Comparison of chemical and physical cold extraction methods of *Moringa oleifera* oil (Moringaceae)

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ABSTRACT

The aim of this study is to establish the best method of cold extraction of Moringa oleifera seeds from Madagascar by comparing chemical and physical method from two types of seeds, unroasted and roasted. This will allows optimizing operating conditions by favouring operation that consumes least energy, economy and time with good efficiency and superior quality. First step is to find out quantitative yield of extracting seed oil using hexane maceration and cold press from unroasted and roasted seeds. Then, organoleptic characteristics description and fatty acid profile analysis of oil by gas chromatography establish oil quality. Comparison of results shows that chemical method with unroasted beans gave highest extraction rate. Solvent promotes lipids transfer to the exterior part of seeds cells. In terms of quality, oil extracted by physical method is comparable to olive oil. Comparing with literature, extraction yields are quite low especially for roasted bean press. This can come mainly from the difference of seeds quality and operating conditions. Thus, for roasted seeds, this low rate may be due to seeds consistency which changes after roasting. On the other hand, quality of oil extracted by press is similar to oils study in literature. Optimization of operating conditions is considered as a perspective of this study.

Keywords: *Extraction, Moringa oleifera, press, hexane, unroasted and roasted seeds*

1. Introduction

Actually, "natural" and "organic" have become the big fashion in terms of nutrition [1]. Madagascar is a country rich in plant biodiversity. Among miraculous plants whose exceptional virtues have already been scientifically proven, tree scientifically named *Moringa oleifera* known by the vernacular name of ananambo remains little consumed and poorly exploited in Madagascar [2]. This plant is native to India but widespread throughout the world, especially in tropical and subtropical countries. It is a drought tolerant tree, practically easy to propagate and fast growing. *Moringa oleifera* is a tree with multiple medicinal, nutritional and industrial virtues [3]. In addition to its many qualities, moringa seeds can purify water effectively because they contain a broad spectrum flocculent for impurities. They can also be used to clarify and purify wastewater as well as water intended for human consumption. Moringa seeds contain 30% to 42% oil and the cake obtained as a by-product during exaction is very rich in protein. Some of these proteins are active cationic polyelectrolytes which are involved in the purification of drinking water, filtration of vegetable oil or sedimentation of fibers in the production of beer and fruit juice [4]. *Moringa oleifera* oil is known worldwide as Ben oil because of its behenic acid content which is not very common in the vegetable oil. Its yield is in the order of 40%. This oil contains all of the major fatty acids found in olive oil and can therefore be used as a substitute for this latter. Moringa seed oil contains about 13% saturated fatty acids and 82% unsaturated fatty acids. It is particularly rich in about 70% oleic acid [5]. Moringa vegetable oil can be used as edible oil and cooking oil [3]. It is a very good edible oil and popular as a seasoning oil in salads in many countries. It is also used in fried foods, due to its high oxidative stability and in the manufacture of margarines either in the fluid state or after

hardening by hydrogenation [5]. Also, it is classified as quality oil in the cosmetics and perfume industry. It is used to clean imperfections on skin; it gives a new radiance to mature skin. Thus, it strengthens and softens hair; it hydrates dry hair with a tendency to dandruff and stimulates hair growth. It can also be used in certain cases of diabetes to stabilize the level of sugars and can stabilize blood pressure [3].

The general objective of this study will therefore be to produce superior quality of *Moringa oleifera* oil in a profitable, efficient and citizen organization in Madagascar by comparing a physical and chemical method from unroasted seeds and roasted seeds.

1.1. Extraction

In chemistry, extraction is an operation which consists in removing from a raw material a set of products that constitute it [6]. In chemical engineering, it is a unitary operation, a process which allows selective separation of one or more compounds from a mixture on their physical and/or chemical properties basis [7]. Chemical method uses an extraction medium, which is solvent, little or immiscible with the main constituent elements of the mixture, i.e. the compound to be extracted or solute. This latter must have a higher affinity with extraction medium than with main constituents of mixture [8]. Physical method consists to extract solutes using physical factors, pressure and heat.

