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Proximate Composition and Quality Characterization of Oil Extracted From *Moringa Oleifera* Kernel Using Different Extraction Method

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Abstract

This study investigates proximate composition and characterization of *Moringa oleifera* seed-oil using four methods of extraction (Cold pressing (CP), Enzyme assisted cold pressing (EACP), Enzyme assisted aqueous extraction (EAAE) and solvent extraction (SE)) and compared with olive oil. The results of proximate analysis of the kernel reveals (7.20 moisture content, 43.12 protein, 33.16 fat, 3.51 Ash, and 6.81 Carbohydrate by difference) %, while the oil yield obtained for CP, EACP, EAAE and SE were (7.75, 12.08, 23.25 and 33.16)%. The fatty acids composition and bioactive compounds were determined through GC-MS analysis. Melting and crystallization temperature ranges: -15.86 to 3.21 and 8.30 to -13.63 °C, respectively. There is a significant difference in term of oil yield, with cold pressing method having the lowest oil yield of 7.8 %. However, the quality of CP extracted *Moringa oleifera* oil was compared with virgin olive oil; it revealed its potentials as a good source of oleic acid making it to be a good substitute for virgin olive oil.

Key words: *Moringa Oleifera*; Extraction Method; Physicochemical Analysis; GCMS

Introduction

Moringa oleifera lamarch belong to the genus Moringaceae with fourteen species. It is known as multipurpose tree and widely used in food and feed industry, as well as for traditional medicine [1]. Almost all the *Moringa oleifera* plant parts are medicinally valuable with multiple applications such as for treating myriads of ailments and diseases including body pains, fever, asthma, cough, blood pressure, arthritis, diabetes, epilepsy, wound, and skin diseases [2-4]. It is cultivated in tropical and sub-tropical climates and has been called with numerous names by different people of the world, including Zogale, "Ewe ile" or Okwe oyigbo among the Nigerian, while in English language, it is called as Benzolive tree [5-7]. The *Moringa oleifera* kernel can be eaten raw when mature or boiled when green [8].

The plant is mainly used for medicinal and nutritional applications for both human and animals due to their rich phytonutrients content. These kernels have various bioactive compounds, exhibited through its antimicrobial, anti-inflammatory, and antitumor properties [9]. It possess zeatin, quercetin, sitosterol, kaempferol and caffeoylquinic acid [10]. Besides, *Moringa oleifera* is a rich source of vitamins (vitamin A, B and C), minerals (calcium, potassium, phosphorus, magnesium) and amino acids [11]. The *Moringa oleifera* oil contain high oleic acid (> 70%), a monounsaturated fatty-acid which has direct link in reducing the risk of clogged arteries and heart diseases, help to boosts metabolism, as well as preventing breast and colon cancers [12-14]. Therefore, *Moringa oleifera* oil can be used to replace polyunsaturated vegetable oil during frying process [15,16].

Several methods have been developed for the extraction of oil from *Moringa oleifera* kernel including solvent extraction (SE), enzyme assisted aqueous extraction (EAAE), cold pressing (CP), ultrasound extraction (UE) microwave extraction (MWE) and supercritical fluid extraction (SFE), [17-24]. The oil yield of *Moringa oleifera* kernel was improved by microwave heat [25], the chemical quality of oil extracted by SC-CO_2 was higher than Soxhlet oil. The quality analysis of microwave–assisted extraction and ultrasound-assisted extraction on *Moringa oleifera* seed was studied [26], and compared with quality of Soxhlet extracted oil. There were no noticeable changes in the fatty acid composition, acyglycerol profile, and thermal properties of oils compared to the solvent extraction oil. Similarly, the storage time was unaffected as well indicting effective enhancement. Other than cold pressing, all the aforementioned methods have demerit of either using flammable and toxic chemicals, or high cost for running equipment [9,27] Meanwhile, CP is a toxic- free method, by pressing or grinding seeds with heavy granite millstones or modern stainless steel press, to breakdown the macrostructure which in turn open up the capillaries through which the oil flows out, with heat friction below 49 °C. Due to the mild condition during pressing, the oil are able to retain its natural composition, with negligible phosphatide content and reduced peroxide value compared to the use of SE method [28].

