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Differential composition of reserves and oil of *Moringa oleifera* seeds cultivated in states of Northeast Brazil

Composição diferencial de reservas e óleo de sementes de *Moringa oleifera* cultivadas em estados do Nordeste do Brasil

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ABSTRACT

Moringa oleifera (Lam.) is an oilseed rich in unsaturated fatty acids. The seed reserve composition can change according to environmental conditions of precipitation and temperature. Thus, this work aimed to characterize the *M. oleifera* seed and its vegetable oil from plants cultivated in different states of Northeast Brazil. Seeds and soil samples were collected in Bahia (BA), Ceará (CE), Paraíba (PB), and Rio Grande do Norte (RN). Regarding climate variables, RN and BA had the lowest (395 mm) and highest (880 mm) precipitation during the collection year, respectively. The size and mass of the seeds and almonds, and the characterization of the flour and the oil of the 'moringa' seeds were measured. The largest seeds and almonds were collected in BA and the smallest in RN. The highest protein and moisture contents were obtained in the seeds from CE. The seeds from RN had the highest oil content, unsaturated fatty acids, ashes, acidity, and saponification levels. Thus, RN presents the smallest seeds of *M. oleifera* with higher content of mineral salts (ashes), oil, and monounsaturated fatty acids.

Index terms: Fatty acid; precipitation; oilseeds; protein content.

RESUMO

Moringa oleifera (Lam.) é uma oleaginosa rica em ácidos graxos insaturados. A composição de reserva da semente pode mudar de acordo com as condições ambientais de precipitação e temperatura. Assim, objetivou-se com este trabalho caracterizar sementes e o óleo vegetal de *M. oleifera* cultivado em diferentes estados do Nordeste do Brasil. As sementes e amostras de solo foram coletadas na Bahia (BA), Ceará (CE), Paraíba (PB) e Rio Grande do Norte (RN). Em relação às variáveis climáticas, RN e BA apresentaram a menor (395 mm) e a maior (880 mm) precipitação durante o ano de coleta. As sementes e amêndoas foram medidas e realizada a caracterização da farinha e do óleo de moringa. As maiores sementes e amêndoas foram coletadas na BA e as menores no RN. O maior teor de proteínas e umidade foram obtidos nas sementes do CE. As sementes do RN apresentaram o maior teor de óleo, níveis de acidez e saponificação e a maior quantidade de ácidos graxos insaturados. Assim, o RN possui as menores sementes de *M. oleifera* com maior teor de sais minerais, óleo e ácidos graxos monoinsaturados.

Termos de indexação: Ácido graxo; precipitação; sementes oleaginosas; teor de proteínas.

INTRODUCTION

Seed storage compounds have several biological functions in seed germination, protection, and seedling development (Ruraż et al., 2020). The reserve material depends on genetic factors, but its quantity is also strongly influenced by environmental conditions, especially during the grain-filling period (Fenner, 1992; Nuttall et al., 2017). Lipids are important components of seed reserves and are present in large quantities in oilseeds. Seed oil is a source of energy and can be used in human food, in the production of fatty acids, glycerin, lubricants, and biodiesel, among other applications (Bhat; Reddy, 2017; Kaseke, Opara, Fawole, 2020; Reda; Carneiro, 2007). The characteristics of crude vegetable oil are extremely important because they determine the destiny and the refining practices to obtain an oil within the quality standards of the current legislation (Gharby, 2022; Ministério da Agricultura, Pecuária e Abastecimento - MAPA, 2006).

Among species whose seeds are rich in oil, *Moringa oleifera* Lam stands out for its multiple uses (Falowo et al., 2018; Granella et al., 2021; Pandey et al., 2012). *M. oleifera*, commonly known as 'moringa', is one of the 14 species from the single genus of the Moringaceae family. It is a fast-growing small tree, native to northern India, well-adapted to a wide variety of soils, which tolerates drought (Fahey, 2005). *M. oleifera* has great nutritional value as its flowers, fruits, leaves, and roots are used in human (Anwar et al., 2007) and animal (Al-Harthi et al., 2022) nutrition. 'Moringa' seeds usually show a high content of proteins (~35%) and lipids (~40%), and around 15% of carbohydrates (Baky; El-Baroty, 2013; Cardoso et al., 2008; Gidde; Bhalerao; Malusare, 2012). The oil of 'moringa' seeds can be extracted by pressing or with solvents is clear yellowish and sweet, and resistant to oxidative degradation (Bhutada et al., 2016). The oil of 'moringa' seeds is commercially known as "ben oil" or "behen oil" due to the high content of behenic or docosanoic acid (Pereira et al., 2016).