1.1.1. Chemical solvent method

Solvent extraction is the most widely used method for obtaining oils from oleaginous materials [9]. This method is based on the solubilization method. It consists in transferring solute in solvent. Solvent can be water, but often it is an organic solvent obtained from petroleum chemistry such as cyclohexane, toluene, petroleum ether, etc. [7]. Different methods exist but the best known are infusion, decoction and maceration [8]. This study is interested in cold solubilization which is maceration. It is cold contact between seed powders and solvent in order to extract oil.

Solvent choice is very important in food or pharmaceutical industry. Standards and rules of hygiene and safety are very strict. It should not remain in final products or traces should be insignificant enough to be harmless. Low viscosity, low density solvent facilitates diffusion in solvent, agitation and mechanical separation [1]. For the extraction to be as fast and complete as possible, the solvent must have large exchange surfaces and short diffusion paths. This can be obtained by grinding the solid material containing the solutes to be extracted. A too small particle size can however cause the formation of lumps and make the passage of the solvent more difficult [7]. For oil extraction, it is often preferable to use hexane because of its properties. It is oil selective, highly volatile, chemically inert and stable at high temperatures [9]. Thus, the evaporation point of hexane being lower than that of the fats to be extracted, it is therefore very easy to separate them by heating [10].

The seed oil content is generally around 40% [10]. An earlier study carried out in Madagascar using unroasted seeds from the southern part of Madagascar gave a yield of around 28% by theoretical single-stage maceration [2].

1.1.2. Physical extraction by press

There are different ways of extracting oil by physical method such as ultrasonic extraction, which uses ultrasonic waves and press extraction which remains the most widely used industrially. It is mainly used for extracting oil from oil seeds [10]. In principle, pressure extraction from a product composed of solids and liquids separates liquids by applying external pressure to them. The product is supported by a wall or a canvas allowing the passage from the solid state to the liquid state. It is therefore a matter of releasing a liquid of a porous mass under the effect of a decrease in the volume of the whole. The basic mechanisms are the deformation of a complex medium (solid-liquid mixture, most often cellular), the flow of liquid through this porous medium and the porous wall. The optimal extraction conditions depend on the press used, the extraction method, the grain size and the duration of the pressing [9].

According to the literature based on the method of Tsaknis et al. [11,12,13], yields of *Moringa oleifera* oil by pressing are of the order of 25.8 ± 0.6.

1.2. Organoleptic characteristics

According to the literature, Moringa oleifera oil is fluid, clear, smooth, yellow in color and with a characteristic odor. Before decanting, the oil obtained by maceration is quite amber, while after filtration, the oil will become clear. Probably, the solvent extracted oil has a clearer appearance than that obtained by cold pressing, because with the solvent there are less entrained particles [9].

1.3. Fatty acid profile

The fatty acid profile is determined by gas chromatography (GC). Analysis by direct introduction of fatty acids into the column is possible, but generally these substances have quite high melting points, while their methyl esters are much more volatile. It is therefore preferable to carry out a prior methylation to be able to work at a lower temperature and to use polar phases which would withstand a lower temperature [14].

According to the literature (Table 1), *Moringa oleifera* seed oil is of the oleic type with a high oleic acid content of around 72%. It is therefore similar to olive oil [15]. The total amount of unsaturated fatty acids in olive oil is around 15.3% and for saturated fatty acids it is around 83.8% [16]. This characteristic is particularly desirable due to the high level of monounsaturated fatty acids. The latter are very stable even in uses such as fried foods [17].

