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Evaluation of *Moringa oleifera* seed oil extracted with different extraction methods

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ABSTRACT

Moringa seed oil composition extracted with different methods such as supercritical fluid extraction (SFE), soxhlet extraction (SOXE) and solvent extraction (SE) was evaluated and compared. The oil yield obtained by SFE, SE and SOXE was 25.25, 27.53 and 29.81%, respectively. There were slightly significant differences among SFE, SOXE and SE oils in their refractive indices, peroxide values, tocopherols, carotenoids, β-sitosterol and campesterol contents. However, SOXE and SE oils showed no significant differences in their TBA, unsaponifiable matter, phenols, flavonoids, oxidative stability, oleic acid, stigmasterol, Δ7- avenasterol and Δ5-avenasterol contents. Furthermore, SFE oil showed significantly higher unsaponifiable matter, phenols, flavonoids, tocopherols, carotenoids, oleic acid, unsaturated fatty acids to saturated fatty acids ratio, sterols contents and oxidative stability. The high content of tocopherol, phenols, and flavonoids of the Moringa oils could be attributed to the higher resistance to oxidation, especially extracted by SFE. These results promote the use of SFE for the extraction of high quality Moringa oil. extraction of high quality.

Introduction

Moringa (*Moringa oleifera* L. *Moringaceae*), commonly known as the 'drumstick' or 'horseradish' tree, comes from the northern India. Nowadays, due to its adapitibility, it is found in the Middle East and worldwide in the tropics and subtropics (Lalas and Tsaknis, 2002; Saini et al., 2016).

Moringa plant (roots, leaves, flowers, green pods, and seeds) is very useful and it used as medicine, food, in water purification, biodiesel, cosmetics and has got many other beneficial applications (Leone et al., 2016; Saini et al., 2016). It is used as feed for livestock and as a traditional medicine (Leone et al., 2016).

Moringa seeds have considerable oil content (up to 40%) with a high content of oleic acid, sterols and tocopherols and, therefore, high oxidative stability. The oil was known as "Ben oil" or "Behen oil". Behenic and palmitic acids are the predominant saturated fatty acids (Tsaknis et al., 1999; Leone et al., 2016). Moringa oil can be used in nutrition as well as for non-diet purposes, like biodiesel, cosmetics and lubricants. Moreover, seed cake (by-product), after oil extraction, is used for waste water treatment or as a fertilizer (Leone et al., 2016).

Seed oil contents varied depending upon variety, climate and extraction method (Lalas and Tsaknis, 2002; Anwar and Bhanger, 2003). The most commonly used chemical techniques for extracting oil from plant matrices include organic solvent extraction, supercritical fluid extraction, accelerated solvent extraction, microwave assisted solvent extraction, ultrasonic assisted solvent extraction and aqueous extraction (Danlami et al., 2014). Thus, hexane is usually used in moringa oil extraction, because of its efficiency and recovery (Saini et al., 2016). However, solvent extraction has main abuse of solvent residue presence in the extracts.

Supercritical fluid extraction (SFE) is a powerful means of extraction of seed oil. It can be considered a technological revolution in the extraction industry (Danlami et al., 2014). SFE is a technique that can overcome these drawbacks of the conventional extraction process which require a large amount of organic solvents and some of them are toxic. In the case of SFE, a co-solvent is essential to enhance the

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solubility of more polar lipids (Danlami et al., 2014). Recently, supercritical fluid extraction (SFE) has gained an increasing attention over the traditional techniques, like solvent extraction in the recovery of edible oils. The commonly used fluid in SFE is CO2, which has several unique characteristics and physico-chemical properties. For example, CO2 is non-toxic, non-flammable, inexpensive, and odourless and has low critical pressure (7.38 MPa) and temperature (31.1 °C). It leaves no solvent residue in the products, thus potentially providing the oil of superior quality. SFE also has advantages over the traditional extraction techniques including operation at low temperatures thus enabling the preservation of the thermally labile compounds in the extracts. Moreover, the selectivity of carbon dioxide in relation to the oil can be easily adjusted by changing the temperature and pressure (Danlami et al., 2014).