Pre-treatment of seeds are often applied to improve the oil yield for CP. The most common pre-treatment methods used for CP of seed oil are through physical modification (de-hulling, size reduction, drying), heating (microwave, roasting) and addition of enzymes. Previous studies revealed that pre-treatments of seeds before CP showed improvement in the oil yield [29-33] However, to the best of our knowledge, no study has reported the use of enzymes as pretreatment, to improve oil yield for cold pressed *Moringa oleifera* kernel. Therefore, this study investigates the effect of using enzyme as pre-treatment to improve the oil yield of cold pressed oil. The results will be compared with other extraction methods namely SE, EAAE and CP. The proximate analysis of the kernel, as well as physicochemical properties, thermal stability and fatty acid composition of the extracted oil will also be documented.

Materials and Methods

Sample Preparation

Matured *Moringa oleifera* seeds were obtained from Akure, South-West of Nigeria. The seeds were de-hulled, sundried and stored at 4 °C. Foreign unwanted materials, rotten and immature kernels were removed. The average sizes of the seed and kernel measured (8.09 and 6.30) ± 0.02 mm using a Vernier caliper. The solvents used are of analytical grade except for methanol with chromatographic grade. Neutrase 0.8L which contain *Bacillus amyloiquefacien* protease with 0.8 unit per gram (U/g) were purchased from Sigma – Aldrich (Dorset, UK). The virgin olive oil was purchased from a local store in Penang, Malaysia.

Proximate Analysis of *Moringa Oleifera* Kernel

Proximate analysis including moisture, crude fat, crude protein, crude fiber and ash content were determined by following AOAC Method 1990. The carbohydrate content was calculated by difference. All determinations were carried out in triplicate.

Physicochemical Analysis of *Moringa Oleifera* Kernel Oil

Free fatty acid (FFA) value, iodine value (IV), saponification value (SV), peroxide value (PV), and color measurement were determined using AOCS(1989), Ca 5a-40, Cd 1.25, Cd 3-25, Cc 7-25, Cd 8-53 and Cc 13c-50 respectively.

Extraction of Oil

Cold Pressing: A cold pressing machine, model – SH-48-100 (Seng Hup Engineering, Lahat, Malaysia) with 100 tons capacity, operated at 1500psi was used to extract oil from *Moringa oleifera* kernel. A stainless steel mold that have (30 x 5 x 147)mm as thickness of the mold head, thickness and diameter of the cylinder. Approximately 750g of *Moringa oleifera* kernel were wrapped inside muslin cloth and placed inside the machine before being subjected to pressing load of 100 kg at 45 °C till oil recovery. The oil obtained was filtered, and centrifuged at 3500 rpm for 20 mins. The collected sample was transferred to an amber bottle.

Solvent Extraction: Petroleum ether was used to extract oil from *Moringa oleifera* kernel using Soxhlet apparatus. Approximately 3g of ground kernel were place into a cellulose thimble and 90ml of petroleum ether with boiling point 55 °C was placed in a round-bottom flask for 6hrs to extract the oil. The collected oil was dried in oven at 60°C for 1hr, transferred into an amber bottle.

Enzyme Assisted Cold Pressing: The procedure of [34] was adopted. A 10g of flaked kernel was measured and poured into a 10 ml of distilled water containing 2% Neutrase enzyme. The mixture was placed inside the oven for 6 hours at 45 oC to activate the enzyme. The treated sample were pressed using pressing machine at 45 °C with 1500 psi and 100 kg load till oil recovery. The collected oil was filtered, centrifuge at 3500 rpm for 20 mins, and transferred into an amber bottle.

Enzyme Assisted Aqueous Extraction: The procedure of [15] and [35] was adopted. A ground kernel was mixed with distilled water (ratio of 1:6 w/v), then boiled for 5mins and allowed to cool to room temperature. The pH was adjusted using 0.5N NaOH to pH of 6.8 (optimal pH for Neutrase enzyme). Later, 2% of the enzyme was added and mix, incubated at 45 °C for 6hrs inside a shaking water bath at 120 rpm. The mixture was transferred into a separating funnel to yield four distinct phases of oily, creamy, aqueous and the meal phase. The water phase was drained off; while the oily phase was remove using a micropipette. The oil was heated at 60 °C in oven to remove the residual water and later transferred into an amber bottle. All samples in amber bottle were labeled, flushed with nitrogen gas and stored at 4 °C for further analysis.

