Physico-chemical Characterization of *Moringa oleifera* Lam (Moringaceae) Seed Oil Harvested from Burkina Faso

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Abstract:
Moringa oleifera seeds are a potential source of oils for food or cosmetic use but also for therapeutic virtues. This study is a contribution to the valorization of this oil by assess the physico-chemical characteristics of the seed oil of *M. oleifera* from Burkina Faso obtained by two extraction methods. Extraction was assessed by Soxhlet with n-hexane and Hot expression extraction. The chemical indices were evaluated by volumetry. Density and refractive index were determined with a pycnometer and an ABBE OPL type refractometer respectively. The density of the oils was 0.904 and 0.907 respectively by organic extraction and by pressing with refractive indexes 1.465 vs 1.467. From these two extraction methods, the chemical index such as the acid value: 3.94±0.19 vs 2.85±0.15 mg of KOH/g of oil ; Peroxide value: 55.33 ± 1.26 vs 2.19 ± 0.56 mEq of O2/Kg ; Iodine value: 37.6 ± 0.64 vs 37.93 ± 0.68 g of iodine/100 g of oil and saponification value: 199,79 ± 1.11 vs 200.39 ± 4.34 mg KOH/g oil were found respectively. *Moringa oleifera* oil obtained by organic extraction is more acidic and more sensitive to oxidation against that obtained by hot press. Thus, organic solvents would negatively impact the quality of the oil.Guinea.

Keywords: *Moringa oleifera*, seeds, organic extraction, Hot expression extraction, chemical index.

Caractérisation physico-chimique d'huile de graines de *Moringa oleifer* Lam (Moringaceae) récoltées au Burkina Faso

Résumé :
Les graines de *Moringa oleifera* sont une source potentielle d'huiles à usage alimentaire, cosmétique et thérapeutiques. Cette étude a pour but de contribuer à la valorisation de cette huile en évaluant les caractéristiques physico-chimiques de l'huile de graines de *M. oleifera* du Burkina Faso obtenue par deux méthodes d'extraction. L'huile a été obtenue par extraction au Soxhlet avec n-hexane et par pressage à chaud. Les indices chimiques ont été évalués par volumétrie. La densité et l'indice de réfraction ont été déterminés respectivement à l'aide d'un pycnomètre et d'un réfractomètre de type ABBE OPL. La densité des huiles était respectivement de 0,904 et 0,907 par extraction organique et par pressage avec des indices de réfraction de 1,465 vs 1,467. Par ces deux méthodes d'extraction, les indices chimiques tels que l'indice d'acidité : 3,94 ± 0,19 vs 2,85 ± 0,15 mg de KOH/g d'huile ; l'indice de peroxyde : 55,33 ± 1,26 vs 2,19 ± 0,56 mEq d'O2/kg ; l'indice d'iode: 37,6 ± 0,64 vs 37,93 ± 0,68 g d'iode / 100g d'huile et l'indice de saponification : 199,79±1,11 vs 200,39± 4,34 mg KOH/g d'huile ont été obtenus respectivement.

Key words: *Terminalia albida*, monographie, activité antiplasmodiale.

Introduction

Plants seeds are potential sources of oils for food or cosmetic use but also for therapeutic virtues.
This is the case of peanut oil (Arachis hypogaea) which occupies a good place in the diet. This is also the case of ''Touloucouna'' oil (Carapa procera), Shea butter (Vitellaria paradoxa) in the field of cosmetology and therapeutic.
However, in recent years, there has been an increase in the use of vegetable oils in traditional medicine as painkillers but especially in cosmetics as active ingredients. The current trend is the Bio label with the use of natural active ingredients. Thus, the future of the industry turns to the valorization of plants and the search for bioactives replacing synthetic. Otherwise, Burkina Faso is endowed with a flora containing many plants that are sources of oils, thus constituting a wealth provided they are valued (Zerbo et al., 2007). Among these plants source, Moringa oleifera Lam., a tropical tree widely cultivated for its many nutritional, therapeutic and cosmetic applications stands out. Indeed, its leaves and seeds are very popular. The seed produces a vegetable oil similar to olive oil in its chemical composition, rich in tocopherols and oleic acid.
That is why the Moringa seeds are ideal for human ingestion and commercial utilisation (Anwar et al., 2005). The seeds, leaves, roots and even flowers of this plant are fit for both human

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and animal consumption (Gharsallah et al., 2021). The leaves are, in particular, a good source of protein, , vitamins, minerals, β-carotene and antioxidants and have ever been used for dietary and medicinal practices (Leone et al., 2016).
According to Gué et al. (2017), its high level of oleic acid gives it health benefits by reducing cholesterol and heart disease. In cosmetology, it is also used in the manufacture of soaps and is also interesting in the perfume industry to stabilize scents (Louni, 2009).