Table 1: Composition of fatty acids

	Moringa oil		Olive oil [15]
Fatty acid	Solvent (hexane) [17]	Press [13]	-
Palmitic acid	6.45	6.36	8.53 à 14.49
Stearic acid	5.5	5.74	1.3 à 3.3
Oleic acid	**73.22**	**71.22**	**64.5 à 80.3**
Linoleic acid	1.27	0.66	3.6 à 16.8
Linolenic acid	0.3	0.17	0.39 à 0.98
gadoleic acid	1.68	2.25	-
Behenic acid	6.16	6.25	0.07 à 0.16
Palmitoleic acid	0.97	1.49	0.26 à 1.76

2. Materials and methods

2.1. Preparation of plant material

Pretreatments include all of the unit operations that prepare the raw material for crushing. For oilseeds, these are mainly: collecting seeds, green and the cracks are not yet in the state of dehiscence. They are stored away from light and humidity at 4 ° cleaning, drying, shelling, crushing. This step may vary depending on the type of seed. For this study, the picking was done in the region of Boeny in the northwest of Madagascar in February and May 2021. The removal of pods, shelling and crushing are carried out manually. The shelled seeds required the drying stage because the fruits collected are not yet fully ripe that's to say the pods are still a little green and the splits are not yet in the state of dehiscence. They are stored away from light and humidity at 4°C, to avoid any oxidation or degradation of the lipids.

2.2. Extraction

1st method: Chemical method (hexane maceration)

This method involves cold letting 200 g of *Moringa oleifera* powder stand in 500 mL hexane to extract the oil, allowing it to macerate for seven days at room temperature. After this period, the solvent becomes charged with oil and meets the almost de-oiled powder. Filtration is then carried out using a nylon fabric. The filtered hexane extract is freed from solvent using a rotary evaporator at 40 ° C to obtain the crude extract. The extraction rate *R (%)* is given by the ratio of the mass of the crude extract m_h (g) and the initial mass of the seed powder m_0 (g) (equation 1). The rate of extraction R of the oil is given by the relation of equation 1.

$$\boldsymbol{R\ (\%) = \frac{m_h}{m_0}}$$

Equation 1

2nd method: Physical method by pressure

This extraction method is used especially for products that contain more than 10% fat, as 7-8% oil remains in the cake. This method uses a CARVER brand manual press in the FOFIFA Ambatobe physicochemical analysis laboratory (Figure 1).

Figure 1: Manual press

200 g of powder from the seeds is placed in a medium mesh fabric cartridge so as not to let the particles of the plant escape. The latter is introduced into the iron cylinder. The assembly is then manually pressed using a jack with a movement from bottom to top under a weight of 20 tons for 15 minutes, the time when the oil is no longer extracted. The oil flows along the scourtins, and comes together in the recovery tray. This principle is carried out for both types of seeds (unroasted and roasted).

The oil content in% is determined by the relation of the weight of the oil extracted by the weight of the test sample (equation 1).

2.3. Analysis by Gas Chromatography (GC)

GC analyzes of the oils were carried out in two different laboratories. The analysis of oils extracted by solvent is carried out in the quality control laboratory of the phytochemistry and quality control department of the Malagasy Applied Research Institute in Madagascar. A CG PE Clarus 580 fitted with an automatic injector was used with an injected volume of 0.5 µL. The type of column used is ELITE-WAX (30 m x 0.32 mm x 0.25 µm) with a head rate of 0.6 bar. The analysis is carried out at 200 ° C with hydrogen as carrier gas. The surfaces (% relative) are calculated on a medium polar column.

For oils produced in the press, the analysis is carried out at the Madagascar pesticide control laboratory.

The chromatograph is equipped with a mega-column: SOLGEL-WAX (30 m x 0.53 mm x 1 µm). The oven temperature is set at 190 ° C, while that of the injector is 260 ° C and the FID detector is 280 ° C. The volume injected is 1 µL. The carrier gas is U nitrogen with a flow rate: 3 $mL.min^{-1}$. The calculation of the contents is made by percentage of areas.

3. Results and discussions

3.1. Extraction efficiency

The extraction rate *R* (%) is calculated by the ratio of the masses of the oils obtained (g) and of the powder (g) of seeds. The extraction results for each method are collated in Table 2.

