

Impact of Extraction on Biochemical Properties and Antioxidant Potential of *Momordica charantia* L. Seeds' Oil

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Abstract

In this study, the influence of provenance and extraction methods on the physicochemical properties and the antioxidant potential of *M. charantia* seeds oil were evaluated. The oil is obtained on the one hand by cold extraction with hexane and on the other hand by hot extraction with soxhlet. The results obtained show that the extraction yield is significantly impacted by the extraction methods and the origin of the seeds. In addition, the soxhlet extraction gives a higher oil yield (32.07 ± 0.01). Cold extraction has made it possible to obtain oils with less attenuated physicochemical characteristics. Indeed, the acid numbers are high in the oils extracted by soxhlet (5.92 ± 0.25 ; 4.25 ± 0.62 and 13.86 ± 0.83) than in those cold extracted with very low peroxide for all the oils obtained. On the other hand, the iodine and refractive indices are high in oils obtained cold (91.58 ± 0.85 ; 100.74 ± 0.03 and 102.08 ± 0.28) (1.53 ± 0.01 ; 1.52 ± 0.01 and 1.52 ± 0.01) with low saponification indices. The polyphenol concentrations and the anti-free radical activity are higher with the oils obtained cold (0.086 ± 0.001 ; 0.08 ± 0.000 and 0.09 ± 0.01 mgEAG/g of oil) and (DPPH) ($55.75\% \pm 1.16\%$; $55.03\% \pm 0.72\%$ and $56.35\% \pm 0.45\%$). The color parameters (L^* , a^* and b^*) of the different oils extracted also vary depending on the extraction method used. Principal Component Analysis (PCA) and correlation analysis were performed on the physicochemical properties and the antioxidant potential of the extracted oils. Therefore, the results suggest cold extraction to obtain a good quality and oxidation resistant oil.

Keywords

Momordica charantia, Oil, Antioxidant Potential, Extraction

1. Introduction

The Cucurbits have a large number of species; they are unconventional oil plants [1]. The bitter gourd (*Momordica charantia*) belongs to the Cucurbitaceae family and is widely cultivated in Asia, South America, India, the Caribbean, East Africa, the Middle East and America [2]. This annual species is better known as bitter melon, bitter gourd, Karela or balm pear [3]. Their fruits are widely used around the world in combination with other foods to meet human protein and fat needs [2]. Previous studies on eco-botanical characteristics [4] and fruit or seed yield [5] of species cultivated in West Africa have been widely published. The whole plant (fruits, seeds, leaves, stems and roots) is used as food and remedy for various types of chronic conditions [6]. Currently, the consumption of bitter gourd has increased enormously day by day not only for their nutritional value but also for their therapeutic value [4]. It has good sources of catechin, gallic acid, chlorogenic acid [7] and saponin compounds [8], but also essential amino acids, vitamin C, vitamin A, carotenoids, folic acid, thiamine, riboflavin, niacin and minerals such as calcium, potassium, magnesium and sodium [2]. Indeed, body weight gain and blood sugar levels could be reduced as well as levels of energy metabolism could be increased in mice fed the powder-rich diet of *Momordica charantia* [9]. Furthermore, the oil from the seeds of *M. charantia* may regenerate tissue without side effects in healing rabbit skin [10]. Likewise the consumption of mature and immature fruits increases the secretion of GLP-1 in order to control glucose homeostasis by incretin effect [11]. In other words, the alcoholic extracts of the seeds could also be used for the treatment of chronic leukemia [12]. There are several ethno-pharmacological indications, such as antidiabetics [13], immune modulator [14], anti-dengue [15] and anti-oxidant activities [16] and hepatic fibrosis [17]. In agriculture, the species is used to promote allelopathic activity [18]. However, studies on the physicochemical characteristics of the oil from Soxhlet and cold extracted seeds are unknown, as are its antioxidant potential and the mineral content of the seed powder. In this context, our study aims to assess the effect of different extraction methods on the yield, physicochemical properties, mineral content and antioxidant potential of different oils of *M. charantia*. Thus, this study should provide sensitive information on the composition of seeds in order to promote their processing and consumption in Senegal.

2. Material and Methods

2.1. Plant Material

The ripe fruits of *M. charantia* L were collected in Fatick, in the Niayes zone (Dakar) and other fruits come to us from Togo during the month of September 2018. A reference specimen is stored and referenced under a name specified with an identification number at the herbarium of the Fundamental Institute of Black Africa (IFAN). After pulping, the seeds were washed and then dried at 65°C for 24 hours in an oven (Figure 1(a)). Then the seeds are crushed using a laboratory



Figure 1. Whole seeds (a) and particles with particle size less than 6000 µm (b).

mortar. The powder thus obtained was also sieved using a 6000 µm mesh sieve to obtain finer particles used for the extraction procedures (**Figure 1(b)**).