However, the physico-chemical characteristics of the oil may vary depending on the variety and country of cultivation of the species. It's in this context that we have chosen to assess the physico-chemical characteristics of the seed oil of M. oleifera from Burkina Faso in order to contribute to the valorization of this oil, on the one hand, and to compare it to oils of various origins in the world on the other hand.

Materials and Methods

- ***Plant material***

The plant material used consisted of whole seeds of Moringa oleifera from Belwet Industrie in Kossodo (Ouagadougou-Burkina Faso). After removing the seed coats, the seeds were crushed manually.

- ***Soxhlet extraction***

The oil from seeds was obtained by Soxhlet extraction for 9 hours. Thus 55 g of seeds powder was extracted with 900 mL of n-hexane. The flask was heated under conditions such that the reflux rate was at least 3 drops per second. After cooling, the solvent was removed using a rotary evaporator and the oil obtained was kept in an oven for 24 hours at 39 ° C to remove the residual solvent.

- ***Hot expression extraction***

The extraction was done using a hot electric oil press (GEENEES type). Thus, 54 kg of seed powder were pressed in different portions. A sieve is placed between the oil outlet and the cakes are collected in a bucket below. The product of the pressing was settled for 72 hours and then the oil that was overswimming was collected.

- ***Density and refractive index***

The density was measured using a pycnometer. The latter was first cleaned neatly with ethanol and acetone before determining its empty mass (m0) using a precision balance. Then the pycnometer was filled with distilled water corresponding to a mass (m1) and then with oil giving a mass (m2). The density was thus calculated using the above formula (Wolff, 1968) :

$$D = \frac{m2 - m0}{m1 - m0}$$

m0 : Mass (g) of empty pycnometer ;
m1 : Mass (g) of pycnometer filled with water ;
m2 : Mass (g) of pycnometer filled with oil

The refractive index of the oils was measured using an ABBE refractometer of the OPL type. Thus, after cleaning the prism of the refractometer with distilled water, few mL of the oil were spread there before hermetically covering the device with the cover in order to read it.

- ***Peroxide value (PV) (AOCS Cd 8b-90)***

A sample 0.5 to 2 g of seed oil was dissolved in 10 mL of chloroform. Then, 15 mL of glacial acetic acid and 1 mL of saturated potassium iodide solution were added to the solution.
After 1 minute of stirring, the vial was incubated at room temperature and protected from light for 5 minutes. Then 75 mL of distilled water were added before titrating the iodine released with 0.01N sodium thiosulphate solution until the yellow color disappeared. Then 4 drops of starch stock were added before continuing the titration until the disappearance of the blue coloration. A blank test was performed under the same conditions. The tests were carried out in triplicate (n = 3).
The peroxide value was expressed as milliequivalent of active oxygen per kilogram (mEQ AO_2/kg) of sample, as a mean ± standard deviation according to the following formula:

$$PV = \frac{(V - Vo) \text{ x } N}{m} \text{ x } 1000$$

V: Volume of sodium thiosulphate for the sample (mL);
Vo: Volume of sodium thiosulphate for blank (mL);
N: Normality of sodium thiosulphate solution;
m: mass of the sample in g.

- ***Acid value (AV)***

The acid value was determined by the slightly modified method of Atolani et al. (2019). One gram seed oil was weighed and mixed with 25 mL ether-ethanol (v/v) in a conical flask followed by the addition of 1 mL of indicator (phenolphthalein 1%). The resulting mixture was titrated with 0.1 N KOH solution. The titration was terminated at appearance of the pink colour. The acid value was expressed in mg of KOH/g of oil while the degree of acidity (Da) was expressed as percentage of oleic acid according to the following formulas:

$$Ia = \frac{(N \times V)}{m} \times 56.1 \quad ; \quad Da = \frac{(N \times V)}{(10 \times m)} \times 282.4$$

N: Normality of the KOH solution; V: Volume of KOH solution ; m: mass of the oil ; 56.1: Molar mass of the KOH ; 282.4: molar mass of oleic acid.

