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Physico-chemical and biochemical characterization of Moringa oleifera seeds from seven areas of Benin

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Abstract

The present study aims to valorize Benin *Moringa oleifera* seeds through the characterization of seeds and oils extracted. Thus, the seeds of seven areas (Sakete, Porto-Novo, Abomey-Calavi, Koutchime, Bassila, Cotonou, Dassa-zoume) of Benin were collected. They were characterized through the evaluation of their color, dimension, dry matter, fats, proteins and ash contents. The quality of the extracted oils from these seeds was then estimated by the determination of their color, acidity, index of saponification and refraction. The results indicated that the seeds have a dimension ranging between 0.27 and 0.34 cm, with a collapsing resistance from 32.16 to 60.23 N and of the colors different from a zone to another. The proximate characteristics of seeds vary respectively on average from 90.67 to 92.80% for the dry matter content; from 37.34 to 44.77 % for the lipid content; from 38.47 to 41.04 % for the protein; from 7.49 to 13.02 for totals sugar and from 3.49 to 3.80% for the ash content. Acidity, the index of saponification and refraction determined on the extracted oils from these seeds vary on average from 3.34 to 5.3, 149.78 to 158.43 of oil and 1.477 to 1.465 respectively. The composition of the fatty acids shows a strong concentration of the mono-unsaturated fatty acids (72.9-78.8%).

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Introduction

In the world and particularly in Africa, the plant occupies a place of choice in survival human being. The majority of the plants find their reason of living, by the fact that they are used as food or in medicine. Therefore, the African flora gets a diversity of plant among which all the parts (leaves, bark, roots, and seeds) show multiple applications. *Moringa oleifera* belongs to these plants which are increasingly known for their importance in the food safety in the rural and urban zones of Africa.

Moringa oleifera is a specy of small tree which can measure up to 10 meters height and is now acclimatized in almost all the tropical areas (Rupjyoti *et al*., 2003). It tolerates a wide range of rainfall with minimum annual rainfall requirements estimated at 250 mm and maximum at over 3000 mm and a pH of 5.0–9.0.This plant is commonly used in popular medicine and food in the african and asian companies. The flowers, the leaves and the roots are used for the treatment of rheumatism and poisonous bites and as cardiac and circulatory stimulants (Anwar and Bhanger, 2003; Anwar *et al*., 2006). Flowers, fruits, leaves, and none mature pods of this plant are edible and form an integral part of the traditional dish in much of tropical and subtropical countries (Siddhuraju and Becker, 2003, Siddhuraju and Becker, 2004; Anwar *et al*., 2005).

The leaves, easy to produce and very rich in proteins, vitamins and minerals, are used and more in projects against malnutrition. However, the majority of the parents do not manage to fill the nutritional insufficiency of the children for a lack of means and the bad control of the use of the natural resources available, which perpetuate malnutrition (Madi *et al*., 2012). The children are generally victims of the diseases due to the deficiencies in nutritive elements such as the vitamin deficiency, the kwashiorchor, stagnation, in short of any kind of diseases related to the malnutrition whose ultimate consequence is death. Ninety five percent (95%) of surveyed of the town of Maroua in North-Cameroun know *Moringa* and 93.4% of the latter use leaves (Madi *et al*., 2012).

In Benin, the request for fresh or dry leaves for the culinary uses grows quickly as information on the nutritional virtues is known, in particular in the North of the country. But the seeds of this plant are less used as food nowadays, in contrast to its leaves which are commonly consumed in all West Africa in vegetable or powder forms of *Moringa* in order to raise the nutritional value of food. The only known utility of these seeds by the Beninese populations is their multiplication. However, in Zimbabwe, the seeds are consumed in various forms: fresh, cooked and fried like peas. They are also used for the extraction of the oil of cooking of food and for various seasonings (Madi *et al*., 2012). *Moringa* fruit has been found to lower the serum cholesterol, phospholipids, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol to phospholipid ratio, atherogenic index lipid and reduced the lipid profile of liver, heart and aorta in hypercholesteremic rabbits and increased the excretion of fecal cholesterol (Mehta *et al*., 2003). Industry uses also the oil of *Moringa* in the manufacture of the cosmetic products. These seeds are useful especially in developing country with the purification of water (Anwar*et al*., 2006; Mougli *et al*., 2005; Kalogo *et al*., 2000).