2.2. Methods

2.2.1. Oil Extraction by Soxhlet

In this part, *M. charantia* oil has been extracted with Soxhlet on particles smaller than 6000 µm. The extraction temperature ranged from 70°C to 75°C and the extraction time was six (6) hours. In order to remove traces of solvents, the extracted oil was evaporated using a rotary evaporator at 45°C, then placed in an oven at 40°C for 24 hours.

2.2.2. Cold Oil Extraction

This method is carried out using a solvent such as hexane or ether. In our work we used hexane as a solvent, the samples, once dried in a well-ventilated medium in the shade, are then ground using mortar until a homogeneous powder is obtained. Then we proceeded by weighing 20 g of the powder, and is collected in a beaker where we have 250 ml glass of hexane. Then the mixture is stirred for 4 hours and then decanted into a flask. Finally, to recover the oil from the hexane phase, the solvent is evaporated using a rotary evaporator at 45°C or by distillation.

2.2.3. Analytical Methods

The physico-chemical characteristics of the extracted oils such as acidity, peroxide number, acid number, refractive index, iodine number, saponification number and color index of the extracted oils were determined. Each analysis was performed in triplicate, and the mean and standard deviation were calculated. The acid number in accordance with standard NF T60-204 and the percentage of free fatty acid (in the form of oleic acid) was obtained by multiplying the acid number by a factor of 0.503 [19]. The saponification index was determined according to the French standard NF T60-206. Whereas the ester number was obtained by differentiating between the saponification number and the acid number [20]. The iodine number is determined according to the French standard NF T60-203; the peroxide index according to the French standard NF T60-220; the

extraction yield according to the standard extraction method on soxhlet (NF V03-905). The polyphenols were assayed according to the method of George and al, [21]. The extinction coefficients at 232 nm and 270 nm (k232 and k270) were determined according to the French standard NF T60-223 with a UV spectrophotometer (SPECORD 200 PLUS). The refractive index was measured with a refractometer (EXACTA-OPTECH, Mod-RMT). A colorimeter (CM-5, Konica Minolta Sensing Americas) was used to determine the L*, a*, b* values, the Y1, c* and h* color parameters of the different oils. The L* component indicating lightness or luminance varies from black to white. The a* component corresponds to the green-red antagonist couple. Component b* corresponds to the antagonistic blue-yellow pair; Y1, c* and h* correspond respectively to yellowing, chromaticity and chromatic tone.

2.2.4. Antioxidant Activity

The antioxidant activity was evaluated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Adaramola *et al.*, [20]. In addition, some of the adjustments were made to his protocol. Thus, 2 mL of DPPH (0.1 mM) prepared in methanol was introduced into a test tube containing 0.5 mL of *Momordica charantia* oil. The mixture was stirred for five (5) minutes then incubated in the dark and at room temperature for 30 minutes. After this incubation period, the absorbance was read at 517 nm against a blank (0.5 mL of *Momordica charantia* oil and 2 mL of methanol) using a UV spectrophotometer (SPECORD 200 PLUS). The absorbance of the control (0.5 mL of DPPH and 2 mL of methanol) is determined at this wavelength. This activity is compared to an antioxidant control (quercetin). The anti-free radical activity is expressed as a percentage of reduced DPPH according to this relationship:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{Absorbance control} - \text{Absorbance echant}}{\text{Absorbance control}} \times 100$$

2.2.5. Statistical Analyzes

To find the best correlations between the random variables of the oil samples, a principal component analysis (PCA) was performed on the physico-chemical data of the oils. To compare the means, analyzes of variance with Fisher's LSD test at 5% significance level was also performed. Thus, all analyzes were performed with R software (version 3.2.4, 2016).

3. Results and Discussion

3.1. Study of Geographic Variability and Mineral Elements of Seeds

Table 1 summarizes the physical parameters studied, such as the mass of seeds, kernels and the shell.

Analysis of the table reveals significant differences at the 5% level for these three parameters. Indeed, the results obtained show that seeds from Togo have the highest average masses of seeds, almonds and hulls. These masses are $0.117 \pm$

Table 1. Distribution of seeds' masses, almonds and husks and minerals compounds of *M. charantia*.