- ***Iodine value (IV)***

In a Erlenmayer flask, was weighed accurately an appropriate quantity (0.3 g) of the dry seed oil. 25 mL of chloroform have been added and agitated for proper mixing. To this solution was added 25 mL Wijs reagent and incubated at room temperature in dark for 1 hour. At the end of reaction, to the flask was added 15 mL of saturated KI solution followed by 100 mL water. Liberated iodine was titrated with standardised sodium thiosulphate solution (0.1 N) using starch as indicator until the blue colour formed disappears after through shaking. A blank test with 2 mL chloroform was performed under the same conditions (Pardeshi, 2020).

The iodine value was expressed in grams of iodine absorbed by 100 grams of oil ($gI_2/100$ g) and was determined as follows.

$$IV = \frac{(Vo - V)}{m} \times 1{,}27$$

Vo: Volume of sodium thiosulphate solution for blank (mL) ; V: Volume of sodium thiosulphate solution for sample (mL) ; m: mass of test portion (g).

- ***Saponification value (SV) (Haïdara 1996)***

In a Erlenmeyer flask, a sample of oil (1-2 g) and 25 mL of 0.5 N potash solution were added. The mixture was boiled under reflux for 1 hour. Then the titration of the excess potash in the solution with hydrochloric acid 0.5 N was carried out, with 4 to 5 drops of a solution of phenolphthalein 1% as a indicator, until the disappearance of the red or pink color. A blank, without the oil, was also treated in the same way as before.

The saponification value was expressed as mg of KOH/g of oil according to the following formula:

$$SV = \frac{(Vo - V) \times N \times 56.1}{m} \times 1000$$

Vo: Volume of KOH for blank ; V: Volume of KOH for the test ; N: Normality of HCl solution; m: mass of the oil ; 56.1: Molar mass of KOH ; 1000: conversion factor from g to mg.

- ***Statistical analysis***

All test was carried out in triplicate and a normal analysis of variance using the Fisher test was performed with the statview software version 4.5. The difference between two values of the same parameter is considered significant if $p < 0.05$.

Results

- ***Extraction, density and refractive index***

One hundred and sixty-five (165) g of Moringa seed powder were extracted with Soxhlet in several steps. Thus 59.58 g of oil were obtained corresponding to a yield of 35.97±3.30 %. For extraction by expression, 1.854 kg oils were obtained from 14 kg of seeds, with a yield of 13.10 ± 2.64 %. However, the oils obtained by these methods had similar densities and refractive indices as shown in next table 1.

Table 1. Extraction yields (mean ± SD), density and refraction index of Moringa seed oil.

Extraction Methods	Yield ± SD (%)	Density	Refractive index
Soxhlet	35.97 ± 3.30 $_a$	0.904	1.465
Hot expression	13.10 ± 2.64 $_a$	0.907	1.467

SD : Standard Deviation a : $p < 0.05$

- ***Chemical values***

The Peroxide value of the oil obtained by organic extraction was significantly higher than that obtained by hot expression (respective values : 55.33±1.26 and 2.19±0.56 mEq of active oxygen/kg of oil ; $p < 0.0001$).

However, their iodine value were statistically similar (respectively 37.60±0.64 and 37.93±0.68 $gI_2/100$ g ; $p > 0.05$). Furthermore, the acid values obtained showed that the oil extracted by Soxhlet was richer in free fatty acids than that produced by expression with 3.94±0.19 and 2.85±0.15 mg KOH/g respectively ($p = 0.0015$).

Thus, these oils have a different acidity degrees corresponding to 1.98±0.09 % (Soxhlet) vs 1.44±0.08 % (expression) of oleic acid. Oil saponification values were also statistically similar with 199±1.11 mg KOH/g oil (Soxhlet) versus 200.39±4.34 mg KOH/g. (Expression) ($p > 0.05$). Next Figure 1 illustrates the chemical parameters of Moringa seed oil according to the extraction method.