Therefore, the main objectives of this research were to extract and characterize the oils present in the seeds of Moringa oleifera using SFE and to compare the results with those using the conventional soxhlet extraction and solvent extraction (soaking) methods.

Materials and methods

Materials

Moringa seeds (*Moringa oleifera*) were collected from trees cultivated in Cairo Governorate, Egypt. Chemicals and standards were obtained from Sigma Aldrich (St. Louis, MO, USA) and El-Gomhoria Co. for Pharmaceutical, Cairo, Egypt.

Methods

Chemical analysis

Moisture, crude protein, crude oil, crude fibers and ash were determined according to Association of Official Analytical Chemists (AOAC) methods (2007). Total carbohydrates were determined by difference.

*Extraction of Moringa seed oil*s

The seeds were grounded by electrical grinder (Moulinex, France). Moringa seeds oils were extracted by soxhlet apparatus (SOXE), by solvent extraction (soaking in hexane for 24 hour) (SE) and by supercritical fluid extraction (SFE).

Soxhlet extraction (SOXE)

Oil was extracted from the ground moringa seeds (100 g) using the Soxhlet apparatus as described in the AOAC (2007) with n-hexane (60-80 °C) as a solvent.

Solvent extraction (SE)

Oil was extracted from the ground moringa seeds (100 g) by soaking in n-hexane for 24hrs at room temperature.

Supercritical fluid extraction (SFE)

SFE was conducted in a lab scale unit (SFE2000; Thar Technologies, Inc., Pittsburgh, PA, USA) equipped with 1000 ml extraction vessel and a high pressure pump according to the described method by Li et al. (2010) and Palafox et al. (2012). 100 g ground moringa seed sample (0.46 mm particle size) was loaded into the extraction vessel. Extraction temperature was set at 40 °C. The extraction pressure was set at 403 bar and time of 30 min. The total flow rate of CO2 was set at 70 g/min and co-solvent (ethanol) (Li etal., 2010; Palafox et al., 2012).

Physical and chemical characteristics of oils

Refractive index (RI), free fatty acids (FFA), peroxide value (PV), unsaponifiable matter and Thiobarbituric acid (TBA) number of moringa oils were determined using AOAC methods, (2007).

Determination of bioactive components and oxidative stability

Oil samples were extracted using 10 ml of a methanol/water mixture (60: 40 V/V) three times. Pooled extracts were washed with n-hexane and solvents were removed with a rotary evaporator (Buchi, Switzerland). Total phenol content as (mg GAE/g) and the flavonoid content as (mg quercetin/g) of extract were determined according to Barakat and Ghazal methods (2016). Tocopherols (α, γ and δ) were extracted using the method described by Gliszczyńska-Świgło and Sikorska (2004) which involved weighing 1 g of sample and transferred to a 10 ml screw capped extraction tube. 4ml n-hexane was added to the extraction tube. The mixture was shaken on a vortex mixer for 0.5 min. rested for 5 min. and shaken another 0.5 min. After centrifugation at 4000 rpm for 5min. 1ml of supernatant was transferred to a 1.5 ml vial and evaporated under nitrogen. The residue was re-dissolved in 0.3 ml n-butanol before being injected into Shimadzu HPLC (CRUA: chromatopac. SCL 6A: System controller. CTO 6A: Column oven. SPD 6AV: Uv-vis detector. LC 20AD UFLC: Pump. Mobile phase used was methanol. Flow rate at 1.5 ml /min. Injected (25 µl) UV (290 nm).

Tocopherols were identified by comparing their retention times with those of corresponding standards and by spiking of samples with appropriate standard. The carotenoids content (ppm) and oxidative stability by rancimat apparatus were determined according to Elsorady et al. method (2017).