Setting	Units	Dakar	Fatick	Togo
Seeds		0.113 ± 0.04 ^a	0.104 ± 0.04 ^a	0.117 ± 0.01 ^b
Almond	g	0.021 ± 0.02 ^a	0.018 ± 0.03 ^a	0.023 ± 0.05 ^b
Shell		0.091 ± 0.03 ^a	0.083 ± 0.03 ^a	0.092 ± 0.05 ^b
Minerals				
Magnesium		198.34	137.12	126.07
Sodium	mg·100 g ⁻¹	37.056	98.76	47.02
Potassium		936.38	882.82	893.67
Calcium		721.21	807.05	803.76

0.01 respectively; 0.023 ± 0.05; 0.092 ± 0.05 g for Togo seeds. However, the seeds of Dakar and Fatick have roughly the same masses. Indeed, some studies have highlighted the morphological, phenological and biochemical differences between populations of *M. charantia*. This study also revealed variability in seed shell mass (0.113 ± 0.023 and 0.098 ± 0.051 g) [22]. The difference in size between seeds from Senegal (Dakar and Fatick) and those from Togo is probably the result of pedoclimatic conditions and genetic variability. Analysis of the composition of mineral elements (K, Ca, Na, Mg) shows very satisfactory potassium (K) and calcium (Ca) contents. The potassium content of seeds of *M. charantia* is respectively 936.38; 882.82 and 893.67 mg·100 g⁻¹, while that of calcium is 721.21; 837.05 and 803.76 mg·100 g⁻¹. These results are consistent with those found in the fruit pulp in which potassium and calcium are the dominant minerals [22]. Therefore the seeds are richer in mineral elements than the fruit pulp. The sodium (Na) content (037.056; 098.76 and 047.02 mg·100 g⁻¹) is the lowest for all samples. In addition, the potassium content of *M. charantia* seeds is significantly lower than that of pistachio melon (*Citrullus lanatus*) (1500 mg/100g) [1] and that of *Nigella sativa* (1180 mg/100g) [23]. Horax *et al.* [24] reported comparable results where the potassium and calcium contents of seeds were much higher than those of fruits and pericarp. In addition, Anjum and al, [25] studied the seeds of two cultivars of bitter melon. Thus, at the end of their work, they reported significant amounts of calcium and potassium from the seeds (425.08 and 399.81 mg/100g) compared to other minerals. The results are further reinforced by the work of Mathew and al, [26] reporting a low amount of magnesium (93.50 mg/100g) in the seeds of some bitter cucurbits. In another report, Islam *et al.*, [6] found a low concentration of magnesium (16 mg/100g) in the bitter gourd. In addition, the results obtained by d'Ullah *et al.*, [27] on the fruits of four cultivars of bitter gourd showed considerably lower magnesium contents with values in the range 0.99 - 1.10 mg/100g.

3.2. Physical Properties of *M. charantia* Seed Oil

The physicochemical properties of *M. charantia* seed oil analyzed in this study

are presented in **Table 2**.

Analysis of variance revealed very variable physicochemical parameters depending on the extraction methods. The table shows a yield in percentage of oil respectively for the cold and hot extraction of the three samples (14.25 ± 0.03 ; 15.02 ± 0.08 ; 11.57 ± 0.06) and (29.70 ± 0.07 ; 32.07 ± 0.01 ; 22.00 ± 0.07). Thus, this yield was higher with hot extraction than with cold extraction. The values obtained in this study are consistent at $40.12\% \pm 0.607\%$ and $30.29\% \pm 0.521\%$ reported by Cissé *et al.* [28] on baobab seeds in Senegal. Differences in extraction yield between samples may be due to parameters used such as temperature and extraction time [29] but also ripening stage, fruit harvest period and extraction methods [20]. The refractive index used to assess the purity of oils is between 1.53 and 1.41. However, analysis of variance indicates that there is a significant difference for all samples. According to Shahidi and al, [30] the lipid refractive index varies with molecular weight, fatty acid chain length, degree of unsaturation and degree of conjugation. The values determined are in agreement with those obtained by Nkafamiya *et al.* [31]. Furthermore, these refractive indices are comparable to those of hazelnut oil *Nigella* (1.46 - 1.47) [32], *Jatropha curcus* (1.468 - 1.469) [33], olive (*Olea europaea* L.) (1.468) [34] and argan tree (*Argania spinosa* L.) (1.468 - 1.471) [35]. For the specific extinction at 232 nm of cold and hot extracted oils, the values are all lower than the limit values set by the International Oleic Council (IOC) and the Codex Alimentarius for virgin olive oils which are respectively 2, 60 and 3.5 [28]. Indeed, this parameter makes it possible at 232 nm to detect the presence of the primary oxidation products of fatty acids (linoleic hydroperoxides, oxidized fatty acids) on the other hand at 270 nm the secondary oxidation products (alcohols, ketones, etc.) are detected.) [36]. Based on analysis of variance, there was no significant difference at the 5%

Table 2. Physical properties of cold *M. charantia* oils and by soxhlet.