Actually, many people have a stake on the use of these seeds in spite of the multiple virtues which they contain. Also, very few scientific work related to the physicochemical characteristics of *Moringa* seeds oil was investigated, the present work aims to characterize seeds of *Moringa oleifera* from seven zones of Benin as well as the oil extracted.

Material and methods

Vegetable material

The seed samples of *Moringa* are gathered directly on the trees. These samples are collected on three sites of production in each zone of study targeted with knowing Calavi, Porto-Novo and Sakete. These zones are selected because of their scale of production raised in Benin.

Physical and mechanical analyses of seeds

Determination of dimensions

The seeds of the various areas were seriated in three groups of 10 seeds according to their sizes (gross seeds, average seeds, and small seeds). The diameter of each seed is measured using a slide caliper of precision 0.01 mm and the average of the diameters of these ten seeds is regarded as the size of this group of seed.

Measure of collapsing resistance

The measurement of the collapsing resistance or breaking load was carried out using dynamometer AMANDUS KHAL of precision 0.001 N on a batch of 50 seeds chosen randomly. Each seed is individually pinch between the two plates of the apparatus, in the direction thickness until it's crushing. The force applied to crush determines the collapsing resistance. The speed of the compression of almond constant and is fixed at 5 mm/mn (Ahmadi *et al*., 2009a; Ahmadi *et al*., 2009b).

Determination the seeds color

The seed color powders of size 600 µm is measured in space L*, a*, b* (CIELAB) using a chromameter (Minolta CR 200 b) calibrated before hand with a white ceramics whose co-ordinates of color are: X = 0.336; Y = 54.5 and Z = 0.343.

The parameters of color which are measured are: brightness or clearness or whiteness (L*) on a scale from 0 to 100 going of the black to the white; saturation in red (a*) which characterizes the intensity of red in the positive values and the green in the negative values; saturation in yellow (b*) which characterizes the intensity of yellow in the positive values and blue in the negative values and the variation of color compared to the white ceramics of reference (ΔE).

Chemical and biochemical analyses of seeds

Determination of the water content and matter volatile (Te) of seeds

The water content and volatile matters were given according to French standard NF V 03-921 of the "Association Française de Normalisation (AFNOR)". It corresponds to the loss of mass undergone by the sample after heating in a drying oven with 103 ± 2°C until constant weight.

Determination of the oil content (T_L)

The seeds of *Moringa oleifera* were crushed and then introduced into an extractor of Soxhelt. After extraction, the solvent was distilled with far vacuum in a rotary evaporator (EYELA, N.N. Series, Co Ltd Tokyo, Japan de Rikakikai).

Totals sugar content

Total sugars were determined according to phenol sulfuric acid method. A standard curve was obtained using the following concentration of sucrose in (mg/ml) 2.5 2.0, 1.25, 1.0, 0.5 g of each sample with 9 ml of distillated water was measured into test-tube. 2 ml of phenol solution (1%) and 1 ml of concentrated H_2SO_4 solution were added. This was shaken for 15 min and boiled for 30 min. It was then allowed to cool. The absorbance was then read on a spectrophotometer (spectrum lab 22) at 700 nm. The sugar concentration was then obtained by extrapolation from the standard curve.

Seed cake analysis

Protein was analyzed by the Microkjedhal nitrogen method, using a conversion factor of 6.25 and fat content was obtained by Soxhlet extraction described as follow: two grams of samples were collected and carbonized. The carbonized material was then incinerated in an electric muffle furnace (EYELA, TMF-2100, Tokyo, Japon) at 550 °C until the constant mass was carried out.

Physicochemical analysis of oil

Index of refraction, saponification index and the acidity of oil were given according to respectively standard methods AOCS standards Cc 7–25, F 9a–44 and Cd 3–25 (Anonymous, 1990). The color of the oil samples was determined as described above.