Extraction Yield

The results obtained from each extraction method were calculated based on the initial weight of oil sample obtained from Soxhlet extraction method. Percentage of oil recovery and yield for all methods are calculated using Equations 1 and 2 [36].

$$\text{Oil recovery \%} = a / b^{*}100 \quad \text{(Equation 1)}$$

$$\text{Oil yield \%} = W_2 / W_1^{*}100 \quad \text{(Equation 2)}$$

Where, a = Weight of Oil Extracted from each of the Extracted Method, b = Weight of Oil Extracted using Soxhlet Extraction, W_1 = Weight of Seed and W_2 = Weight of Oil Extracted from Seed.

Thermal Behavior

The melting and crystallization temperatures of the oil were determined using Differential Scanning Calorimetry (DSC) (Perkin Elmer Diamond, Norwalk, USA). The DSC was calibrated using indium, operated using 99.99% nitrogen as purge gas with a flow rate of 100ml/min and a pressure of 20psi. Oil sample (9-10mg) were placed in aluminum pan and sealed hermetically while reference sample was prepared by placing an empty aluminum pan inside the DSC equipment. The samples was cooled from -60 °C and held for 2mins, then heated to 70 °C, at rate of 5 °C/min and held for 2mins at 70 °C, before subjecting it to cooling from 70 °C to -60 °C at the rate of 5 °C/min. The melting and crystallization thermograms were recorded to determine onset, peak and offset temperatures.

Gas Chromatography Mass Spectrophotometer (GCMS) Analysis

Fatty acid compositions of the extracted oil were analyzed using GCMS model 6890-5972 (Hewlett Packard, Atlanta, USA). Prior to injection, the oil were converted into their fatty acid methyl esters (FAME) according to AOCS 2009. The gas chromatography was equipped with DB-WAX capillary column (30m × 0.25 mm × 0.25µm film thickness). The inlet temperature was maintained at 250 °C. The initial temperature was set at 50 °C with a hold of 2min, followed by 4 °C – 250 °C with a hold of 6 min. 1 µl of sample was injected with split ratio 100:1, using auto sampler. The carrier gas was helium, with a constant flow of 0.5 ml/min. The mass spectrophotometer transfer line temperature was set at 250 °C with source temperature of 230 °C. The samples were analyzed at electron energy 70 eV and average linear velocity 19cm/sec at 50 °C. The mass analyzer range was set to 50-650 amu, with scan rate 5 scans/sec. The retention indices, mass spectra and the obtained data were compared with the database of National Institute Standard and Technology (NIST) with a MS library version 2011.

Result and Discussion

Proximate Analysis

Proximate analysis of the *Moringa oleifera* kernel in the present study is tabulated in Table1. The data was compared with the available literatures. The moisture content obtained in the present study is 7.2%; the lowest among those reported in literature. The difference in their moisture content values can be contributed to the maturity index of the seed, drying method and climatic condition [37]. The low moisture content of the seed (<10%) shows that it can resist microbial growth, hence has a longer shelf life. The percentage of crude protein obtained is 43.1% being the highest, while crude fat and fiber values are in accordance with the values reported in literature. The ash content value obtained is low (3.5%), indicating a low level of inorganic residues. The calculated total carbohydrate is 6.8%, considerably lower than the reported values [5,15,38].

Proximate Parameter (%)	Present Study	[39]	[38]	[15]	[40]	[41]	[42]	[43]
Moisture content	7.20±0.03	4.1	-	7.9	5.3	4.7	7.3	10.0
Crude Protein	43.12±0.9	38.4	36.7	38.3	37.6	28.0	38.5	36.0
Crude Fat	33.16±0.05	34.7	41.7	31.4	39.3	45.9	32.8	39.0
Ash Content	3.51±0.07	3.2	3.8	6.5	4.2	4.1	9.0	3.9
Crude Fiber	6.20±0.02	3.5	4.8	4.5	3.2	6.7	7.5	3.4
CHO (By diff)	6.81±0.08	17.1	17.8	16.5	13.6	11.0	4.9	8.7

Values are Means ± SD of Triplicate Determination; CHO -Carbohydrate

Table 1: Proximate Composition of *Moringa Oleifera* Seed

Physicochemical properties

Physicochemical properties of the extracted oil from *Moringa oleifera* kernel oil were summarized in Table 2.