Settings	Dakar1	Fatict1	Togo1	Dakar2	Fatick2	Togo2
Yield (%)	14.25 ± 0.03^a	15.02 ± 0.08^b	11.57 ± 0.06^c	29.70 ± 0.07^d	32.07 ± 0.01^e	22.00 ± 0.07^f
I.R	1.53 ± 0.01^a	1.52 ± 0.01^b	1.52 ± 0.01^b	1.41 ± 0.01^d	1.43 ± 0.03^b	1.43 ± 0.02^b
I.J	87.88 ± 0.02^a	88.52 ± 0.01^b	95.17 ± 0.02^c	95.22 ± 0.07^d	95.65 ± 0.04^e	92.95 ± 0.04^f
L*	79.98 ± 0.02^a	80.25 ± 0.02^a	77.40 ± 0.04^c	69.82 ± 0.07^d	69.51 ± 0.04^d	81.75 ± 0.04^f
a*	-2.07 ± 0.02^a	-4.48 ± 0.01^b	-11.33 ± 0.05^c	-12.78 ± 0.01^d	-12.18 ± 0.05^c	-3.31 ± 0.04^f
b*	83.64 ± 0.01^a	86.36 ± 0.02^b	97.03 ± 0.01^c	94.28 ± 0.05^d	95.63 ± 0.00^e	98.95 ± 0.01^f
c*	95.65 ± 0.01^a	92.36 ± 0.01^b	94.77 ± 0.00^c	93.07 ± 0.05^d	92.22 ± 0.05^b	92.87 ± 0.01^d
h*	94.53 ± 0.02^a	94.11 ± 0.02^a	93.56 ± 0.01^c	94.11 ± 0.01^a	96.23 ± 0.01^c	95.39 ± 0.01^f
k232 nm	0.23 ± 0.02^a	0.26 ± 0.05^a	0.28 ± 0.03^a	0.29 ± 0.04^a	0.24 ± 0.03^a	0.20 ± 0.03^a
k270 nm	0.22 ± 0.03^a	0.32 ± 0.40^{bc}	0.32 ± 0.03^{bc}	0.37 ± 0.02^b	0.38 ± 0.01^b	0.26 ± 0.00^b

(Dakar1; Fatick1; Togo1) = cold extraction; (Dakar2; Fatick2 and Togo2) = extraction by soxhlet; I. R: refractive index; I.J: Yellowing index.

cut-off at 232 nm for all samples and all extraction methods. Therefore, regardless of the extraction method used, the primary oxidation compounds do not vary. In addition, our values at 232 nm of the extracted oils agree with that (1.73) reported by Gharby *et al.* [37] for sesame oil. In addition, the primary oxidation products are weak for all oils. This trend was also reflected by the peroxide number which was low for all samples of extracted oil. Ultimately, the extracted *M. charantia* oil is said to be more resistant to oxidation. On the other hand, the specific extinction at 270 nm between 0.28 and 0.33 remains high but still below the limit value accepted for virgin olive oils [38]. Indeed, the combination of the peroxide number with the extinction coefficients (k_{232} and k_{270}) shows that the oxidative stability of cold-extracted oil is relatively better, which could be explained by the presence of natural antioxidants such as tocopherols, sterols, carotenoids and phenolic compounds [39] [40]. **Table 2** summarizes the color parameters (L^* , a^* , b^* , $Y1$, c^* and h) of hot and cold extracted oils. The values of L^* are respectively (79.98; 80.25 and 77.40) and (69.82; 69.51 and 81.75) for cold and hot extraction. The analysis of variance shows that there is a significant difference between the oils extracted with these two extraction methods. Likewise, the color parameter a^* indicating the shade of color between green and red is (-2.07; -4.48; -11.33) and (-12.78; -12.18 and -3.31) respectively for the oils obtained with the cold and hot extraction. In fact, oil extracted hot with hexane tends more towards red coloring than those extracted cold. The parameter b^* corresponding to the shade of color between blue and yellow varies according to the different oils extracted. The highest b^* value was obtained with the hot-extracted sample from Togo which is 98.95. This value indicates that this oil is more yellow than those of the others obtained cold. On the other hand, the values of the $Y1$ yellowing index of hot-extracted oils are very similar. However, the analysis of variance shows a significant difference between the samples and the extraction methods. The oil from the hot-extracted Togo sample is higher. Consequently, the latter would contain a greater quantity of carotenoids [41]. The difference in coloration observed between hot and cold obtained oils could be attributed to the temperature and duration of extraction. In addition, Al-Farga *et al.* [42] reported with oil from the seeds of *Boerhavia elegans* the values of 65.44 (L^*), 1.11 (a^*) and 28.33 (b^*).

3.3. Chemical Properties of *M. charantia* Seed Oil

The chemical characteristics of the oil of different seeds of *M. charantia* are shown in **Table 3**. Data processing by analysis of the variance revealed a significant difference from all studied parameter.

The acid number indicates the state of degradation of the oil by evaluating the amount of free fatty acids formed during extraction or storage. The lower the acid number, the less free fatty acids it contains, making the oil less susceptible to rancidity. The acid values for the three samples according to the two extraction methods (cold and hot) are respectively $(3.89 \pm 0.37 \text{ mg KOH/g})$; $(6.73 \pm 0.37$

Table 3. Chemical characteristics of oils extracted cold and by Soxhlet.