Table 2: Yield of extractions

	Maceration		Press	
	Unroasted seeds	Roasted seeds	Unroasted seeds	Roasted seeds
Mass of the powder (g)	200	200	200	200
Mass of oil (g)	50	42	38	11
Oil volume (mL)	58	57	39.5	12.5
Yield (%)	25	20	19	5.5

The oil yields per press are quite low compared to the literature which is around 25 %. The differences can come mainly from the locality, the nature and properties of the seeds and the mode of conditioning of the raw materials. But also, climatic conditions and the acid-base character of the soil. In contrast, for hexane extraction, the results approach the national benchmark of around 28 %.

These results show that the oil contents extracted by solvent are the highest (20 and 25 %). The solvent's ability to dissolve lipids promotes transfer out of cells. But also, the solvent can take out all the soluble solutes. By diffusion, hexane takes on oil and can extract almost all of the oil from the seeds. In contrast, press extraction records a much lower yield (19 and 5.5 %) than that found in the literature. This can be explained by the type of press, its performance and the operating conditions.

Press extraction has the lowest extraction rate. This means that some of the oil is trapped in the meal. It was therefore necessary to re-treat the cake obtained after extraction by cold press for a second extraction by solvent in order to extract any extractable oil. For roasted bean pressure, this 5.5% extraction rate shows that much of the oil remains absorbed by the roasted beans. In fact, compared to the maceration of the roasted seeds, for 200 g of powder, a mass of about 31 g of oil is absorbed by the cake. The cake therefore still contains a fairly large amount of fat. This can be explained by the consistency of the seeds. Indeed, the texture of the seeds changes after roasting.

3.2. Organoleptic characteristics of crude oils

The description of the organoleptic characteristics of the oils obtained by the two extraction methods are presented in Table 3.

Table 3: Organoleptic characteristics of crude oils

		Aspects	Color	Odeur
Maceration	Unroasted seeds	Bit frozen, less fluid, Clear, low viscosity	Yellow (light)	characteristic
	Roasted seeds	Bit frozen, Clear, low viscosity	Yellow (quite amber)	Characteristic
Press	Unroasted seeds	Fluid, Clear, low viscosity	Yellow (light)	Characteristic
	Roasted seeds	Fluid, Clear, very viscous	Yellow (quite amber)	characteristic

Usually, the oils produced by both methods exhibit the characteristic identities of *Moringa oleifera*. They are fluid and clear with a moringa odor comparable to that of peanut oil. The difference is in color and appearance. The oils obtained from the unroasted seeds are clear. On the other hand, those obtained by roasted seeds are quite amber. But after settling, the latter become clearer. Thus, the solvent extracted oil is frozen at room temperature. Roasting changes part of the nature of the seeds. They turned brown when roasting.

In terms of quality, the oils extracted with the press present the best aspects and appearances compared to those obtained by maceration. They are more fluid, while oils produced by solvents have gelled properties. Indeed, these differences are often due to the presence of the solvent.

3.3. Fatty acid profile of crude oils

The fatty acid composition of the oils produced, determined by gas chromatography after esterification, is given in Table 4.

Table 4: Fatty acid profile of crude oils

	Solvent (hexane)				Press			
	Unroasted seeds	Roasted seeds	**IMRA Madagascar**	**[18]**	Unroasted seeds	Roasted seeds	**[13]**	**LCP Madagascar**
Palmitic acid	**24.7**	**23.4**	5.5 à 5.8	6.45	6.15	6.09	6.36	4.44 à 8.68
Stearic acid	0.6	1.1	4.1 à 4.9	5.5	5.05	5.40	5.74	0.76 à 6.06
Oleic acid	**32.9**	**45.8**	77.3 à 78.8	73.22	75.15	74.17	71.22	72.19 à 86.32
Linoleic acid	**13.1**	**11.2**	0.6 à 0.7	1.27	0.92	1.34	0.66	0.32 à 2.79
Linolenic acid	1.5	0.7	0.1 à 0.2	0.3	0.16	0.13	0.17	-
Arachidic acid	0.1	0.2	2.2 à 3.0	1.68	3.3	3.53	2.25	1.02 à 4.88
Gadoleic acid	0.1	0.2	2.2 à 2.5	6.16	-	-	6.25	-
Behenic acid	0.2	0.1	2.0 à 4.1	0.97	5.51	5.94	1.49	1.41 à 7.91
Gondoic acid	-	-	-	-	1.90	2.05	-	0.95 à 3.27
Palmitoleic acid	-	-	-	-	1.15	1.00	-	0.00 à 2.05
Total	73.2	82.7		**95.55**	99,29	99.65	**94.14**	