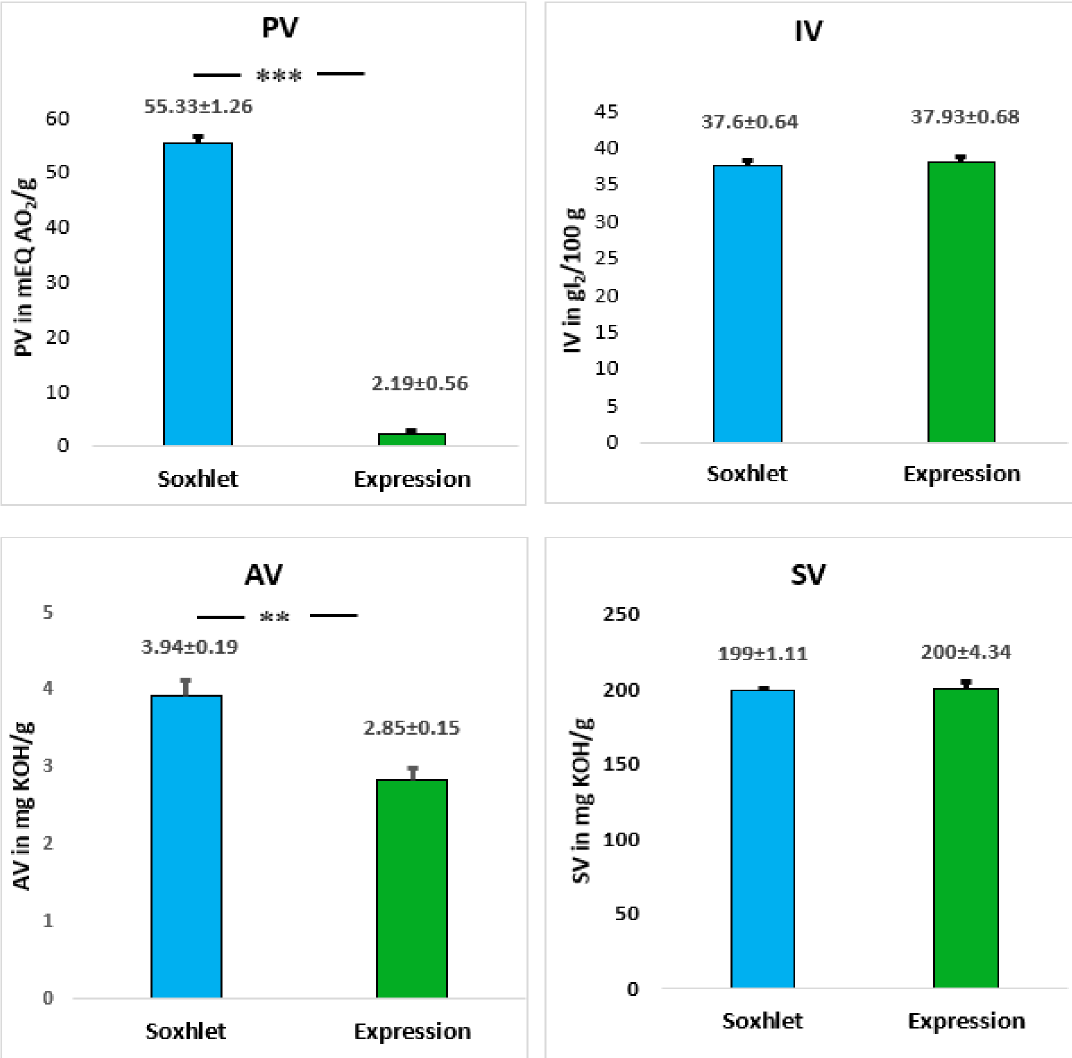

Figure 1 : Chemical parameters of *Moringa oleifera* seed oils
PV: peroxyde value; **IV**: Iodine value; **AV**: Acid value; **SV**: Saponification value;
**: $p < 0.01$; ***: $p < 0.001$; $n = 3$.

Discussion

The seed oil of Moringa was obtained by two types of extraction: by an organic solvent and hot expression. The organic extraction was done with n-hexane. The choice of this organic non-polar solvent was justified by its ability to solubilize fats such as oils. It is commonly used cause of its ability to extract oil but also its low boiling point allowing to work at low temperatures (Agroconsult, 2016).

Comparing the two types of extraction, the yield of the oil obtained by organic extraction was higher than that by expression (35.97±3.30 % vs 13.10±2.64 %). Despite the heating, the expression recorded the lowest efficiency. This could be due, in part, to the fact that much of the oil remains in the cakes while n-hexane is known for its ability to deplete the seeds of their oil. This corroborates with several studies with yields between 25.6 % and 41.5 % (Leone et al., 2016; Eman et Muhamad, 2016 ; Zongo et al., 2016). These high values were consistent with the typical profile of seeds oleaginous (Magalhães et al., 2021).

