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PHYSIOCHEMICAL CHARACTERISTICS AND FATTY ACIDS COMPOSITION OF *MORINGA OLEIFERA* OIL OF FAR NORTH CAMEROON

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ABSTRACT

In this work, a comparative study on Moringa oleifera (MO) oil extraction techniques were conducted. MO seeds were collected from the Far North Region of Cameroon and oil was extracted using Soxhlet (SO), Ultrasonic (US) and Cold press (PR) methods. Physicochemical characteristics and fatty acid composition were determined for each MO oil extracted. The SO seems to be the effective method for oil extraction with a yield equal to 38.94 ± 0.28%, followed by US 35.11 ± 1.45% and at least PR (23.00 ± 1.00%). Moreover, myristic and lignoceric acids were found at low concentration in all extracted oil. However, the unsaturated fatty acid composition of the extracted oils follows PR>US>SO order. Oleic acid was the main unsaturated fatty acid with high rate obtained using PR and SO extractions (75.37%, 75.31% respectively) which is not very different from that obtained by US extraction (74.85). A higher value of acidity was noted in PR extraction method and lower oxidative state value was noted in PR extracted oil comparing to US and SO methods. No major differences were observed for the other physic-chemical parameters. Major and minor saturated fatty acids were palmitic and myristic acids, respectively. Lower total saturated fatty acids were recorded in PR extracted MO oil. We found also linoleic and linolenic acids in the group of unsaturated fatty acids of MO oil.

Keywords: *Moringa oleifera* oil; physicochemical properties; Soxhlet; Ultrasonic; Cold press extraction; Oil composition

INTRODUCTION

Moringa oliefera Lam. belongs to the kingdom of Plantae, the class of Magnoliopsida, the genus of Capparales and the family of Moringaceae **(Laleye *et al.*, 2015; Muhammad *et al.*, 2016)**. It is native to India, in the south valleys of the Himalayas and it can be found now in many countries of Asia, South America, and Africa **(Madi *et al.*, 2012; Dalei *et al.*, 2016)**. It is called «life tree» because of its exceptional virtues and properties. All plant parts including kernels seeds are used in various fields such as nutrition, where it could represent an effective solution in the context of the fight against malnutrition. The plant is consumed as food and / or used in traditional medicine for the treatment of metabolic, inflammatory, infectious, tumor, respiratory diseases, arthritis, atherosclerosis, pain relief etc, **(Elgamily *et al.*, 2016)**. It is also used in the cosmetics in the form of products with added value, such as Moringa oil and then it is used in water purification **(Bidima, 2016; Jaja-Chimedza *et al.*, 2017).**

The Moringa seeds are oleaginous (oilseeds). They contain approximately 35–40% depending on the plant variety and climate **(Oliveira *et al.*, 2016; Chen *et al.*, 2019)**. The Moringa oil is yellow, brilliant and edible with pleasant taste and aroma. It has been used for several decades for culinary, cosmetics (hairdressing, soap, body milk and perfume industries) **(Ghazali and Mohammed, 2011)**agriculture and medicinal purposes due to its healthful properties and its excellent oxidative stability which confers a great thermal stability **(Abdulkarim *et al.*, 2007;Nwidu *et al.*, 2018).**It would therefore be a natural antioxidant, renowned for its antibacterial, antimicrobial and anti-inflammatory properties **(Dayrit *et al.*, 1990; Xu*et al.*, 2019; Musarat *et al.*, 2019)**. It is also an excellent exfoliant, it softens, nourishes and gives a new radiance to skin, making it an ideal beauty oil for people with skin prone to blemishes **(Foidl *et al.*, 2001; Gué *et al.*, 2017)**. It also strengthens and softens the hair. But its benefits are not limited to skin and hair, Moringa oil is also rich in vitamins A, B and C and it seemsto have beneficial effects for health by stimulating the immune system and reducing cardiovascular diseases **(Abdulkarim *et al.*, 2007; Ojiako and Okeke, 2013; Randriamboavonjy *et al.*, 2016)**.