There is a difference in the fatty acid composition contents of the oils extracted by chemical and physical methods. This may come from the batches because the picking seasons have been shifted by several weeks. The results for oils obtained by pressing are in agreement with the fatty acid composition of previous studies [13]. While oils produced by solvent are very different from references [18]. Probably, the virgin oil obtained in the press does not undergo any chemical reaction, as well as it does not contain any chemical substance which could vary the content of its chemical constituents. For the two extraction methods studied, moringa oil is a very good source of unsaturated fatty acids with a highest content of around 77 %, of which oleic acid is the majority.
This oil is thus similar to olive oil and is classified in the category of oleic oils.
For maceration and pressing, the highest total amounts of saturated fatty acids found in our produced moringa oils are 25.6 % and 20.01 %, respectively. They are higher than that found in olive oil, which is around 15.3 %. The highest total amounts of unsaturated fatty acids found are 57.9 % for the hexane extraction and 77.38 % for the press, respectively. They are lower than that found in olive oil which is around 83.8 %.

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4. Conclusions

This work constitutes a contribution to a partial knowledge of *Moringa oleifera* seed oil in order to promote its valorization.

The extraction of the oil by methods of cold chemical extraction by solvent and physical by pressure, as well as the use of two types of dry seeds, unroasted and roasted, made it possible to determine the highest extraction rate. The highest oil yields are obtained by chemical method. Extraction by hexane maceration with the unroasted seeds has the highest yield, at 25 %. These results show the importance of the choice of the type of seeds and the method for the extraction of *Moringa oleifera* oils. Indeed, the extraction yield varies according to the extraction method and the nature of the seeds.

In terms of quality, the oils extracted by pressing have the best organoleptic characteristics compared to those obtained by maceration. They verify the sensory characteristics of *Moringa oleifera* oil, which are fluid, clear, and yellow in color and have a characteristic odor. Solvent-extracted oils change due to the presence of other solvent-extracted molecules.

The analysis of the fatty acid profile by GPC highlights on the one hand the richness of the oil in monounsaturated fatty acids, of which oleic acid is the majority. Thus, among the saturated fatty acids, palmitic acid is predominant. On the other hand, polyunsaturated fatty acids are present in trace amounts. The fatty acid composition of the oils obtained in the press is similar to references [13]. On the other hand, those which are extracted by solvent present a difference compared to the references [18]. Thus, it is the oils obtained from the press that have a composition of stearic and oleic acid almost similar to olive oil, which gives *Moringa oleifera* oil interesting nutritional properties, particularly in terms of favorable action exercised by monounsaturated fatty acids on the evacuation of cholesterol.

In short, there are several ways to extract oil from Moringa oleifera seeds, but the best is still the cold-pressed method. This way of obtaining Moringa oil promises to retain maximum nutrients and better quality in taste and appearance. But also, it is preferable to work with unroasted seeds to have a good yield and maintain the quality of the oil. Thus, the operation is less profitable for solvent extraction because it requires high consumption of time and money. Otherwise, the chemical method gives the highest extraction rate.

In perspective, it would be desirable to optimize the cold extraction method, for example the use of a more powerful hydraulic press. It is therefore necessary to study other cold methods such as the use of another solvent for the chemical method and the use of another type of press for the physical method.

5. References

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