Parameters	(CP)	(EACP)	(EAAE)	(SE)	OL
Color L	94.5±0.11^{a}	90.8±0.09^{c}	91.4±0.07^{c}	93.7±0.04^{b}	94.6±0.14^{a}
A	4.6±0.06^{a}	4.1±0.03^{a}	4.2±0.03^{a}	4.2±0.08^{a}	4.4±0.08^{a}
B	53.8±0.05^{b}	58.3±0.03^{a}	52.1±0.01^{c}	53.7±0.04^{b}	29.8±0.07^{d}
FFA (%)	2.8±0.02^{c}	2.8±0.04^{c}	2.9±0.02^{b}	3.1 ±0.14^{a}	0.9±0.01^{d}
PV (Meq/kg)	3.5±0.12^{c}	3.6 ±0.15^{c}	3.6±0.11^{c}	4.2±0.02^{b}	7.5±0.12^{a}
I V (gI_2/100g)	61.1±0.06^{d}	66.2±0.02^{c}	68.4±0.01^{a}	67.9±0.01^{b}	71.1±0.01^{a}
SV (mgKOH/g)	180.4±0.01^{b}	180.9±0.04^{b}	181.6±0.01^{b}	185.7±0.03^{a}	182.6±0.04^{b}
Viscosity (mPa.s)	66.9±0.03^{a}	61.4±0.11^{b}	62.3±.010^{b}	42.6±0.09^{c}	66.6±0.07^{a}
Acid value (mg/KOH)	5.6±0.04^{c}	5.3±0.07^{c}	5.9±0.08^{b}	6.2±0.05^{a}	5.9±0.05^{b}

Parameters	(CP)	(EACP)	(EAAE)	(SE)	OL
Oil yield %	7.7±0.07[d]	12.1±0.03[c]	23.3±0.03[b]	33.1±0.05[a]	-
Oil recovery %	23.4	36.4	70.1	99	-

Mean Values in the Same Row Followed by the Superscript Letters Are Not Significantly Different (p> 0.05). Values are Means ± SD of Triplicate Determination. CP: Cold Pressing, EACP: Enzyme Assisted Cold Pressing, EAAE: Enzyme Assisted Aqueous Extraction, SE: Soxhlet Extraction, OL: Olive Oil, L: Lightness; A: Red-Green, B: Yellow-Blue, FFA: Free Fatty Acid, IV: Iodine Value, SV: Saponification Value

Table 2: Physicochemical Properties of *Moringa Oleifera* Kernel Oil Using Different Types of Extraction Methods

Oil Yield and Recovery: Among all the extraction methods used, CP has the least oil yield and recovery. The finding is similar to those reported for canola, mango and Chilean hazelnut in literatures [24,33,37]. Meanwhile, the oil yield and oil recovery using EACP obtained are higher than CP counterpart and within the range reported in other studies [34,44] for enzyme assisted aqueous extraction (EAAE) oil, the oil yield and recovery obtained are 23.3% and 70.1%. These values are in close agreement with other reported *Moringa Oleifera* oil values of 22.6% and 72.0% [15], 25% and 77% [35] and 28.9% and 70% [45] respectively.

The use of enzyme at optimum temperature proven to enhance the oil extraction process by reacting with the broken cell membrane of the seed, as seen in both EACP and EAAE method. The oil yield and oil recovery values obtained using solvent extraction method are the highest among the tested methods, however still within the reported ranges in literatures [15,35,36,46,47]. Higher oil recovery for SE could be attributed to the use of inorganic solvent and high temperature during the extraction process.

Variations observed between the present study and data from published literature could be due to various factors such as different species used, climatic condition, and location of cultivation, ripening stage, harvesting period, pre-treatment methods applied before extraction, particle size of milled seed and method of extraction [36,48,49]. However, the variation could be control if the seed oil used reached the same maturity index, undergone the same drying process and size reduction of kernel into smaller particle sizes.