Moringa oil has become popular, therefore, very essential for researchers to develop more effective methodologies for oil extraction and above all to characterize it in order to standardize it. This will enhance its value in the world as is the case with several other vegetable oils such as olive, sunflower and rapeseed oils. Since there is insufficient information on the quality of *M. oleifera* oil in Cameroon, this study will therefore focus on characterizing this oil and comparing the influence of different extraction methods on fatty acid composition and oil characteristics.

MATERIAL AND METHODS

Raw material

Mature MOkernels were collected in Maroua city in the Far North Region of Cameroon and air-dried for a week,cleaned and shelled to obtain good quantity of kernels. The obtained kernels were crushed using a brand blender and the resulting powder was used for the extraction of Moringa oil.The resulted powder was kept in sealed polyethylene bags at 25° C for further experiments.

Extraction of *Moringa oleifera* oil

Soxhlet extraction

Sixty grams of MO dried kernels powder wereplaced in an extraction cartridge and introduced into the Soxhlet extractor. The oil extraction was realized at 45° C using 340 mL of hexane. After 8h extraction time, the mixture obtained was carried in the rotary evaporator (Rotavapor R-100, BUCHI) to separate the oil from the

solvent by evaporation at a temperature of 45°C. The recovered oil waskept in sealed brown bottles under refrigeration (4°C) for further analysis.

Ultrasonic extraction

An ultrasonic device (Ultrasonic processor UP100H) was used for MO oil seeds extraction with a frequency of 30 kHz. The ultrasound power level was set at 100% by amplitude controller. Then,30 g of kernels powder was mixed with 170 mL of hexane in an Erlenmeyer which was covered with aluminumfoil and placed below the apparatus for 20 min sonication time. When the set time has elapsed, the resulting mixture was filtered using filter paper in order to separate the solid residues from the solution. Then, the resulting filtrate was placed on a rotary evaporator equipped with a vacuum pump to separate the solvent from the oil. The oil obtained was analyzed after the yield was calculated.

Cold press extraction

Extraction was initiated by gradually introducing 400g of uncrushed hulled seeds into the press machine, which had been turned on for 20 minutes, in order to heat it up. The extracted oil was stored in sealed bottles under refrigeration (4°C) for later analysis. With respect to the other methods, the yield was also calculated and oil composition and physicochemical characteristics were analyzed.

Extraction yield

The yield of different extracted MO oils was calculated using the following formula:

$$\textbf{Yield (\%)} = (W_1 - W_2) \times 100$$

Where:
W_1 is the weight of the extracted oil
W_2 is the mass of the initial kernel powder sample.

Determination of the physicochemical properties

The parameters analyzed for Moringa oil of Cameroon were humidity, density, acidity, peroxide and saponification values.

Density

The density was determined according to the ISO 279 (1998) standard method. After cleaning and drying the pycnometer in the oven for one hour, the mass of the empty pycnometer was determined. Using a pipette, the pycnometer was filled with distilled water and its mass determined. Then, the pycnometer was cleaned and dried again in the oven for one hour, then using a pipette, MO oil was filled this time and its mass determined. The density was determined using the following formula:

$$\textbf{Density} = (W_1 - W_0)/(W_2 - W_0)$$

Where:
W_0: Weight (g) of empty pycnometer
W_1: Weight (g) of pycnometer filled with water
W_2: Weight (g) of pycnometer filled with oil.

Acid value

To measure the acidity, 2g of MO oil was dissolved in 50 mL of a mixture of ethanol: ethyl ether 95: 5 (v/v). The mixture was titrated with 0.1 N potassium hydroxide solution using 1mL of phenolphthalein as indicator. The acidity was expressed as a percentage of mg KOH g^{-1} according to the following formula:

$$\textbf{Acid value} = (V_b - V_s) \times 5.61/W$$

Where:
V_b: NaOH volume poured in blank assay;
V_s: NaOH volume poured in sample assay;
W: Weight of sample in grams