Fatty acids composition of moringa seed oils

An aliquot of fatty acids after saponification and acidification of moringa oil, about 10 mg, was dissolved in 2mL hexane and then 0.4 mL of 2N KOH in anhydrous methanol was added (Elsorady et al., 2017). After 3 minutes, 3 mL water was added. The organic layer, separated by centrifugation, was dried over anhydrous sodium sulfate, and then concentrated, with a N2 stream to around 0.5 mL for GC analysis of fatty acids methyl esters (FAME), as described below. Agilent 6890 series GC apparatus provided with a DB-23 column (60 m × 0.32 mm × 0.25 μm) was used for the identification and quantification of fatty acids.

Determination of sterols composition

The identification and determination of sterols by GC were according to the method described by Elsorady and Eid. (2011).

Statistical analysis

Statistical analyses were done using SPSS program version 16.0. The data were expressed as means ± SD. Results were compared by two-way ANOVA. Differences were considered significant if $p < 0.05$.

Results and discussion

Table 1 shows proximate composition of moringa seeds, which are a good source of proteins and oils. It has 34.97 and 29.81%, respectively. It also containes approximately 7.13% moisture, 4.88% ash and 30.32% total carbohydrates. The obtained results agree with Barakat and Ghazal (2016). Anwar and Bhanger, 2003; Anwar and Rashid, (2007); Leone et al. (2016) reported that moringa seeds had more oil than protein content. Variations in proximate composition may result from several factors such as cultivation factors and growing and extraction conditions (Barakat and Ghazal, 2016). Results in Table 2 reveal that the extraction methods significantly affected the moringa oil yield ($p < 0.05$). The yields of soxhlet extraction, solvent extraction and SFE were 29.81, 27.53 and 25.25% (w/w), respectively. These results agree with Li et al. (2016), who found that soxhlet extraction method had higher oil yield than SFE-CO_2. The higher oil yield recorded with Soxhlet extraction is due to the temperature of extraction (65 °C), which leads to cell walls breaking. This, together with the organic solvent used and continuous recycling of the hot solvent through the Soxhlet extraction process, promotes better extraction efficiency by penetrating into the cell wall of the seed, which was also broken during the process of grinding, solubilizing all the oil present (Daroch et al., 2013; Li et al., 2016). The obtained results do not agree with Dinesha et al. (2018), who reported that oil extraction with SFE was 37.76%, observed to be higher than those of soxhlet extraction (29.12%), and significantly lower than solvent extraction (22.12 %). Physico-chemical properties of the extracted oils are shown in Table 2.

Table 1. Proximate composition of moringa seeds (mean ± SE)

Characteristics	Content (%)
Moisture	7.13±0.34
Crude oil	29.81±0.35
Crude protein	34.97±0.70
Ash	4.88±0.09
Total carbohydrates	30.32±0.27

Table 2. Effect of extraction methods on oil yield and physico-chemical properties of moringa seed oils

Parameters	Solvent extraction (SE)	Soxhlet extraction (SOXE)	Supercritical fluid extraction (SFE)
Oil yield (%)	27.53±0.31 ^{b}	29.81±0.35 c	25.25±0.44 ^{a}
Refractive index (at 20° C)	1.4587±0.000 ^{a}	1.4611±0.000 ^{b}	1.4729±0.006 ^{c}
FFA % (as oleic acid)	0.26±0.02 b	0.18±0.02 a	0.22±0.01 b
Peroxide value (meqO_2/kg oil)	0.92±0.02 ^{c}	0.78±0.03 ^{b}	0.72±0.02 ^{a}
TBA	0.08±0.01 ^{b}	0.08±0.01 ^{b}	0.06±0.00 ^{a}
Unsaponifiable matter (%)	0.56±0.02 ^{a}	0.60±0.01 ^{a}	0.77±0.02 ^{b}

*a-c different superscripts indicate significant differences ($p<0.05$)

Refractive index ranged from 1.4587 to 1.4729, with significant differences among different extracted oils. The lowest refractive index was obtained for solvent extraction (1.4587), followed by soxhlet extraction (1.4611) and the highest value of 1.4729 was obtained in SFE extracted oil. These results agree with Dinesha et al. (2018).