Settings	Dakar1	Fatict1	Togo1	Dakar2	Fatick2	Togo2
I. A	3.89 ± 0.37 ^a	6.73 ± 0.37 ^b	3.33 ± 0.37 ^c	5.92 ± 0.25 ^d	13.86 ± 0.83 ^e	4.25 ± 0.62 ^f
I.AGL	1.82 ± 0.14 ^a	3.53 ± 0.14 ^b	1.53 ± 0.19 ^c	3.03 ± 0.04 ^d	6.97 ± 0.41 ^e	2.13 ± 0.13 ^f
I. E	29.35 ± 1.05 ^a	45.14 ± 0.59 ^b	48.00 ± 0.20 ^b	88.51 ± 0.96 ^a	81.99 ± 0.10 ^e	96.43 ± 0.36 ^d
I. P	0.31 ± 0.08 ^a	0.15 ± 0.05 ^b	0.22 ± 0.04 ^b	0.67 ± 0.04 ^c	0.87 ± 0.07 ^b	0.94 ± 0.03 ^b
I.I	91.58 ± 0.85 ^a	100.74 ± 0.03 ^b	102.08 ± 0.28 ^b	94.62 ± 0.60 ^a	90.95 ± 0.89 ^b	80.67 ± 0.30 ^b
I.S	94.43 ± 1.96 ^d	96.68 ± 1.93 ^b	100.38 ± 1.38 ^c	33.51 ± 0.62 ^a	51.58 ± 0.99 ^b	50.21 ± 0.99 ^c

mg KOH/g; 3.33 ± 0.37 mg KOH/g) and (5.92 ± 0.25 mg KOH/g; 13.86 ± 0.83; 4.25 ± 0.62 mg KOH/g). This value is much lower than that obtained by Oluwole *et al.* [43] on unripe pear oil (17.82 ± 0.22 mg KOH/g) but higher than that of shea butter (1.79 mg KOH/g) reported by Afolayan *et al.* [44]. In fact, the oil from the Togo seeds showed the lowest level of acid (3.33 ± 0.37 mg KOH/g). Therefore, hydrolysis of the ester bonds of triglycerides is stronger in this oil. At the same time, increasing the acid value leads to a change in the content of glycerol and free fatty acids in the oil. From these results, it can be said that the quality of cold-extracted *M. charantia* oils (4.65 mg KOH/g) is less impaired than that extracted hot (13.86 mg KOH/g). In addition, these values are very high compared to those reported by Nkafamiya *et al.* [31] (0.33 mg KOH·g⁻¹) and Birnin-Yauri and Garba [45] (3.14 mg KOH·g⁻¹). These recorded differences could be reasonably explained by the pretreatment applied to the seeds of *M. charantia* or the presence of a highly active lipase which would lead to rapid acidification of the oil during extractions [28]. In addition, these important values can be attributed to the heating temperature. According to Tchiégang *et al.* [36] the temperature induces the hydrolysis of one or two ester bonds of triglycerides favoring the formation of free fatty acids. The percentage of free fatty acids (FFA) in the oil is a crucial parameter for determining the quality of the oil, because the lower the content of free fatty acids, the higher the quality of the oil, in particular with regard to its edibility. The percentage of free fatty acid of (2.293 ± 0.156) and (4.043 ± 0.193) obtained by oil from the seeds of *M. charantia* in this study is low compared to 4.88 ± 0.03, 2.45 ± 0.2, 2.38 ± 0.18 and 38.85 ± 3.34 obtained by the oil of *Citrullus vulgaris* [45]. The low GLA content of an oil is also an indicator of low sensitivity to enzymatic hydrolysis and could be an advantage over oils with high free fatty acid value which can degenerate during storage [20]. The ester number represents the number of milligrams of potassium hydroxide required to saponify the esters present in 1 g of the oil. It is obtained as the difference between the saponification number and the acid number. The ester values of (88.51 ± 0.96; 81.99 ± 0.10; 96.43 ± 0.36) and (29.35 ± 1.05; 45.14 ± 0.59; 48.00 ± 0.20) obtained in this study are much higher than those obtained by Adaramola *et al.* (31.26 ± 0.03 mg of KOH/g) on avocado seeds. However, these values are lower than those of rubber seed oil (191.93