Fatty Acid Composition

Fatty acid methyl esters were prepared according to standard IUPAC method 2.301 and analyzed on a

Perkin-Elmer gas chromatograph model 8700 fitted with a methyl lignoserate coated (film thickness) 0.22 ím), polar capillary column SP-2340 (60 m, 0.25 mm), and a flame ionization detector. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. Other conditions were as follows: initial oven temperature, 130 °C; ramp rate, 5 °C/min; final temperature, 220 °C; injector temperature, 260°C; detector temperature, 270 °C. The internal standard used was nonadecanoic acid. Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of authentic standards of fatty acid methyl esters. All of the quantification was done by a built-in data-handling program, provided by the manufacturer (Perkin-Elmer) of the gas chromatograph.

Statistical analyses

The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA).

Results and discussion

Tables 1 and 2 respectively showed the physical and biochemical characteristics of *M. Oleifera* seeds collected from seven different areas of Benin. According to results, the size of seeds varies from 0.27 cm to 0.34 cm (Table 1). Indeed, seeds collected from Porto-Novo and Dassa-zoumé showed practically the same sizes and those from Koutchimé and Calavi were in the same range. In the same order, seeds from Cotonou and Bassila have also the same sizes and finally those of Sakété were the smallest. Values obtained were lower than those reported by Lina *et al.* (2010) who obtained 1.037cm.

Table 1. Physical and mechanical analysis on seeds of *M. Oleifera*

Parameters		Calavi	Porto-Novo	Sakete	Bassila	Koutchime	Cotonou	Dassa-zoume
Sizes		0.31±0.60ab	0.33 ±0.06cd	0.27 ±0.02^{e}	0.30±0.03a	0.32±0.30bc	0.30±0.25a	0.34±0.07d
Collapsingresistance		32.16±12.48a	46.46±7.16b	60.23±12.89c	50.46±11.34d	46.57±8.14e	46.52±5.33f	46.73±3.42g
Color	L*	63.93 ± 0.91d	63.71 ± 0.26c	56.91 ± 1.72a	60.73±0.24b	63.73±0.51c	64.04±0.42e	64.31±0.26f
	a*	1.93 ± 0.05a	2.35 ± 0.34b	5.78 ± 0.28^{e}	5.13±0.03d	2.38±0.23bc	2.36±0.21bc	2.38±0.05c
	b*	18.06 ± 0.33c	17.80 ± 0.28b	18.68 ± 0.40g	18.43±0.34^{e}	18.14±0.12d	17.78±0.18a	18.52±0.11f

In addition, the collapsing resistance of seeds significantly varies from an area to another. Its values are between 32.16 and 60.23N and the hardest seeds were those from Sakete with the collapsing resistance of 60.23N which is significantly higher than those of seeds from others areas.

The whiteness, the saturation in red and the yellow saturation of seeds of the various areas of Benin were respectively from 56.91 to 64.31; 1.93 to 5.78 and 17.78 to 18.68. Whiteness and saturation in yellow of seeds significantly varied, except for the samples collected from Porto-novo and Koutchime. Highest values of whiteness and saturation in yellow were obtained respectively with the seeds from Dassa-zoume and Sakete; and samples from Sakete and Cotonou. Concerning saturation in red, it is practically the same value with the samples of Porto-Novo, Koutchimeand Cotonou on the first hand, and with the seeds of Koutchime, Cotonou and Dassa-zoume on the other hand. The values obtained for this parameter in these areas were significantly lower than those of Sakete and Bassila but were significantly higher than those from Calavi, which have the least values of saturation in red.

The seeds from Porto-Novo and Sakete have relatively the same dry matter contents and the same results were also obtained for those from Koutchime and Cotonou. However, there is a significant difference between the seed samples of the other areas.

Fat content is significantly varied according to sampling zone. The major yields were obtained in

seeds collected from Bassila (42.80%) and the lowest value of fat content is obtained in seed collected from Abomey-Calavi (37.34%). This variation of the fat content could be in relation with the agro-pedological structure of ground in the different sampling zones. These values were also higher than those reported by Abdulkarim *et al.* (2005) in *Moringa oleifera* seeds from Pakistan. In opposition, the seed collected from Abomey-calavi and Porto-Novo, which significantly have the lowest fat contents, are also lower than those reported by Anwar and Bhanger (2003) and Anwar *et al.* (2006) in Pakistan.