The search for alternative vegetable oils grows every year and 'moringa' oil is a viable option due to its several uses in the food, biofuel, and pharmacological industries (Magalhães et al., 2020). 'Moringa' oil is considered a substitute for olive oil as a result of its high oleic acid and low linoleic acid concentrations, which provide oxidative stability (Anwar et al., 2007; Ruttarattanamongkol et al., 2014; Zhong et al., 2018). 'Moringa' oil has also been considered a candidate for the production of biodiesel because it does not dispute land with crops and other agricultural products (Magalhães et al., 2020; Mofijur et al., 2014).

In the same species, the oil content and composition can vary depending on climatic conditions, stress, soil fertility, plant age, quality of the raw material, extraction method, and refining procedures (Galvão et al., 2013). Therefore, this work aimed to chemically characterize the seed and oil of *M. oleifera* from four states of Northeast Brazil.

MATERIAL AND METHODS

Characterization of the collection sites and plant material

Moringa oleifera seeds were collected in Sebastião das Laranjeiras (Bahia, BA) (14º57'01"S 42º93'94"W), climate Aw according to the Köppen-Geiser classification, 880 mm average precipitation, 24.5° C average temperature; Juazeiro do Norte (Ceará, CE) (7º22'40"S 39º33'00"W), climate Aw according to the Köppen-Geiser classification, 640 mm average precipitation, 26.7° C average temperature; São Mamede (Paraíba, PB) (6º86'67"S 37º06'85"W), with climate BSh according to the Köppen-Geiser classification, 416 mm average precipitation, 26.87° C average temperature; and Parelhas (Rio Grande do Norte, RN) (6º69'68"S 36º63'99"W), climate BSh according to the Köppen-Geiser classification, 395 mm average precipitation, 26.9° C average temperature. All these municipalities are located in the Northeast of Brazil and their climatic characterization was obtained from the National Institute of Meteorology (INMET, Brazilian acronym) and Ceará Foundation of Meteorology and Water Resources (FUNCEME, Brazilian acronym).

Seeds were collected from about 30 'moringa' plants in each collection site, and the moringa material was the same in all areas. The *M. oleifera* plants from Sebastião das Laranjeiras were on average 18 months old. The area had supplementary irrigation in the dry period and was fertilized to correct soil deficiencies. Plants from Juazeiro do Norte were on average five years old and were fertilized only during the seedling phase. Plants from São Mamede were on average ten years old, the fertilization was carried out only in the seedling phase, and the area had supplementary irrigation in the dry period. In Parelhas, the plants were on average six years old, and fertilization occurred only in the seedling phase. Thus, we analyzed four collection locations (Sebastião das Laranjeiras-BA, Juazeiro do Norte-CE, São Mamede-PB, and Parelhas-RN) with five replications, totaling 20 experimental plots.

Seed biometry and flour characterization

The diameter of seeds and almonds (n = 10 per experimental unit) was measured using the Western Ws8 Dc-6® electronic digital caliper. To determine the mass, five replicates of 100 seeds and almonds were weighed in an analytical balance with a precision of 0.0001 g.

The almonds were removed from the seeds and crushed in low rotation in a food processor (Philco®). The obtained flour was packaged in glass jars and protected from light with aluminum foil. The protein content was analyzed by the modified Kjeldahl method of nitro sulfuric digestion as described by the Instituto Adolf Lutz (2008). The initial values of nitrogen (N) quantification were multiplied by 6.25 for obtaining the protein percentage. For the moisture content, 2g of flour was dried with infrared radiation on a Shimadzu® moisture balance. The flour was also weighed and incinerated in a muffle furnace until constant mass to calculate the percentage of ashes (Instituto Adolf Lutz, 2008).

Seed oil extraction, quantification, and composition

The oil from the *M. oleifera* flour was extracted in a Soxhlet-type apparatus with boiling hexane under reflux for two hours. Afterward, the hexane was separated from the oil in a rotary evaporator at a temperature of 70º C. The oil content was determined by the percentage of the ratio between the amount of oil and the total flour mass of the seeds.

The acidity and saponification indexes of *M. oleifera* oil were determined by titration. For the acidity index, a solution of ethyl alcohol and ethyl ether (1:2 v/v) and phenolphthalein was added to the oil and titrated with 0.1 N potassium hydroxide. For the saponification index, a 4% alcoholic potassium hydroxide solution was added and refluxed, followed by titration with 0.5 N hydrochloric acid (Instituto Adolf Lutz, 2008).