Color: Generally, *Moringa oleifera* oil color was analyzed with a spectrophotometer, to indicate lightness, redness and yellowness. There is a significant difference between CP and SE, however, no significant difference was observed, which contradicted to the result of EAAE and EACP. In terms of the variation in the oil color intensity, the use of enzyme and solvent contributes to higher release of color pigments from the seed during extraction [27,49]. The lightness ranges from 94.69 to 90.89, whereas the values of redness show no significant difference, while yellowness values ranges from 58.32 to 29.58 with olive oil having the lowest value.

FFA: The FFA values obtained are 2.8, 2.8, 2.9 and 3.1% for CP, EACP, EAAE and SE methods respectively. All values are within acceptable limit for edible vegetable oil of maximum 7% [8].

PV: The PV of the extracted oil with different extraction methods ranges from 3.50 to 4.20meq O_2/kg, which is within the permitted level of > 10 Meq O_2/kg for edible vegetable oil. There is no significant difference between oil sample extracted with enzyme assisted methods and cold pressing. On the contrary, the value of 7.50 Meq O_2/kg was obtained for olive oil, within the permissible level of 20 Meq O_2/kg [8,50,51].

IV: Higher iodine values show higher degree of unsaturation level of the oil [9,52]. The results range from 61-68g of I_2/100 g oil.

SV: The saponification value obtained is in the range of 180-185 mgKOH/g for the four extraction methods used. There is no significant difference in the oil extracted using CP, EACP and EAAE except for SE.

Viscosity: The viscosities obtained for extracted oil are 66.9, 61.4, 62.3 and 42.6 for CP, EACP and EAAE and SE. There is variation between the obtained results is due to different extraction method used. SE method shows the lowest viscosity because of the temperature and solvent used, since an increase in temperature denoted exponential decreases in viscosity [40,53-56] reported a range between 42 - 64 for crude, neutralize, deodorized and bleached oil of *MO* kernel extracted using solvent extraction from Mexico origin, while [57], reported a range of 43.8 and 43.6 for both oil sample extracted using CP and SE respectively, from India origin.

Thermal Analysis

Thermal behavior of the extracted *Moringa oleifera* oil using DSC are presented Table 3 and Figure 1a and b, showing the melting and crystallization behavior of the oil using different extraction method.

	Melting temperature (°C)			Crystallization temperature (°C)			
	Onset	Peak	End	Onset	Shallow peak	Deep peak	End
CP	-15.9	-3.8	3.2	5.9	4.6	-38.7	2.7
EAAE	-13.8	-4.9	0.6	7.3	3.9	-43.9	3.2
EACP	-10.3	-3.2	2.0	5.9	5.9	-40.8	1.3
SE	-15.2	-9.3	1.9	8.3	3.6	-41.5	-13.6
OL	-14.5	-4.2	1.5	-10.9	-12.8	-41.1	-23.2

CP: Cold Pressing; EAAE: Enzyme Assisted Aqueous Extraction; EACP: Enzyme Assisted Cold Pressing; SE: Soxhlet Extraction and OL: Olive Oil

Table 3: Thermal Behavior of *Moringa Oleifera* Kernel Oil Using Different Extraction Methods and Olive Oil