The FFA, peroxide value (PV), TBA and unsaponification matter of extracted moringa oils by different methods, the major parameters for oil quality are depicted in Table 2. SFE oil showing slightly higher FFA content (0.22%) as compared with soxhlet extraction oil (0.18%) (Table 2). This may be related to the use of polar solvent which could extract these FFA. Free fatty acids are mainly those of short hydrocarbon chains which make them more polar than triglycerides and thus readily extracted with the polar solvents. Bruhl and Matthaus, (1999) reported that SFE oils showed significantly higher FFA content than their soxhlet extraction; however, the high contents of FFA were not an indicator for hydrolysis during extraction. However, the higher FFA values may be accounted for through several explanations. O'Brien (2009) suggests that the presence of carbon dioxide may contribute to elevated FFA values. It is not likely that the oils would have been hydrolysed during processing with SFE because reported studies, which have evaluated the effect of SFE on FFA value, indicateed that the oils obtained via SFE-CO2 have lower FFA values compared to traditional extraction methods (Martinez et al., 2008; Jahurul et al., 2014). Jahurul et al. (2014) noted that the FFA values of mango kernel oil extracted with SFE-CO2 were lower compared to values of oils obtained with solvent extraction. Similarly, Martinez et al. (2008) also reported low FFA values for walnut oils.

The peroxide value is an indicator of the initiation stage of oxidation and primary oxidation products in oil. The significantly lower peroxide value (0.72meqO2/kg oil) of the SFE oil could be related to working under CO2, therefore minimizing oxidation reactions as compared to soxhlet and solvent extractions. The peroxide values of the oils extracted by different extraction methods were within the limit value (15 meqO2/kg oil) as recommended by Codex (1999). The same trend was observed for TBA.

Also, data in Table 2 showed that unsaponifiable matter of the SFE oil was slightly higher than other oils. The significantly higher unsaponifiable matter of the SFE oil showed the ability of SFE to extract more phospholipids, sterols, chlorophylls, carotenoids, fat-soluble vitamins, tocopherols and polyphenols with potent antioxidant properties. The SFE oil, therefore, is expected to pose better quality and stability.

Phenolic and tocopherols compounds are responsible for antioxidant activity on moringa oil (Leone et al., 2016). High oxidative stability of moringa seed oil is related to high content of tocopherols and phenols (Lalas and Tsaknis, 2002).

The antioxidant activity of δ -tocopherol exceeds that of γ -, β-, and α -tocopherol. Thus, tocopherols present in high concentrations in moringa seed oil are expected to offer some protection during storage and processing (Tsaknis et al., 1999). Naturally, oils have tocopherols as antioxidants to prevent oils oxidation (O'Brien, 2009). Results in Table 3 reveal that SFE oil has the highest content of tocopherols, phenols, flavonoids and carotenoids as compared with those extracted with soxhlet and solvent extractions. These results agree with Dinesha et al. (2018). This may be related to polar co-solvent (ethanol) in the SFE. Martinez et al. (2008) noted that oils extracted with SFE had higher carotenoid content than those extracted with screw pressing. The presence of these components, with their known antioxidant potential in SFE oil, is an indication of their superiority.

