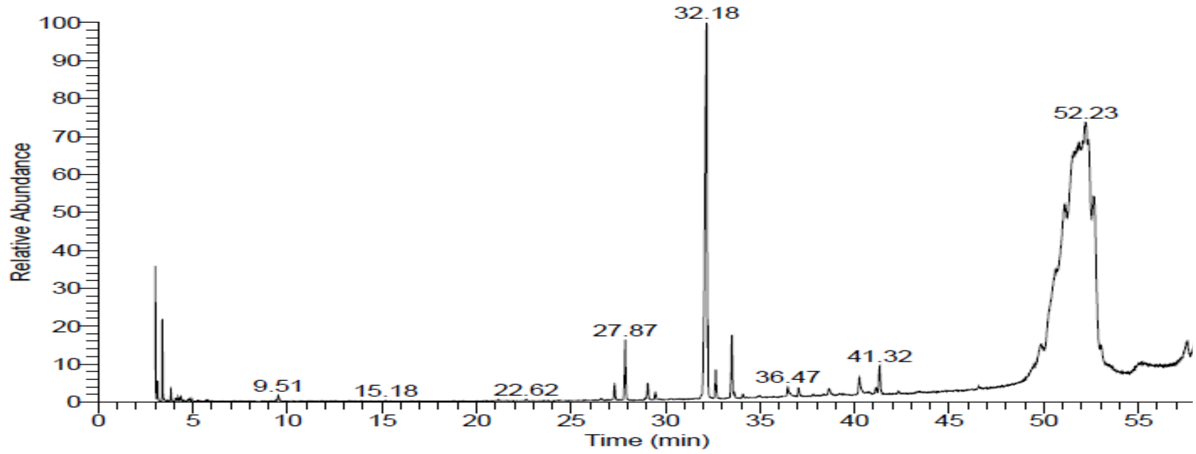

Fig.6. Chart of Gl chromatogram of *Moringa oleifera* fixed-seed oil from the dry-pod stage of untreated control plant extracted with organic-solvent method during 2020 season.

4. Discussion

Concerning harvest dates

Results obtained here confirmed that harvest dates had a positive effect on fruit-pod characteristics of *Moringa oleifera*. It enhanced pod weight, pod length, seed weight, and seed yield/plant.

These improvements might have occurred because of the higher temperature with more sunshine hours during the major part of the vegetative growth period that enhances the photosynthesis process and leads to securing a high amount of carbohydrate assimilates and ultimately the protein content that improves plant productivity, seeds yield and quality [21]. In addition, adequate moisture availability through plant growth (as was provided here by the recommended optimum doses of irrigation water) and higher relative humidity during the post-anthesis period helps in the development of a larger sink and efficient utilization of resources during the reproductive phase [22].

Moreover, the accumulation of the oils and their chemical characteristics: (free fatty acid, acid number, ester number, and saponification number) increased gradually during the maturation of the fruit as was seen and proved in this study where more fatty acids get liberated from their ester linkage with the parent triglyceride molecule. This increase may be due to the daily temperature (22-23°C) during grain filling which was suitable for increasing seed accumulations of oil and protein content.

Concerning methods of seed-oil extraction

The result data of this research study on *Moringa oleifera* oil have established the fact that the different extraction methods (cold press, warm press, and petroleum ether solvent) had affected not only oil yield, but also the physical/chemical characteristics of the extracted oil and not to mention the chemical composition (quantity and ratio to each other) in the resultant oil.

As for oil yield, extraction methods could have exerted influence through two factors, namely: heating (temperature and time) and solvent type used. A sufficiently high temperature at or near the boiling point and its duration can increase the oil yield and vice versa for lower temperature, extraction conditions, and efficient expression [23, 24]**.** An organic solvent like petroleum ether might have exerted its effect on oil yield by the extraction time and/or the physical/chemical properties of the solvent (polarity).

For the physical/chemical properties of the extracted oil, extraction methods might have caused their influence through heating temperature, screw-head pressure, and physical/chemical properties of the solvent used (polarity and temperature). A high temperature might cause hydrolysis of one or more ester bonds in triglycerides which are greater in oils produced from pressing than that produced from solvent. Screw-head pressure in both cold and warm press methods has a great effect on oil yield where increasing head pressure leads to an increase in barrel temperature by increasing the friction between the compressed materials. In general, the more pressure applied, the higher is then the oil yield but if the temperature raised above the limits the produced oil will decrease in quality parameters [25].

The polarity of the petroleum ether solvent could have exerted a positive effect on the length of the fatty acid chain in the oil and also on fatty acid chains esterified to glycerol by limiting the lipids' solubility thus leading to lower oil yield [26, 27].

5. Conclusion

Harvest *Moringa oleifera* pods after 90 days from blooming (on the 3rd harvest date) in combination with oil extraction by cold press gave lower oil yield with rather high-quality features (very low acid number and free-fatty acids) excellent for human consumption. While the oil extracted by both warm press and petroleum ether solvent scored higher, more or less, equal oil yield with lesser quality features, and still acceptable in the case of warm-press for human consumption and suitable in the case of petroleum ether solvent for use in many industrial purposes.

6. Conflicts of interest

There are no conflicts to declare.

7. **Funding**

None

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