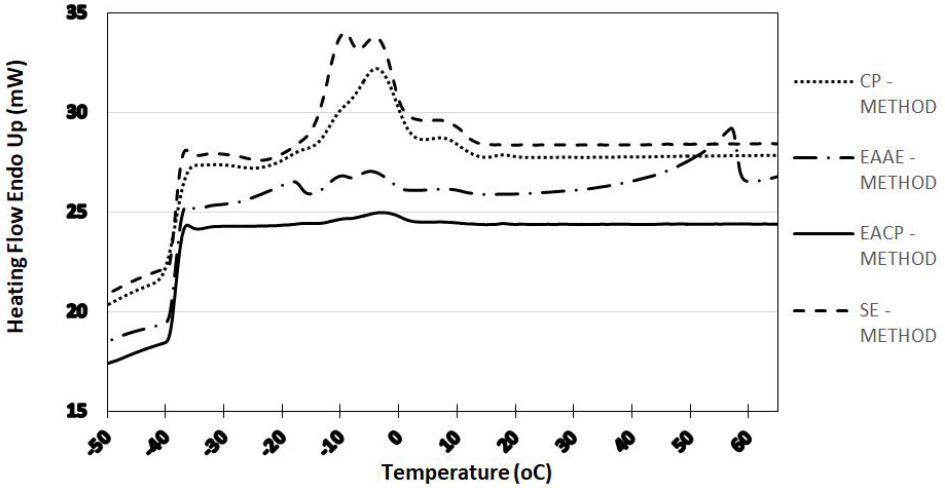

Figure 1a: *Moringa oleifera* Kernel Oil DSC Heating Curve

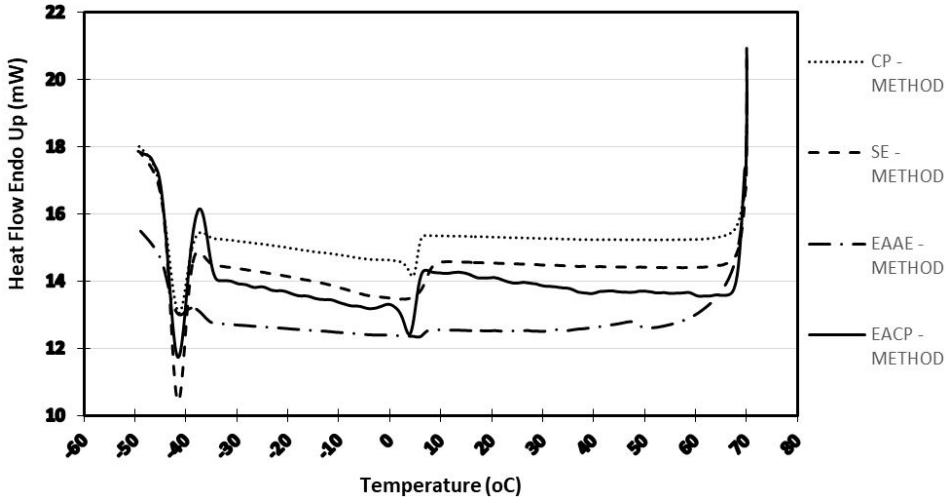

Figure 1b: *Moringa oleifera* Kernel Oil DSC Cooling Curve

The peak temperature of EACP at crystallization behavior is at onset temperature of -10.3 °C and melts at higher temperature of -43.9 °C, compared to other extracted oil. The onset of crystallization and melting for others extracted methods are CP of -15.9 °C and -38.7 °C, EAAE of -13.8 °C and -40.8 °C, SE of -15.2 °C and -41.5 °C and olive oil of -14.5 °C and -41.1 °C. The work of Abdulkarim, *et al.*, [15] reported melting peak of EAAE and SE extracted oil at -38.1 °C and -37.5 °C respectively.

Fatty acid composition: Table 4 describes the identified components, retention time, molecular weight, retention index, molecular formula and the percentage composition of fatty acid methyl esters components.

S/N	Component	Retention Index	Mole cular weight	Reten tion Time	% Composition of occurrence					Molecular formula	Similarity Index
					Extraction methods						
					CP	EACP	EAAE	SE	OL		
1	Propanoic acid (2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	1347	216	22.660	2.66	-	-	-	-	$C_{12}H_{24}O_3$	92
2	Propanoic acid (2-methyl, 3hydroxy-2,4,4-trimethylpentyl ester)	1331	216	23.810	6.03	-	-	-	-	$C_{12}H_{24}O_3$	94
3	Propanoic acid (2-methyl, 1-(1,1-dimethylethyl) -2-methyl-1,3propanediyl ester	1605	286	25.352	12.17	-	-	-	-	$C_{16}H_{30}O_4$	95
4	Hexadecanoic acid	1878	270	31.067	1.64	7.01	6.03	7.17	8.14	$C_{17}H_{34}O_2$	87
5	Octadecanoic acid	2077	298	34.727	0.75	3.97	4.27	6.16	-	$C_{19}H_{38}O_2$	85
6	9-octadecenoic acid	2085	296	35.270	12.70	74.21	-	-	-	$C_{19}H_{38}O_2$	95