mgKOH/g) [44] and *African pear* (128.48 mg KOH/g) [46]. The iodine number is used to determine the degree of unsaturation in a vegetable oil and to assess the stability during storage. The recorded iodine value varies significantly with different samples and extraction methods. In fact, according to the two extraction methods (cold and hot), this index is between 100.74 ± 0.03 and 102.08 ± 0.28 mgI₂/100g and seems to be affected by the latter. In addition, the lowest iodine value (90.95 ± 0.89 mgI₂·100g⁻¹) was recorded with the Fatick sample. Therefore, this high quality oil would be more resistant to oxidation and have a longer shelf life. In other words, cold-extracted oil is said to contain the lowest amounts of oleic and linoleic acids. Iodine values less than 100 mgI₂·100g⁻¹ will classify the oil of *M. charantia* as a non-drying oil. These high levels of iodine suggest the high content of unsaturated fatty acids in these oils. The results obtained are in agreement with those indicated by Nkafamiya *et al.* [31] and Danbature *et al.* [47]. The peroxide number helps to understand the degree of oxidation of unsaturated fatty acid products. In fact, the oxidation of oils leads to the formation of hydroperoxides, the primary products of oxidation. It is a very sensitive criterion for evaluating the early stages of oxidative deterioration of an oil during production and during storage [48] [49]. This standard method is included in the specifications for “fatty substances” with a threshold value of 10 meq O₂ per kg of material for a refined oil [48] [50]. Analysis of variance indicates that there are significant differences between the peroxide values. In other words, all the peroxide values of the *M. charantia* oils obtained are below this limit value of 10 mEq·kg⁻¹ set by the Codex Alimentarius [38]. These values of peroxides obtained are much lower than those of palm (16.08 mEq·kg⁻¹) and sorrel (*Hibicus sabdariffa*) (5.00 ± 0.01 mEq·kg⁻¹) oils reported respectively by Birnin -Yauri and Garba [51], and Betiku and Adepoju [52]. In other words, the oxidative deterioration of oils during hot extraction operations has been extensively studied [37] [53]. The measured saponification index values are (94.43 ± 1.96 ; 96.68 ± 1.93 ; 100.38 ± 1.38 mg of KOH·g⁻¹) and (33.51 ± 0.62 ; 51.58 ± 0.99 ; 50.21 ± 0.99 mg of KOH·g⁻¹) respectively for cold and hot extracted oils. This significant difference observed between the oils extracted can be attributed to the extraction methods, the extraction time and/or the origin of the samples. Cold-obtained *M. charantia* seed oil has a higher saponification index. Therefore, this oil would contain more free fatty acids for stability during storage. These indicate a predominance of acids long chain fat in these oils [54] which is very important for the food and cosmetic industries. Oils with high saponification would be less susceptible to deterioration [55]. The results also indicate that the saponification values are higher than those of *Persea americana* oil (35.76 mg KOH/g) [20], but lower than those of *argan* oil (190.88 mg of KOH/g) and olive oil (97.94 mg KOH/g) [53].

3.4. Antioxidant Potential of Extracted Oils

To assess the quality of the extracted *M. charantia* oils, parameters such as po-

lyphenol content and antioxidant activity were measured. The results obtained are summarized in **Table 4**.

Analysis of variance data processing shows that there is a significant difference at the 5% level depending on the oil extraction methods. According to the results obtained, the oil from the cold-extracted sample from Togo was richer in polyphenols with 0.09 ± 0.001 mg EAG/g of oil followed by the sample from Fatick (0.087 ± 0.000 mg EAG/g of oil). These values are lower than those obtained by Salih *et al.* [56] on baobab seeds (*Adansonia digitata* L.) with a polyphenol content of 6.689 ± 0.086 mg EAG/g. With the results of the latter, we can also say that the amount of polyphenols available in *M. charantia* oils remains very low. It emerges from this study that the oil obtained when cold contains a higher content of polyphenols than when it is hot. Therefore, this oil would be more beneficial dermatologically. According to Ranalli *et al.* [57] this difference in terms of phenolic compounds between cold extracted oil and hot oil results from the method, the duration or the temperature of extraction. Galvano *et al.* [58] reported that natural antioxidants (tocopherols, carotenoids, etc.) influence the oxidation of lipids. As a result, cold-extracted oils will be less susceptible to oxidation and therefore easier to store. However, by comparing the contents in polyphenols reported by Adaramola *et al.* [20] on the oil of *Persea americana* (8.27 ± 0.06 mg EAG/g of oil), we can say that the oil of the seeds of *M. charantia* displays a low polyphenol content. The antioxidant activity of the extracted oils varied depending on the extraction process. The oil from the seeds of Dakar and Fatick exhibits greater anti-oxidant activity than the sample from Togo. Therefore the consumption of this oil would be very beneficial against oxidative stress. According to Rahimi *et al.* [59] oxidative stress damages cellular components, such as proteins, lipids and nucleic acids [60] and ultimately leads to cellular apoptosis [61]. Rezaeizadeh *et al.* [62] reported that various herbal remedies are used in traditional therapies today only because of their antioxidant properties. In addition, the use of fruits and vegetables containing antioxidant agents decrease the possibility of chronic diseases such as diabetes [63], cancer and cardiovascular diseases [49]. Previous studies have shown that cucurbits have been identified as useful sources of antioxidants which can protect against oxidative stress and therefore have a primary role to play against injury from lipid peroxidation [64]. Furthermore, Wu *et al.* [64] [65] mentioned that *M. charantia* contains potent antioxidant and free radical scavenging activity, which can be extracted from compounds such as flavonoids and phenols. There is a correlation between the content of phenolic compounds and the antioxidant

Table 4. Polyphenol and antioxidant activity of oils cold-extracted or by Soxhlet's method.