Table 2. Chemical and biochemical analysis of seeds of *M. Oléifera.*

	Samples							Literature		
Parameter	Calavi	Porto-Novo	Sakete	Bassila	Koutchime	Cotonou	Dassa-zoume	1	2	3
Dry mater	92.80±0.02e	92.56±0.27d	92.55±0.34d	90.67±0.05a	92.11±0.07b	92.13±0.04b	92.21±0.03c	-	-	-
Fat content	37.34±0.03a	37.93±0.40b	38.57±0.12c	42.80±0.04f	39.15±0.07d	41.52±0.13e	44.77±0.05g	40.39±1.15	30.80±1.03	38.37±0.90
Protein	41.04±0.34g	39.68±0.73c	40.63±0.37e	38.47±0.37b	40.73±0.33f	39.84±0.26d	37.76±0.14a	29.36±1.50	38.30±1.03	31.36±0.65
Total sugars	12.94±0.42f	13.02±0.35g	7.49±0.32a	9.54±0.31c	10.11±0.26e	9.84±0.12d	8.53±0.26b	-	-	-
Ash	3.59±0.02a	3.80±0.27a	3.49±0.34a	3.68±0.03a	3.69±0.05a	3.72±0.11a	3.45±0.04a	6.60±0.50	6.50±0.15	5.46±1.19

1=(Anwar and Bhanger, 2003); 2= (Abdulkarim*et al.*, 2005); 3= (Anwar *et al.*, 2006),
The values relating of the different letters to the same line are significantly different with the threshold from 5%.
For the chemical and biochemical parameters, the values carrying of the different letters in the same column are significantly different with the threshold from 5%.

However, these fat content values recorded in *Moringa oleifera* seed collected in Benin were ranged from 37.34 to 42.80%. These values were however lower than those reported in others oleaginous such as peanut (48.1%) (Fachman and Kraut, 2000) and were higher than those reported in cotton seed (15.0-24.0%), soya (17.0- 21.0%) and were similar to those reported in mustard (24.0 to 40.0%).

Table 3. Physicochemical analysis of *M. Oleifera* seeds oil.

Parameters		**Calavi**	**Porto-Novo**	**Sakete**	**Bassila**	**Koutchime**	**Cotonou**	Dassa-zoume
Index of acide (%)		5.30±0.07a	4.67±0.06a	3.34±0.12b	4.52±0.13a	5.21±0.17a	3.52±0.35b	4.68±0.42a
Index of saponification (mg of KOH/g of oil)		153.28±4.47a	154.37±0.94a	158.43±7.35a	152.12±3.22a	159.11±4.53a	153.47±5.11a	151.47±3.33a
Index of refraction		1.47±0.0005a	1.46±0.001a	1.46±0.0005a	1.45±0.0002a	1.43±0.004a	1.48±0.0001a	1.42±0.0003a
Color	L*	49.77±1.55a	71.80±1.72b	56.74±2.20c	49.74±1.34a	71.85±1.62b	72.11±1.12b	49.68±3.2a
	a*	3.96±0.05a	5.53±0.48b	1.51±1.21c	3.93±0.23a	3.92±0.51a	1.54±1.34c	5.56±0.21b
	b*	21.95±0.86a	33.49±1.88b	23.25±1.40a	21.92±0.57a	22.47±0.36a	35.36±0.26b	24.11±0.13a

The values relating of the different letters to the same line are significantly different with the threshold from 5%.
•For oil characteristics,the values carrying of the different letters in the same column are significantly different with the threshold from 5%.

The content of protein of the collected samplesis ranged from 37.76 to 41.04%. These proteins content values are in accordance with those reported by Abdulkarim *et al.* (2005) at Pakistan; but are significantly higher than those reported by Anwar and Bhanger (2003) and Anwar *et al.* (2006). Theses finding justified the report of Anwar and Bhanger (2003) who explained that the flour the seeds of *Moringa oleifera* represents a significant source of protein and could be incorporate as ingredients in food baby.