S/N	Component	Retention Index	Molecular weight	Retention Time	% Composition of occurrence					Molecular formula	Similarity Index
					Extraction methods						
					CP	EACP	EAAE	SE	OL		
7	2n-Pentylcyclopropane	1544-	226	35.805	0.23	-	-	-	-	$C_{14}H_{26}O_2$	59
8	Methyl10, 11-Octadecadienoate	-	294	36.285	0.83	-	-	-	-	$C_{19}H_{34}O_2$	73
9	2-Furancarboxaldehyde	-	126	36.512	1.03	-	-	-	-	$C_6H_6O_3$	68
10	2--Dodecenol	1465	184	37.178	1.20	-	-	-	-	$C_{12}H_{24}O$	62
11	Ethylmalononitrile	1541	176	37.565	1.61	-	-	-	-	$C_{11}H_{16}N_2$	61
12	Di-(9-octadecenoyl)	-	621	37.885	2.02	-	-	-	-	$C_{39}H_{72}O_5$	70
13	Hexadecanoic acid	3031	418	38.140	2.48	-	-	-	-	$C_{26}H_{42}O_4$	59
14	2-Pentanal	905	126	38.445	2.24	-	-	-	-	$C_8H_{14}O$	62
15	11-dodecenoic acid	1471	212	38.592	2.43	-	-	-	-	$C_{13}H_{23}O_2$	62
16	7-oxabicyclo	956	112	38.925	2.36	-				$C_7H_{12}O$	71
17	5,6,8Trio-o-acetyl	2057	330	39.112	2.23	-	-	-	-	$C_{15}H_{22}O_8$	54
18	4-Hexenoic acid	1425	186	39.258	2.63	-	-	-	-	$C_9H_{14}O_4$	70
19	4-Primidinol	1078	126	39.485	2.93	-	-	-	-	$C_5H_6N_2O_2$	73
20	14-Pentadecenoic acid	1859	240	40.802	0.79	-	-	-	-	$C_{15}H_{28}O_2$	67
21	Phthalic acid	2434	332	42.565	0.76	-	-	-		$C_{20}H_{28}O_4$	70
22	9-borabicyclo	-	138	42.818	0.52	-	-	-	-	$C_8H_{15}BO$	58
23	6H-Furo	1967	226	43.818	0.45	-	-	-	-	$C_9H_{10}N_2O_5$	64
24	Methyl2-methyoxyoct-2-enoate	1244	186	44.072	1.12	-	-	-	-	$C_{10}H_{18}O_3$	52
25	Tiglate	1325	182	44.378	2.43	-	-	-	-	$C_{11}H_{18}O_2$	60
26	4-nonenal	1112	140	44.553	3.26	-	-	-	-	$C_9H_{16}O$	64
27	Hexacecanyl	4740	586	44.925	2.37	-		-	-	$C_{28}H_{58}O_{12}$	53
28	Di- (9-octadecenoyl	-	621	54.753	0.58	-	5.37	-	-	$C_{39}H_{72}O_5$	70
29	9-octadecenal	2007	266	59.775	22.01	-	-	-	-	$C_{18}H_{34}O$	89
30	2,3-octadecadien-1-ol	2069	266	61.312	5.54	-	-	-	-	$C_{18}H_{34}O$	90
31	9-octadecenoic acid	2085	296	35.372	-	5.51	65.38	79.91	-	$C_{19}H_{36}O_2$	91
32	Methyl 18-methylnonadecanoate	2212	326	38.031	-	1.25		-	-	$C_{21}H_{42}O_2$	76
33	Cyclopropane octanoic acid	1941	282	38.553	-	2.14	2.20	-	-	$C_{18}H_{34}O_2$	80
34	Docosanoic acid	2475	354	41.126	-	2.64	4.23	3.67	-	$C_{23}H_{46}O_2$	88
35	Pentaerythritol	-	404	60.608	-	0.95	-	-	-	$C_{23}H_{42}B_2O_4$	43
36	Pregn-4-ene	2527	362	62.448	-	1.01	-	-	-	$C_{21}H_{30}O_5$	41
37	Fumaric acid	1799	346	64.368	-	1.32	-	-	-	$C_{18}H_{31}ClO_4$	38
38	10-octadecenoic acid	2085	296	35.415	-	-	5.92	-	56.58	$C_{19}H_{36}O_2$	95
39	Eicosanoic acid	2212	326	38.063	-	-	2.39			$C_{21}H_{42}O_2$	94
40	Oleic acid, 3-hydroxypropyl ester	2527	340	59.228	-	-	3.22	-	-	$C_{21}H_{40}O_3$	53
41	1-methyl-4-nitro-5-[(3-chloropropyl) amino]-(1H) - Imidazole.	1799	218	59.548	-	-	0.99	-	-	$C_7H_{11}CN_4O_2$	43
42	Fumaric acid	2650	382	53.582	-	-	-	0.96		$C_{23}H_{42}O_4$	35
43	Uridine	-	244	59.102	-	-	-	1.19		$C_9H_{12}N_2O_6$	39
44	1,11-bis (trimethylsiloxy) undecan	1683	332	60.625	-	-	-	0.95		$C_{17}H_{40}O_2Si_2$	38
45	9,12-octadecadienoic acid	2093	294	36.282	-	-	-	-	6.84	$C_{19}H_{34}O_2$	91
46	Squalene	2914	410	42.571	--	-		-	4.93	$C_{30}H_{50}$	81
47	Atis-16-ene	1789	272	52.965	-	-	-		3.18	$C_{20}H_{32}$	43