However, the density of *Moringa oleifera* oil varies very slightly with the extraction method (0.904 and 0.907). It is very close to that of sunflower oil (with a high oleic acid content) which varies from 0.909- 0.915 according to the WHO Codex Alimentarius (2017). The refractive index tells us about the purity and group of the oil. Thus, the oil could be classified as non-siccative with a refractive index that varies between 1.465 and 1.467 depending on the extraction method. The values are close to those of olive oil and sunflower oil with refractive indexes of 1.467 and 1.471

respectively. The peroxide value is used to assess the degree of oxidation of the unsaturated fatty acids of oils. It can be related to the storage conditions but also to the extraction modes and makes it possible to detect the first steps of oxidation of the oils (Judde, 2004). Thus, the peroxide value of the oil obtained by expression (2.19±0.56 mEq O_2/kg) was in the standards of most conventional oils according to the Codex Alimentarius (<10 mEqO_2/Kg). Moreover, this result corroborates that of Louni (2009), who had found a peroxide index of 2.92 mEq O_2/Kg of Moringa oil obtained by the press. This could be explained by the richness of *M. oleifera* oil in tocopherols, which are natural antioxidant substances (Genot et al., 2004 ; Louni, 2009). However, the peroxide value of the oil obtained by Soxhlet was much higher (55.33±1.26 mEq O2/Kg of oil). Thus, n-hexane or the long contact time with heat during extraction would influence the oxidation of fatty acids of oils obtained by Soxhlet.

The iodine value highlights the degree of unsaturation of the oil. The higher it is, more the oil contains unsaturated fatty acids. The iodine values (37.60±0.64 and 37.93±0.68 g I_2/100 g of oil respectively for those obtained by Soxhlet and pressing), allow it to be classified among non-siccative oils (98.78 to 109.48 g I_2/100 g of oil) according to Aranha and Jorge (2013). This confirms the results obtained previously with the refractive index. In this group non-siccative oils belong in particular olive, peanut and castor oils.

However, these IV values found are much lower than those found by several authors with values between 62 – 70 g iodine/100 g *M. oleifera* oil (Louni, 2009 ; Gué et al., 2017). This variation in the iodine index may be related to several factors such as the variety of the plant, the climate, the harvest period, and the degree of maturity of the seed. Thus, this oil could be more stable because less rich in unsaturated fatty acids.

As for the acid value, it defines the quality of the oil. It characterizes the purity and stability of oils at room temperature. *Moringa oleifera* oil extracted by Soxhlet gives a slightly higher value (3.94±0.19 mg/g oil) than that obtained by hot pressing (2.85±0.15 mg KOH/g oil). This suggests that the solvent extraction method has a negative influence on the quality of the oil, probably due to heating and chemical interactions between the oil and solvent. Non-polar solvents such as hexane, give an oil with a relatively high acid number (Louni, 2009).

According to the standards of the Codex Alimentarius CXS 210-1999, oil obtained by cold pressing must have an acid value ≤ 4.0 mg KOH/g of oil. By comparing our results to this standard, we see that the samples analyzed are compliant. *Moringa oleifera* oil therefore has good stability.

Acidity measures the amount of free fatty acids present in a fat. We obtained values of 1.98±0.09 % for soxhlet and 1.44± 0.08 % oleic acid for hot press. This acidity is less than the maximum value recommended (3%) for an oil of consumption (Novidzro et al., 2019).

The saponification values tells us about the length of the fatty acids in the oil. Thus the saponification indices obtained were 199.79±1.11 and 200.39±4.34 mg of KOH/g of oil for Soxhlet and the press. This suggests that *Moringa oleifera* oil is easily saponifiable because according to Andrianirina (2009), more the saponification index is higher, the saponification reaction or the fatty substance is more easily saponified. Comparing it to palm oil (193-206 mg KOH/g), thus they will be able to have approximately the same foaming power (Akinola et al., 2010 ; Ali et Tay, 2013).

Conclusion

Moringa oleifera oil obtained by organic extraction is more acidic and more sensitive to oxidation against that obtained by hot press. Thus, organic solvents would negatively impact the quality of the oil.

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