Peroxyde index

The peroxide value was determined according to ISO 3960(2007) standard method. In a flask, oil sample (2 g) was dissolved in 10 mL of chloroform. 15 mL of acetic acid and 1 mL of potassium iodide (saturated aqueous solution) were added. The flask was then immediately stirred vigorously for 1 minute, then left in the dark for 5 minutes at room temperature. Then 75 mL of distilled water was added to the mixture. The whole content was titrated with 0.01N sodium thiosulfate ($Na_2S_2O_3$) solution to color fewer end points using starch as indicator. The peroxide value is expressed asmeq of O_2 / Kg of sample and determined by the following formula:

$$\textbf{Peroxide value} = (V_b - V_s) \times M \times 10^3 / W$$

Where:
V_b: $Na_2S_2O_3$ volume consumed in blank assay
V_s: $Na_2S_2O_3$ volume consumed in sample assay
M: Molarity of $Na_2S_2O_3$ solution
W: Weight of sample in grams

Saponification value

The saponification value was determined according to ISO 3657 (2002) standard method. Ethanolic potassium hydroxide (0.5 N) was pipetted into conical flasks containing 1.0 g of sample. The content of each flask was reflux for 45 min or until clear with occasional shaking, then cooled to room temperature, after which it was titrated with sulfuric acid (0.5 N) using phenolphthalein as indicator. A blank was subjected to the same treatment. Results were expressed as mg KOH g^{-1}. Calculation of the saponification value of oil sample was as follows:

$$\textbf{Saponification value} = (V_b - V_s) \times 28.05 / W$$

Where:
V_b: H_2SO_4 volume poured in blank assay
V_s: H_2SO_4 volume poured in sample assay
W: Weight of sample in grams.

UV extinction coefficient

The specific extinction coefficient was determined according to the official method described by the OIC (International Olive Concil,2011). After filtering the oil samples is passed through anhydrous sodium sulfate and 1% solution of oil in cyclohexane was prepared. The reading was taken in quartz cuvette with1 cm optical path at wavelength of 270 nm. The specific extinctions reported at specified wavelength are calculated as follows:

$$\varepsilon = A_\lambda/(C \times l)$$

Where:
ε: Specific extinction coefficient
A_λ: Absorbance measured at 270 nm
C: Concentration of the solution
l: Optical pathin centimeters (1cm)

Gas Chromatography for Fatty Acids composition by GC-MS

The free fatty acid (FFA) content was determined using the ISO 660 (2009) standard protocol. In order to identify and quantify FA profiles, the esterification of lipid was carried out with organic solvents. Therefore, the isolated lipid was transformed to fatty acid methyl esters (FAMEs) and subsequently, the FAMEs were quantified by gas chromatography. The Agilent gas chromatograph, which was equipped withon-column injection in a capillary column BAC1, (0.25mm×0.25µm×30m) with detector: FID, Injector: SPLIT 1/100, T = 280°C, Carrier gas: Helium was employed for FA profile analysis. The following parameters as temperature program: 130° C, 170° C, 215 °C, 230° C and times 1 min, 3 min and 10 min with injection temperature 280°C were used. Fatty acids were identified by comparison with the retention time of appropriate standards and their content was determined by calculating the areas of the corresponding peaks.

Tocopherols contents determination by HPLC

Tocopherol contents of Moringa EO's were determined according to the ISO 9936 (2006) standard method using an HPLC equipped with a fluorometric detector (excitation wavelength 290 nm – emission wavelength 330 nm) on a silica column (25 cm 4 mm). The elution is carried out with a mixture of (isooctane: isopropanol) (99:1) with a flow rate of1.2 mL/min during the analysis time (20 min). Quantification was carried out by external standard curves of four tocopherols and daily reference of quantitative and qualitative tocopherol standards.