Settings	Dakar1	Fatick1	Togo1	Dakar2	Fatick2	Togo2
P.T	0.08 ± 0.01^a	0.08 ± 0.00^a	0.09 ± 0.01^b	0.07 ± 0.01^c	0.07 ± 0.01^c	0.07 ± 0.00^d
AAR (%)	55.75 ± 1.16^a	55.03 ± 0.72^a	56.35 ± 0.45^b	52.00 ± 0.01^c	49.00 ± 0.63^d	50.00 ± 0.01^c

P.T: total polyphenols; AAR: Anti-radical activity.

activity of the various oils obtained. This positive correlation between these two parameters is consistent with the results obtained through several studies [58] [66]. Recent studies have shown that, polyphenolic compounds from fruits and seeds such as flavonoids are one of the main groups that indicate a wide range of biological activities which are mainly attributed to their antioxidant property [67].

3.5. Statistical Analyzes

3.5.1. Principal Component Analysis

In order to assess the impact of the fruit collection area and the extraction methods on the physico-chemical characteristics and the antioxidant potential of the extracted oils, the principal component analysis (PCA) was carried out (Figure 2 and Figure 3).

The first two dimensions (Dim 1 and Dim 2) express 79.95% of the total variance. Indeed, the first dimension (Dim 1) contributes to 50.39% and the second (Dim 2) to 29.56%. The variables, yield, peroxide number, saponification number, acid number, anti-free radical activity, specific extinction coefficient (k270 nm), a* (shade of color between green and red), b* (color shade between

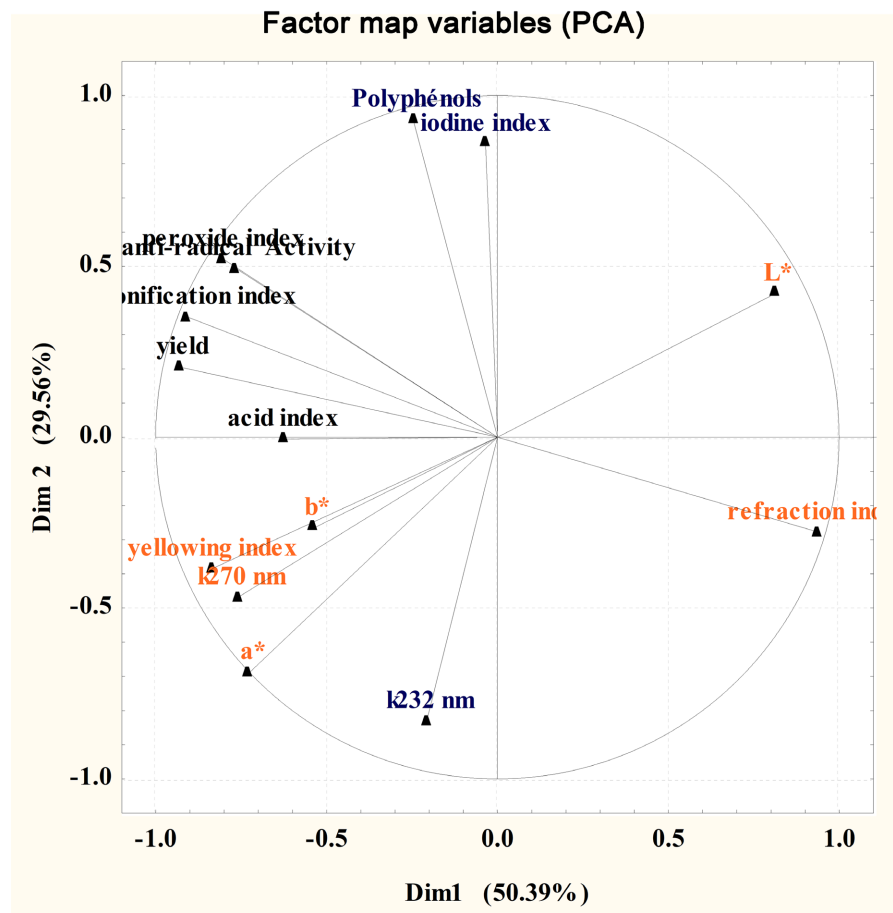


Figure 2. Correlation between the physicochemical properties and the antioxidant potential of oils, the first two dimensions of PCA.