S/N	Component	Retention Index	Molecular weight	Retention Time	% Composition of occurrence					Molecular formula	Similarity Index
					Extraction methods						
					CP	EACP	EAAE	SE	OL		
48	Heptadecane	-	296	53.398	-	-	-	-	2.20	$C_{21}H_{44}$	38
49	Cyclohexane propionic acid	1517	198	53.648	-	-	-	-	4.32	$C_{11}H_{18}O_3$	49
50	1-Hydroxytridecan-5-one	-	214	53.738	-	-	-	--	5.66	$C_{13}H_{26}O_2$	46
51	9-octadecenoic acid	-	282	54.342	-	-	-	-	2.84	$C_{18}H_{34}O_2$	48
52	Cyclopropane butanoic acid.	2528	374	60.042	-	-	-	-	5.31	$C_{25}H_{42}O_2$	52

CP: Cold Pressing; EAAE: Enzyme Assisted Aqueous Extraction; EACP: Enzyme Assisted Cold Pressing; SE: Soxhlet Extraction and OL: Olive Oil
Table 4: GCMS Chemical Components of Oil Extracted from *Moringa oleifera* Kernel.

The analysis identifies aldehyde, fatty acid and its methyl esters with chained, branched, saturated, unsaturated and polyunsaturated hydrocarbons. Some primary constituent identified are Oleic, Palmitic, Arachidic, Behenic and Stearic. While the secondary constituents identified are Fumaric acid, Phthalic acid, Uridine, 2 furancarboxaldehyde, 1-methyl-4-nitro-5-[(3-chloropropyl) amino]-(1H) – imidazole, and 1, 11-bis (trimethylsiloxy) un-decane. The primary and secondary constituents have application in pharmaceutical and food industries, for example, the Fumaric acid can substitute for tartaric and citric acid use.

This constituents can also be used as sedative, antifungal and anticancer by interfering the DNA activities in the cell with little quantity of Cyclopropaneoctanoic acid that are found in human adipose tissue and serum. Notably, the oil obtained from CP retains their natural bioactive compounds and taste because the extraction was carried out at low temperature 45 °C without any interaction with other chemical/solvent. The by-product has no residue or after effect of extraction method.

Conclusion

Quality evaluation of oil extracted from *Moringa oleifera* kernel using different extraction method has been investigated. All the extraction process conducted; except for SE are environmental friendly option with mild operational conditions to preserve the nutritional composition, physicochemical properties, and fatty acid composition. The oil yield percentage for the cold press extraction was relatively lower compared to the other extraction methods. This low yield condition can be enhanced by applying enzymatic treatment as a pretreatment method; therefore the produced oil can retains its natural composition while producing chemical free waste for animal feed use. The GCMS analysis reveals the various compound present in the of the extracted *Moringa oleifera* kernel oil. The thermal behavior of the extracted *Moringa oleifera* kernel oil exhibited two peaks for crystallization and melting point, with values identical to those reported in literatures. The oil quality analysis shows that *Moringa oleifera* kernel oil is less susceptible to rancidity, which implies longer shelf life for cold pressed *Moringa oleifera* kernel oil and its potential to be used as an alternative for virgin olive oil.

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