RESULTS AND DISCUSSION

Oil extraction yields

Moringa seeds oil extraction was performed using tree different extraction modes giving different yield values. Yields recorded in Soxhlet, US and cold press (PR) were of 38.94±0.28, 35.11 ± 1.45 and 22.00 ± 1.00 % respectively (Table 1). The result obtained by Soxhlet extraction corroborates with previous studies where the yield ranged between 32 and 41% **(Lalas and Tsaknis, 2002; Ogbunugafor *et al.*, 2011)**. This value is higher than that obtained by Tsaknis for MO Mbololo variety from Kenya (35.7%), by Malaysian MO (30.8%) **(Abdulkarim *et al.*, 2005)** and by Orhevba for Nigerian MO (33-37%) **(Orhevba *et al.*, 2013)**, but lower than that yield obtained by Ogbunugafor *et al.*, (2011) for variety in Nigeria (41.47%) and

Saudi Arabia (45%) **(Basuny *et al.*, 2016)**. This is also in line with results ofAbdulkarim *et al.* **(2005)**, who gave the value of oil yield from mature seeds of any plant to be between 22–43%. However, the best yield remains that of Moringa peregrine kernels (49.80%) reported from Saudi Arabia **(Tsaknis, 1998; Manzoor *et al.*, 2007)**. This variation in oil yield would depend on species, geographic area and climate **(Manzoor *et al.*, 2007; Ogbunugafor *et al.*, 2011; Oliveira *et al.*, 2016; Chen *et al.*, 2019)**.

Ultrasonic assisted extraction applied to Moringa seeds seems to give less oil yield than Soxhlet extraction. Nevertheless our result of 35% obtained is better than Janakiand Yamuna Devi **(2015)** who had obtained 31.2% for the same seed species. To get better yield Thirugnanasambandham **(2017)** had optimized his extraction to 59% using ultrasound assisted experiment with methanol while Mohammadpour *et al*., **(2019)** had optimized the extraction of *Moringa peregrina* oil to 54% using ultrasound assisted extraction with response surface methodology. The result obtained by cold press extraction corroborates with previous studies where the yield was between 20 and 25% **(Xiaona *et al.*, 2021; Athikomkulchai *et al.*, 2021)**. Our oil yield of 23% is lower than that obtained by Lalas and Tsaknis,**(2002)** for the Indian variety (24.5%), by the Chinese OM (25.5%) **(Xiaona *et al.*, 2021)** and by Ogunsina for the Indian OM (24.2%) **(Ogunsina*et al.*, 2014).**However, the observed difference in yield is not huge and remains within the range mentioned in the literature. This small variation in oil yield would depend on the species, geographical area and climate **(Oliveira *et al.*, 2016; Chen *et al.*, 2019)** and also on the apparatus used.

The cold press extraction (PR) gave lowersyield compared toSoxhlet (SO) and ultrasonic method (US). The latter still reduces oilextraction time thanks to the solutes which diffuse rapidly into sample.Parameters used for US extraction such as temperature, time and frequency should be optimized to increase oil yield. In solid liquid extraction, ultrasounds play an important role in mass transfer process through enhancing capillarity effects of solvent. The observation on the effectiveness of a given method should not be based only on the yield but also on the composition and the quality criteria of this oil.

Physicochemical characteristics

According to data collected in our study (Table 1), the density, the saponification value and UV extinction values of Moringa oils extracted by PR, SO and US are approximately similar. As has been reported in literature, density, acidity, UV extinction and saponification values are not affected by the extraction method **(Rzhepakovsky *et al.*, 2022; Eman and Muhamad, 2016)**. However, a slight difference was noted in the oil extracted by cold press (2.12 ± 0.21) compared to soxhlet (1.71 ± 0.03) and ultrasound (1.91 ± 0.42). This could be explained by the fact that there is a weak action of lypolytic enzymes for *Moringa oleifera* oils extracted by Soxhlet and by Ultrasonics **(Özcan *et al.*, 2019)**.

The oxidative state of oils is estimated by peroxide index and UV extinction value. In general, the values remain low for the three oils (1.28 ± 0.28, 1.24 ± 0.45, 0.67 ± 0.02meq O_2kg^{-1}) respectively for SO, US and PR. The peroxide value of the oil extracted by the press cold technique is very low than the others (Table 1). Indeed, the use of hexane as a solvent, the effect of extraction temperature, and even the power and frequency applied for ultrasound extraction would be responsible for the increase in peroxide values of *Moringa oleifera* seed oils extracted by SO and by US **(Özcan *et al.*, 2019)**. The humidity and the mode of extraction could enhance the oxidation phenomena.