The fatty acids of the vegetable oil of *M. oleifera* were transformed into methyl esters (Encinar; González; Rodríguez-Reinares, 2005). Methanol and sodium hydroxide were added to the oil. This mixture was refluxed for three hours, distilled water was added, and the mixture was transferred to a separatory funnel. The mixture was then washed three times with ethyl ether and the ethereal phase was collected. An alkaline hydroalcoholic phase was acidified with hydrochloric acid to pH 2-3 and then washed three times with ethyl ether, extracting a second ethereal phase that was mixed with the first. Methanol and hydrochloric acid were added to the ethereal mixture and refluxed for 10 minutes. After this period the mixture was cooled to room temperature and distilled water and ethyl ether were added. The ethereal fraction was separated and filtered with anhydrous sodium sulfate.

The ethereal fraction was subjected to fractionation in a chromatographic column containing silica gel using hexane and chloroform as eluents in a 1: 1 mixture. The esters were separated by thin layer chromatography until a behavior similar to that of the normal pure substance was obtained. After this procedure, the esters were analyzed by gas chromatography (Shimadzu®) for 47 minutes with an injection temperature of 280 ºC, pressure of 65.2 kPa, using H_2 as carrier gas at a speed of 36.8 cm s^{-1} and total flow of 54 mL m^{-1} (Eder, 1995).

Statistical analyses

Due to the heterogeneous nature of the plant material used, the data obtained were analyzed in a non-parametric Kruskal-Wallis test ($\rho < 0.05$) and the means compared by the Holm test ($p < 0.05\%$). Canonical analysis of variables and confidence ellipses ($p \leq 0.01$) were performed to study the interrelationship between variables and factors through the candisc package (Friendly; Fox, 2017). The statistical analyzes were performed with the program R (R Core Team, 2022).

RESULTS AND DISCUSSION

Chemical characterization of the soil from the sampling regions

The soil collected in BA showed the highest amount of organic matter, sum of bases, and cation exchange capacity, but the lowest amount of phosphorus (Table 1). The soil of the PB region showed the presence of aluminum, whereas the one from the RN region had a high amount of phosphorus and sodium.

Diameter and mass of seeds and almonds, and centesimal composition of *M. oleifera* flour

Seeds from BA had the highest mass of 100 almonds (Table 2), which did not statistically differ from PB and CE seeds. The mass of 100 seeds was significantly higher in seeds from BA. The largest diameter of seeds and almonds were found in seeds from BA, without significant differences from the diameter of seeds from CE.

These results demonstrated a proportionality between the almond and the seed sizes in 'moringa' fruits from different production sites in Northeast Brazil. The environment in which the mother plant is inserted probably influenced the size of the seeds produced (Li; Li, 2016; Nguyen et al., 2021). Seed filling is a crucial growth stage, sensitive to environmental changes (Sehgal et al., 2018, Singer; Zou; Weselakem, 2016; Wang et al., 2020). This process involves biochemical alterations that prevent or stimulate the accumulation of various seed constituents through the modification of enzymatic activities (Sehgal et al., 2018) or epigenetics (Teng et al., 2022). The smallest seeds were collected in RN, where the annual precipitation was below 500 mm (without supplementary irrigation). This environmental condition can be detrimental to seed production in this species (Adebayo et al., 2017; Ayerza, 2011; Leone et al., 2016, Melo; Benitez; Barbosa, 2020). *Moringa oleifera* plants subjected to a water regime of 300 mm $year^{-1}$ had a rapid initiation of flowering, but the fruit set was delayed (Mashamaite et al., 2021; Muhl et al., 2013). Thus, low water availability during grain development reduces the potential seed size (Melo; Benitez; Barbosa, 2020).

Table 1: Soil chemical attributes of the collection sites.

State	pH	P	K^+	Na^+	H^++Al^{3+}	Ca^{+2}	Mg^{+2}	SB	CEC	OM
	H_2O	mg dm^{-3}---------------------------			cmol$_c$ dm^{-3}	-------------------------------			g kg^{-1}	
BA	6.4	4.3	0.54	-	0	8.6	2.3	11.4	11.4	22.0
CE	7.1	41.9	0.57	0.06	0	1.05	0.36	2.04	2.04	7.86
PB	6.9	50.2	0.49	0.07	1.07	1.29	0.40	2.25	3.32	11.3
RN	7.7	238.4	0.50	0.26	0	1.72	0.70	3.18	3.18	12.2

SB - the sum of bases; CEC - Cation Exchange Capacity; OM - Organic Matter.

Table 2: Average diameter, mass of 100 seeds/almonds, and flour centesimal composition of *M. oleifera*.