The oxidative stability of the extracted moringa seed oils by different extraction methods, as measured by the rancimat method, is shown in Table 3. SFE oil has got the highest oxidative stability (32.51 hrs), followed by those soxhlet extraction (29.39 hrs) and solvent extraction (28.54 hrs). Xia et al. (2013) found that the SFE extracted bayberry kernel oil had a greater oxidative stability. The higher resistance to oxidation could be attributed to the high content of tocopherol, phenols and flavonoids of the moringa oils

Fatty acid profile of the obtained moringa seed oils is shown in Table 4. Moringa oil is rich in unsaturated fatty acids, which amounted to about 75% of the total fatty acids. The fatty acid compositions of moringa oil extracted by the three methods are similar and there is a slight difference among them. The major fatty acids of moringa oil were C18:1, C22:0, C18:0 and C20:0, which accounted for 69.28–70.64%, 7.35–7.63%, 5.45–5.99% and 5.44–5.73%, respectively. Oleic acid is a principal unsaturated fatty acid. These results agree with Tsaknis et al. (1999). High oleic acid in moringa oil makes it desirable in the term of nutrition and high stability cooking and frying oil (Leone et al., 2016). Therefore, Moringa oils with high proportion of oleic acid are more stable than the others. Also, oleic acid is less susceptible to oxidation than polyunsaturated fatty acid from the n-6 series (linoleic acid). Anwar and Rashid, (2007) reported oleic acid as being the dominant fatty acid present in moringa seed oil.

Table 5. indicates sterol profiles of extracted moringa seed oil by different extraction methods. Among the phytosterols, β-Sitosterol was the most prominent,

which accounted for 43.25–51.23% of the total phytosterols, followed by stigmasterol and campesterol for all moringa oil samples extracted with different methods. These results agree with Tsaknis et al. (1999) and Anwar and Bhanger, (2003). SFE oils have got the highest content of sterol components, except for campesterol and β- sitosterol. Lalas and Tsaknis (2002) found that M. oleifera oil showed high content of β-sitosterol, stigmasterol and campesterol. For campesterol and β-sitosterol, the highest amounts are found for the soxhlet extraction oil (Table 5), which likely results from the higher temperature used for soxhlet extraction.

Table 3. Effect of extraction methods on bioactive components and oxidative stability of moringa seed oils

Parameters	Solvent extraction (SE)	Soxhlet extraction (SOXE)	Supercritical fluid extraction (SFE)
α-tocopherol (mg/100g)	16.26±0.15 [a]	18.35±0.06 [b]	20.34±0.18 [c]
γ-tocopherol (mg/100g)	4.10±0.25 [a]	4.88±0.09 [b]	5.98±0.14 [c]
δ-tocopherol (mg/100g)	1.03±0.02 [a]	1.12±0.03 [b]	1.28±0.04 [c]
Total phenols (mg/g)	39.96±0.48 [a]	40.55±0.19 [a]	44.54±0.81 [b]
Total flavonoids (mg/g)	17.26±0.18 [a]	17.32±0.16 [a]	19.03±0.35 [b]
Total carotenoids (ppm)	14.81±0.16 [a]	15.20±0.14 [b]	16.63±0.10 [c]
Oxidative stability	28.54±0.94 [a]	29.39±0.15 [a]	32.51±0.90 [b]

*a-c different superscripts indicate significant differences ($p<0.05$)

Table 4. Effect of extraction methods on fatty acids composition of Moringa seed oils