The saponification value of the Cameroon MO SO oil (130 ± 0.86 mg of KOH g^{-1}) is lower compared to those released from MO oils of Kenya **(Tsaknis *et al.*, 2002)**, Nigeria **(Abiodun *et al.*, 2012)** and Brazil **(Pereira *et al.*, 2016)** (178, 180.31 and 179.4 mg of KOH g^{-1} respectively). It is the same for the peroxide value (1.28 ± 0.28 meqO_2 kg^{-1}) and the acidity (1.71 ± 0.03 mg of KOH g^{-1}) of this oil which are lower than those of Nigeria **(Abiodun *et al.*, 2012)** and Brazil **(Pereira *et al.*, 2016)** and higher than those of Algeria **(Boukandoul *et al.*, 2015)**, Kenya and Saudi Arabia **(Basunfy *et al.*, 2016)**.

Table 1 Physicochemical parameters of US, SO and PR extracted Moringa oils

Extraction mode	Yield of oil (%)	Density	Acidity (mgKOHg^{-1})
SO	38.94 ± 0.28	0.92 ± 0.05	1.71 ± 0.03
US	35.11 ± 1.45	0.91 ± 0.05	1.91 ± 0.42
PR	23.00 ± 1.00	0.91 ± 0.05	2.12 ± 0.21
	Peroxyde index (meq O_2kg^{-1})	**Saponification value**	**UV extinction value**
SO	1.28 ± 0.28	130 ± 0.86	0.21
US	1.24 ± 0.45	130 ± 2.22	0.19
PR	0.67 ± 0.02	129.30 ± 0.57	0.20

The acidity of Cameroonian MO oil extracted by cold press (2.12±0.21 mg KOH g^{-1}) corroborates with previous studies of **Xiaona *et al.*, 2021** (2.35±0.01 mg KOH g^{-1}). It is lower than that of India variety **(Ogunsina*et al.*, 2014)** with a value of 3.5±0.12 mgKOHg^{-1} and higher than those of Saudi Arabia and Soudan **(Özcan *et al.*, 2019)** and Tunisia **(Gharsallah *et al.*, 2021)** respectively 0.14 and 1.5±0.21 mg KOH g^{-1}. The value of saponification (129.30 ± 0.57) is lower than all the values found in the previous studies **(Ogunsina *et al.*, 2014; Özcan *et al.*, 2019; Gharsallah *et al.*, 2021; Xiaona *et al.*, 2021)**. Ogunsina *et al.*, **(2014)**, Gharsallah *et al.*, **(2021)** and Xiaona *et al*., **(2021)** found very high values of peroxide index while **Özcan *et al.*, 2019** had a lower value (0.14meq O_2kg^{-1}) compared to that obtained with MO oil in our study (0.67 ± 0.02meq O_2 kg^{-1}).

The low value of acidity reflects low hydrolysis during the extraction and storage of the oil. This means that, MO oil is edible oil and low peroxide signifies the absence of hydroperoxides, relatively unstable compounds and indicators of the early-stage oxidation, catalyzed mainly by the joint action of oxygen, temperature and light. These low values of the peroxide number show that the oil was extracted quickly after harvest, and it was stored in good conditions which would suggest that the oil does not oxidize prematurely and will be kept over time. On this basis, some authors have therefore qualified Moringa oil as an oil that can be used in cosmetics and food industries, for biofuel generation, medical applications (paint), varnishes manufacturer **(Anwar and Rashid, 2007; Manzoor *et al.*, 2007; Ogbunugafor *et al.*, 2011)**.