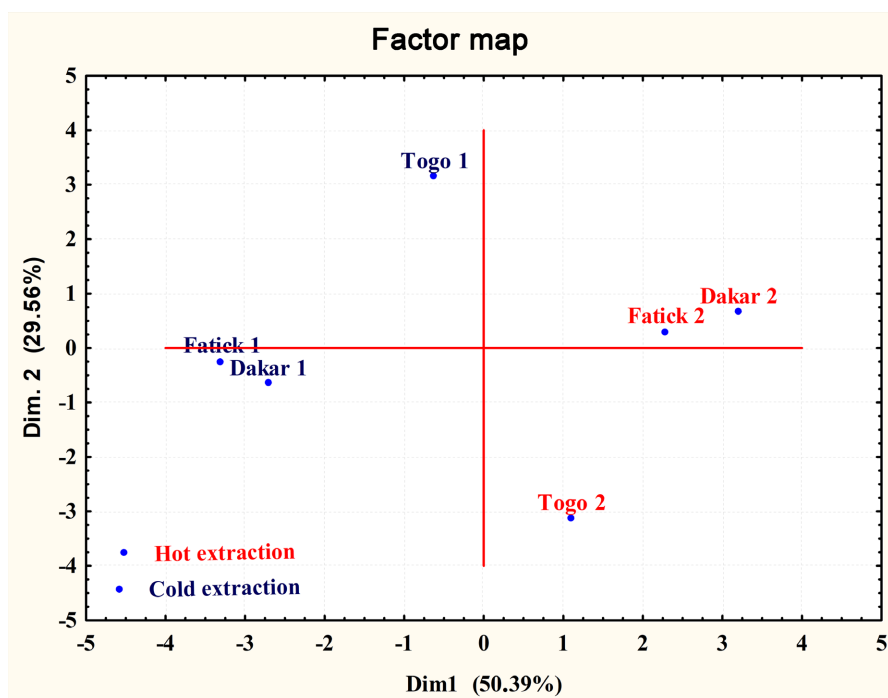


Figure 3. Projection of the different extraction methods on the physicochemical properties and the antioxidant potential in the factorial plane of PCA.

blue and yellow) and yellowing index Y1 are negatively well correlated with the first axis, while the luminescence variables L^* and refractive index are positively correlated with the first axis. Indeed, the parameters making it possible to evaluate the oxidation state of the extracted oils are well aligned with this first dimension (Dim 1), which could be considered as an axis of the quality of the oils obtained. In addition, the polyphenol and iodine number variables are positively correlated to the second dimension (Dim 2). On the other hand, the extinction coefficient variable is negatively correlated with this second dimension (Dim 2). In other words, the parameters representing the antioxidant potential, the state of preservation and the identification of the oils obtained are well aligned with this second dimension. The dimension axes (Dim1 and Dim 2) define the origins of the samples and the oil extraction methods used. The position on the Dim 1 axis differentiates the oil with the peroxide index, the specific extinction coefficient at k_{270} nm, the shade of color between green and red (a^*), the shade of color between blue and yellow (b^*) and the yellowing index Y1 (cold extraction) to other oils characterized by the color parameters (L^*), and the refractive index (hot extraction). On the other hand, the position on the second axis (Dim 2) opposes oils with a high polyphenol content, and a high iodine number (hot extraction oil), to oils (cold extraction with hexane) with a coefficient extinction at low k_{232} . Thus, based on the physicochemical properties of oils, it is very clear that cold-extracted oil with hexane retains its quality at best. In addition, this extraction method (cold with hexane) makes it possible to obtain oils with anti-free radical activity and higher color indices. In addition, the oil extraction

methods were grouped into two classes according to the figures obtained. The first class is oil hot extracted with soxhlet. The latter is characterized by high yield, acid index, extinction coefficient and color parameters (a^* and b^*). The second class is oil cold extracted with hexane. This class is characterized by an important refractive index and luminescent L^* . From these results, it is clear that cold-extracted oils are less spoiled during extraction. In addition, choosing to produce *M. charantia* oil from this extraction method would preserve the quality and stability of the oils obtained.

4. Conclusion

The impact of provenance and extraction methods on the physicochemical characteristics, polyphenol content and radical elimination activity of the extracted *M. charantia* oils was determined. The results obtained show that the cold-extracted oil retains its physico-chemical properties as much as possible and contains a very high content of phenolic compounds and anti-radical activity. However, soxhlet extraction provides the best oil yield. In addition, cold extraction makes it possible to obtain oils whose physicochemical characteristics are less attenuated. This same cold-extracted oil has acceptable antioxidant activity. Taken together, these results provide fundamental information regarding the extraction method to be used for a specific use of this *M. charantia* oil. Therefore, further studies would be necessary to determine the optimal conditions for oil extraction and to identify the bioactive compounds present in the oil.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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