Fatty acids	Solvent extraction (SE)	Soxhlet extraction (SOXE)	Supercritical fluid extraction (SFE)
$C_{14:0}$	0.15±0.03 [b]	0.11±0.02 [ab]	0.08±0.01 [a]
$C_{16:0}$	5.20±0.09 [b]	5.14±0.08 [b]	4.39±0.14 [a]
$C_{16:1}$	1.32±0.02 [a]	1.35±0.07 [a]	1.50±0.18 [a]
$C_{17:0}$	0.08±0.00 [a]	0.07±0.01 [a]	0.06±0.01 [a]
$C_{17:1}$	0.07±0.00 [a]	0.08±0.02 [a]	0.09±0.01 [a]
$C_{18:0}$	5.99±0.10[b]	5.75±0.34 [ab]	5.45±0.13 [a]
$C_{18:1}$	69.28±0.32 [a]	69.54±0.91 [a]	70.64±0.29 [b]
$C_{18:2}$	0.59±0.08 [a]	0.65±0.05 [a]	0.80±0.03 [b]
$C_{18:3}$	0.23±0.02 [a]	0.25±0.04 [a]	0.34±0.02 [b]
$C_{20:0}$	5.73±0.09[b]	5.68±0.17 [ab]	5.44±0.10 [a]
$C_{20:1}$	2.09±0.04 [a]	2.10±0.08 [a]	2.26±0.03 [b]
$C_{22:0}$	7.63±0.12 [a]	7.50±0.25 [a]	7.35±0.02 [a]
$C_{22:1}$	0.04±0.01 [a]	0.08±0.01 [b]	0.09±0.02 [b]
$C_{24:0}$	1.65±0.17 [a]	1.70±0.09[a]	1.50±0.07 [a]
SFA	26.37±0.41 [b]	25.95±0.76 [b]	24.27±0.42[a]
USFA	73.63±0.41 [a]	74.05±0.76 [a]	75.72±0.42 [b]
U/S	2.79±0.06 [a]	2.85±0.11 [a]	3.11±0.07 [b]

*a-b different superscripts indicate significant differences ($p<0.05$), SFA: Saturated fatty acids; USFA: Unsaturated fatty acids

Table 5. Effect of extraction methods on sterols composition of Moringa oils

Sterols profile	Solvent extraction (SE)	Soxhlet extraction (SOXE)	Supercritical fluid extraction (SFE)
24-Methylenecholesterol	0.75±0.03 [a]	0.78±0.02 [a]	0.92±0.03 [b]
Campesterol	14.62±0.05 [a]	16.35±0.15 [c]	15.85±0.13[b]
Campestanol	0.29±0.01 [a]	0.33±0.02 [b]	0.51±0.02 [c]
Δ7-campestanol	0.55±0.02 [a]	0.55±0.01 [a]	0.79±0.03 [b]
Stigmasterol	16.71±0.05 [a]	16.63±0.07 [a]	17.60±0.13 [b]
Clerosterol	1.80±0.02 [a]	1.89±0.03 [a]	2.32±0.11 [b]
Stigmastanol	0.52±0.03 [a]	0.61±0.02 [b]	0.84±0.03 [c]
β-sitosterol	43.25±0.24 [a]	51.23±0.14 [c]	48.32±0.12 [b]
Δ7- avenasterol	0.79±0.04 [a]	0.90±0.05 [a]	1.16±0.08 [b]
Δ5- avenasterol	8.52±0.04 [a]	8.64±0.09 [a]	8.96±0.09 [b]
28- isoavenasterol	1.11±0.02 [a]	1.16±0.10 [a]	1.64±0.06 [b]
Δ7,14- stigmastanol	0.42±0.04 [a]	0.40±0.05 [a]	0.66±0.03 [b]

*a-c different superscripts indicate significant differences ($p<0.05$)

High temperatures likely assist in the extraction of campesterol and sitosterol, which are also less susceptible to thermal degradation (Zanqui et al., 2015). Tsaknis et al. (1999) indicated that the high oxidative stability of moringa oil should be attributed to other constituents of the non-glyceride fraction of the oil, which possess antioxidant properties.
For Δ5- avenasterol was 8.96, 8.64 and 8.52 for SFE, soxhlet and solvent extraction oils, respectively. The high oxidative stability (Table 3) of Moringa oils might be explained by the presence of Δ5- avenasterol.

Conclusions

The supercritical fluid extraction (SFE) showed technical viability in the extraction of Moringa oil from seeds. The lowest oil yield was obtained by SFE extraction method. It had the highest oil quality compared to soxhlet and solvent extraction methods with its higher unsaponifiable matter, tocopherols, phenols, flavonoids, oleic acid, sterols contents and oxidative stability.

*Conflicts of Interest***:** No conflict of interest.

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