Fatty acids composition by GC/MS

Analysis of the fatty acid profiles of different MO oils were performed using gas chromatography. Results of Table 2 showed that Moringa oils contain 80.11, 79.66, 80.05% of unsaturated fatty acids and 19.59, 20.09 and 19.65 %of saturated fatty acids using PR, US and SO techniques, respectively. Oleic acid was the major compound in all tested oil as reported in previous works. It is about 75% but remains higher than all varieties from Kenya **(Tsaknis *et al.*, 1999)**, Malaysia **(Abdoulkarim *et al.*, 2007)** and Saudi Arabia **(Basuny *et al.*, 2016)** for Soxhlet extraction (Table 3). The high percentage of oleic acid in the oil makes it desirable in terms of nutrition and high-stability cooking and frying oil **(Ghazaliet, 2011)**. About US extraction, the values of our results are higher compared to those of Zhong *et al.*, **(2018)** who obtained 66.28% of oleic acid with 70.20% of unsaturated fatty acids and 22.08% of saturated fatty acids. The Table 4 shows the fatty acid profiles of *Moringa oleifera* from some countries compared to ours for cold-pressed oil. It appears that the percentage of oleic acid in our study is lower than that of Ogunsina *et al.*, **(2014)** and higher than those of India (**Athikomkulchai *et al.*, 2021)**, China **(Xiaona *et al.*, 2021)** and Tunisia **(Gharsallah *et al.*, 2021)**. The unsaturated fatty acid composition of our variety corroborates with those of Ogunsina *et al*.**, (2014)** which had obtained 80.7%.

Table 2 Fatty acids composition of Moringa oils extracted by different techniques

Fatty acids (%)	Formula	SO extracted oil	US extracted oil	PR extracted oil
Myristic	**C 14:0**	0.10	0.10	0.10
Palmitoleic	**C 16:1**	1.82	1.75	1.84
Palmitic	**C 16:0**	6.76	6.69	6.86
Linolenic	**C 18:3**	0.17	0.17	0.17
Linoleic	**C 18:2**	0.66	0.74	0.66
Oleic	**C 18:1**	75.32	74.95	75.37
Stearic	**C 18:0**	5.20	5.12	5.28
Paullinic	**C 20:1**	2.08	2.05	2.07
Arachidic	**C 20:0**	2.81	2.80	2.86
Behenic	**C 22:0**	4.27	4.88	4.08
Lignoceric	**C 24:0**	0.51	0.50	0.41
∑Saturated fatty acids		19.65	20.09	19.59
∑Unsaturated fatty acids		80.05	79.66	80.11

Soxhlet extracted oil contains on one hand palmitoleic acid (1.82%), linoleic acid (0.66%), linolenic (0.17%), paullinic acid (2.08%) and on the other hand palmitic (6.76%), stearic (5.20%), myristic (0.10%) and arachidic acid (2.8%). The percentage of behenic acid (4.27%) was lower comparing to other saturated fatty acids. It is approximately with the results of Tsaknis' team work **(Tsaknis *et al.*, 1999).** This oil can therefore be used as a natural source of behenic acid. It has been used in margarine, shortening, and foods containing semi-covered and solid fats, as a structuring and solidifying agent in oil, eliminating the need to hydrogenate the oil. **(FDA 2001; Adoulkarim *et al.*, 2005).**

Press cold extracted oil contains on one hand palmitoleic acid (1.84%), linoleic acid (0.66%), linolenic (0.17%), paullinic acid (2.08%) and on the other hand palmitic (6.86%), stearic (5.28%), myristic (0.10%) and arachidic acid (2.86%).
The US extraction revealed 1.75% of palmitoleic acid, 0.17% of linolenic acid, 0.74% of linoleic, 2.05% of paullinic acid while we found palmitic (6.69%), stearic (5.12%), myristic (0.10%) and arachidic acid (2.80%). This goes in the same direction as previous studies **(Adoulkarim *et al.*, 2005)**. The percentage of behenic acid is 4.08% and 4.88% respectively for PR and US extracted oil. This acid is not mentioned in some works **(Xiaona *et al.*, 2021; Gharsallah *et al.*, 2021)**. The same saturated fatty acids were found in oils regardless of the extraction method.
There is no real difference in the fatty acid composition of oils extracted by Soxhlet, Ultrasonic and even by cold press. This would simply mean that the extraction method does not alter the fatty acid composition of the oil.
With these values, *Moringaoleifera* oil would be comparable to olive oil or sunflower oil in terms of oleic acid **(Ruttarattanamongkol *et al.*, 2014)**. Normally, vegetable oils from corn, rice bran, palm, peanut, safflower, soybean, sunflower contain less than 40% oleic acid while Moringa oil has a tremendous concentration of it that ranges from 70% to 75% **(Lalas and Tsaknis, 2002; Anwar *et al.*, 2006)** depending on climate and genetic variation. On the other hand, the percentage of behenic acid is higher than varieties from Kenya **(Tsaknis *et al.*, 1999)**, Malaysia **(Abdoulkarim *et al.*, 2007)** and Brazil **(Pereira *et al.*, 2016)**.
Tables 3 and 4 reports the percentage of fatty acids of Soxhlet and Press cold extracted MO seed oil from Cameroon and other regions. Results shows a difference between the compositions of the oil studied with the other oils. The composition in C16:0, C16:1 and C18:0 is close to that from Malaysia. While it contains less C18:1 than other countries. Oil from Cameroun MO seeds is richer in C22:0 comparing to other seed oils from MO collected in Kenya, Malaysia, Brazil, India, China or Saudi Arabia.