The carbohydrates and ash content of seed are ranged respectively from 7.49 to 13.02% and 3.45 to 3.80 %. These ash content are lower than that reported by Oliveira *et al.* (1999); Abdulkarim *et al.* (2005) and Farooq and Umer (2007).

The physicochemical characteristics of the oil of the various seed samples are presented in Table 3.

The acidity, saponification index and refraction values are ranged respectively from 3.52 to 5.30%; 151.47 to 159.11 and 1.42 to 1.48. The composition of the fatty acids of the oil samples **(**Table 4**)** revealed that the total content of the fatty acids saturated are ranged from 20.5 and 26.3% with the presence of C16:0, C18:0, C20:0, C22:0 and C24:0 . There values are respectively ranged from 6 to 7.2%; 4.1 to 7.2%; 2.8 to 4.4 and from 1.1 to 1.4% with the prevalence of palmitic and stearic acids.

Table 4. Composition in fatty acid of the oil of seeds of *M. oleifera*.

Fatty acid	Calavi	Porto-Novo	Sakete	Bassila	Koutchime	Cotonou	Dassa-Zoume
C16:0	6.2±0.4a	6.1±0.32b	6.l2±0.45c	6.6±0.50d	6±0.25e	6.3±0.43f	7.2±0.37g
C16:1	1.2±0.08a	1.3±0.11b	1.3±0.13b	1.2±0.07a	1.3±0.14b	1.4±0.16c	1.6±0.10d
C18:0	4.6±0.39c	4.1±0.35a	4.6±0.43c	5.9±0.51e	7.2±0.47f	4.5±0.41b	4.9±0.27d
C18:1	74.6±0.84a	74.8±0.81b	74.4±1.01c	71.1±0.93d	69±0.76e	73.7±1.12f	71.8±0.85g
C18:2	0.6±0.12a	0.6±0.15a	0.6±0.21a	0.7±0.24b	0.7±0.17b	0.7±0.11b	0.6±0.13a
C 20:0	3±0.31b	2.8±0.32a	3±0.27b	3.7±0.23d	4.4±0.33e	3.2±0.21c	3.2±0.18c
C20:1	2.5±0.06b	2.7±0.05d	2.5±0.11b	2.1±0.07a	2.6±0.12c	2.6±0.08c	2.7±0.13d
C 22:0	6.1±0.33a	6.4±0.35c	6.1±0.45a	6.3±0.41b	7.3±0.32d	6.5±0.28e	6.6±0.30f
C 24:0	1.2±0.02b	1.1±0.11a	1.2±0.08b	1.3±0.05c	1.4±0.03d	1.2±0.07b	1.3±0.12c

The values relating of the different letters to the same line are significantly different with the threshold from 5%.
• For the fatty acid composition, the values carrying of the different letters in the same column are significantly different with the threshold from 5%.

The oils were also characterized by the presence of mono-unsaturatesfatty acids C18:1 (69-74,8%) and poly-unsaturated fatty acids C18:2 which is in small proportion (0.6-0.7%). The high concentration of the fatty acids obtained in the oil from *Moringa oleifera* seed sampling in Benin is in accordance with the results of Tsaknis *et al.*(1999); Anwar and Bhanger (2003); Anwar *et al.* (2006) and Abdulkarim *et al.* (2005). The presence of long saturated acids in oil from *Moringa oleifera* seed is also in accordance with the report of Anwar and Bhanger (2003) and the proportion of oleic acid in these oils are in accordance with that reported by Tsaknis, (1998) from seeds of *Moringa peregrine* in Saudia Arabia . The composition in fatty acid of the oil from seeds of *Moringa oleifera* is relatively near that reported in olive oil.

Conclusion

This study upgrades the knowledge on the importance of seeds of *Moringa oleifera* from Benin and their oils contents. The various analyses performed on theses seeds and results obtained, allowed to classify these seeds in the group of potential oil and protein content seeds and could be allowed to recommended their used in the human consumption to reduce nutrient deficiency in food.

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