Table 3 Comparison of fatty acids composition of Soxhlet extracted MO oil from Cameroon and other countries.

Fattyacids (%)	C 16:0	C 16:1	C 18:0	C 18:1	C 20:0	C 20:1	C 22:0	C 24:0	References
Cameron	6.76	1.82	5.20	75.32	2.81	2.05	4.88	0.40	Our study
Kenya	6.04	1.4	4.14	73.6	2.76	2.40	6.73	/	Tsaknis *et al.*, (1999)
Malaysia	7.8	2.2	7.6	67.9	4.0	1.9	6.2	1.3	Abdoulkarim , (2007)
Brazil	5.8	1.4	6.2	70.2	3.7	1.9	5.6	/	Pereira *et al.*, (2016)
Saudi Arabia	12.3	2.10	5.10	65.0	/	/	/	/	Banusy *et al.*, (2016)

On the basis of the results obtained, the fatty acid composition of the seed oil of MO Cameroon variety has been shown to be part of the category of oleic acid oils **(Tsaknis *et al.*, 1999; Eman and Muhamad, 2016)**. This oil has roughly the same oleic acid content (C18: 1) as olive oil, which allows it to be classified as high oleic oils. Due to its high oxidation stability, MO oil remains better than peanut, soybean and palm oil for frying and cooking **(Eman and Muhamad, 2016; Saa et al, 2019)**. In addition, the fatty acids in *M. oleifera* seed oil are known to decrease the risks of cardiovascular disease and stroke **(Schwingshackl and Hoffmann, 2014)** and high cholesterol problems **(Eman and Muhamad, 2016)**. MO oil obtained by PR technique contains less of saturated acid comparing to SO and US methods. In fact, it contains highest rate of oleic acid (75.37%). Cameroon MO oil seems to not contain polyunsaturated fatty acids. However, it gives the oil a low reactivity to oxygen. The more unsaturated the oil, the faster it oxidizes **(Parker *et al.*, 2003)**. The performance of ultrasonic extraction could be improved by optimizing certain parameters such as the granulometry of Moringa seed powder, the time and frequency of utrasonication as well as the seed: solvent ratio.

Table 4 Comparison of fatty acids composition of Cold press extracted MO oil from Cameroon and other countries.

Fattyacids (%)	C 16:0	C 16:1	C 18:0	C 18:1	C 20:0	C 20:1	C 22:0	C 24:0	References
Cameroon	6.86	1.84	5.28	75.37	2.86	2.07	4.08	0.41	Our study
India	5.8	1.2	3.9	79.5	2.2	/	5.1	/	Ogunsina *et al*., 2014
India	8.22	1.57	5.25	71.57	2.92	/	4.15	/	Athikomkulchai *et al*., 2021
China	5.43	1.35	5.04	70.85	/	2.90	8.38	1.29	Xiaona *et al*., 2021
Tunisia	6.11	1.4	) 5.37	73.36	3.26	2.21	5.71	0.66	Gharsallah *et al*., 2021

Tocopherols composition by GC/MS

Table 5 shows the content of tocopherols in *Moringa oleifera* seed obtained by differents methods of extraction. The seed oil only contains alpha, beta, gamma and delta tocopherols. The main component was alpha-tocopherol (54.61, 50.46, 62.35%) followed by gamma–tocopherol (29.22, 31.84, 25.99%), beta-tocopherol (4.77, 7.94, 3.38%) and delta-tocopherol (6.73, 3.10, 4.56%) respectively for SO, US and PR extraction. The α-tocopherol content for all the extractions is under the range of the literature finding **(Tsaknis*et al.*, 1999; Ozcan*et al.*, 2019; Garshall *et al.*, 2021; Xiaoni *et al.*, 2021)** for the cold-pressed Kenyan, Indian, Chinese and Algerian Soxhlet extraction **(Boukandoul*et al.*, 2015)** Moringa seed oil. However, the latter reported a higher content of γ-tocopherol (86.87 mg/g, 65.73 and 69.84 mg/g) for a Indian **(Garshall*et al.*, 2021)**, Saudi Arabia and Soudan **(Ozcan*et al.*, 2019)** and Algerian **(Boukandoul*et al.*, 2015)** MO seeds oil and δ-tocopherol (10.13, 62.27 and 65.27 mg/g) for Chinese **(Xiaoni*et al.*, 2021)**, Saudi Arabia and Soudan **(Ozcan*et al.*, 2019)** and Algerian**(Boukandoul*et al.*, 2015)** MO oil in comparison to that found in the Cameroonian SO, US, PR Moringa seed oil (31.84, 29.22, 25.99 mg/g) vs. (3.10, 6.73, 4.56 mg/g) respectively for γ and δ-tocopherol. The total tocopherols are 156.1 mg/g, 76.66 mg/g and 374.34 mg/g respectively for SO, US and PR oil extracted. We find that the total tocopherols of oil extracted by PR is very high compared to SO and US. This could be explaining by the fact that solvent extractions destroy some tocopherols while PR extraction releases the maximum amount of tocopherols. Moringa seed oil is an ideal dietary source of total α- and γ-tocopherols.

Table 5 Tocopherol contents of Moringa oil from Cameroon extracted by different techniques.

Tocopherols (%)	Soxhlet extraction	US extraction	Press cold extraction
α-tocopherol	54.46	50.47	62.35
γ-tocopherol	29.22	31.84	25.99
β-tocopherol	4.77	7.94	3.38
δ-tocopherol	6.73	3.10	4.57
ND	4.59	6.64	3.74
Total Tocopherol (mg/g)	**156.1**	**74.66**	**374.8**

***ND : Non determined**

Tocopherolscommonly known as vitamin E, prevent heart diseases and cancer and delaying Alzheimer's disease **(Garshall*et al.*, 2021)**. Tocopherolshave antioxidant properties physiological effects such as inhibiting cholesterol synthesis and tumor cell
growth **(Xiaoni *et al.*, 2021)**. The presence of tocopherol in vegetable oil is also of great significance to the oxidative stability of oils.

CONCLUSION

A comparative study between three extraction methods of kernels oil of Moringa collected in Cameroon has been conducted. Fatty acid composition and physicochemical characteristics of different obtained MO oils were compared. Although the yield of PR is lower compared to other techniques, the oil extracted by this method presents satisfactory results in terms of saponification, peroxide index, acidity and UV extinction values.
Furthermore, low saturated fatty acids rate in US extracted oil showed the importance of such technique to enhance the nutritional quality of edible oil. PR extracted MO oil contains more oleic acid than SO and US extracted oils. In addition, the ultrasonic method considerably reduced the extraction time. In developing countries where the consumption of vegetable oils such as olive oil, sunflower oil, rapeseed oil and palm oil is high and where resources are dwindling, MO oil represents a potential alternative provided that cultures of *M. oleifera* are available with using cold press extraction. This method of extraction gives the good and bio quality of oil, and it is respectful of the Environment. Future research can be carried out in order to improve the extraction parameters in the US and to increase the yield with a view to obtaining oil with more efficient nutritional criteria.

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