State	Variable						
	Mass of 100 almonds (g)	Mass of 100 seeds (g)	Seed diameter (cm)	Almond diameter (cm)	Protein (%)	Humidity (%)	Ashes (%)
BA	16.88 a	23.89 a	0.75 a	0.66 a	35.06 b	6.17 a	4.47 d
CE	14.56 ab	21.76 b	0.72 a	0.61 b	37.31 a	6.20 a	15.06 b
PB	15.96 a	20.99 c	0.67 b	0.56 c	31.56 d	5.20 b	13.87 c
RN	14.25 b	20.13 d	0.58 c	0.43 d	32.32 c	6.12 a	15.88 a
χ^2	9.63	16.71	16.23	17.45	17.85	10.95	17.85
α	0.022	0.0008	0.001	0.0005	0.0005	0.012	0.0005

Significant differences (Holm test for comparison of the means, $p < 0.05\%$) within columns are indicated by letters.

The flour from 'moringa' seeds collected in PB showed the lowest percentage of moisture, whereas the flour from the seeds collected in CE presented the highest protein content (37.31%) (Table 2). The amount of mineral salts (ashes) was higher in the flour from seeds collected in RN (15.88%) (Table 2). The lower concentration of protein in the seeds from PB can be explained by the presence of H^++Al^{3+} in the soil. This ion can reduce the availability of nutrients, such as nitrogen essential for protein biosynthesis (Borges et al., 2020; Pal'ove-Balang; Mistrik, 2011; Souza et al., 2016; Zhao; Shen, 2018). The reduction in protein content was not so severe because the toxicity of Al^{3+} is greater in pH below 5.5 (Rahman; Upadhyay, 2021; Xiao; Yu; Xu, 2014). The phosphorus content can explain the proportion of mineral salts (ashes) in seeds from CE, PB, and RN because an excess of Ca, Mg, P, and Fe can increase the percentage of mineral salts in the samples (Brosse et al., 2012; Jorgensen, 1997; Stavridou et al., 2017). The soil of Sebastião das Laranjeiras (BA) had the lowest amount of phosphorus and the flour of the seeds from this area showed the lowest ash content among all the collection states.

Characterization of *M. oleifera* oil

The highest and the lowest acidity index (Table 3) were found in the oil from seeds collected in RN and PB, respectively. This index is directly influenced by the seed moisture content that positively affects the deterioration rate of fatty acids, increasing the release of H^+ and, consequently, the acidity index (Salaheldeen et al., 2014; Wiltshire et al., 2022). Vegetable oils can be degraded through several processes such as hydrolytic reactions, enzymatic oxidation, photo-oxidation, and self-oxidation, besides the storage temperature (Oliveira et al., 2018; Owuna, 2020; Salimon et al., 2014). The oils with the highest saponification index were those from seeds collected in RN and BA. Although the highest oil content was obtained in the seeds of RN (51.06%), these seeds were smaller. Eman and Muhamad (2016) and Afzal et al. (2020) also reported higher oil content in seeds smaller than 1 cm. An inverse relationship was observed in seeds collected in BA, as the supplementary irrigation contributed to larger seeds, which had a lower oil content. This may be due to delayed seed maturation as water is excessively consumed during vegetative growth (Mostafa; Afify, 2022). Furthermore, when investigating the seed and oil yield of *M. oleifera* trees from arid and sub-humid regions, a significantly higher oil percentage was reported for seeds produced in arid regions. However, when this percentage was related to seed/tree productivity, the yield from sub-humid regions was higher than in arid regions, as well as the higher oil yield in regions with lower rainfall at the time of *M. oleifera* flowering (Ayerza, 2012; Faisal et al., 2020; Leone et al., 2016). Thus, environmental variables such as light, temperature, soil type, and available nutrients directly affect the yield of moringa oil (Ayerza, 2011; Faisal et al., 2020).

Table 3: Characterization of *M. oleifera* vegetable oil.

State	Variables		
	Acidity index (mg KOH g^{-1})	Saponification index (mg KOH g^{-1})	Vegetable oil content (%)
BA	5.132 c	180.06 a	36.40 d
CE	6.970 b	161.39 b	46.98 b
PB	0.730 d	153.26 b	42.65 c
RN	9.918 a	181.06 a	51.06 a
χ^2	17.85	15.00	17.86
α	0.0005	0.0018	0.0005

Significant differences (Holm test for comparison of the means, p < 0.05%) within columns are indicated by letters.

The chromatogram of 'moringa' seed oil from BA revealed the presence of five classes of chemical substances, whereas only three were found in the seed oil from seeds of PB, RN, and CE. The highest amount of unsaturated fatty acids in the vegetable oil of *M. oleifera* was found in the seeds of RN (88.53%), followed by seeds of PB (75.33%), CE (70.90%), and BA (59.36%) (Table 4).

The unsaturated fatty acids present in the 'moringa' oil were mostly C18: 1 (Table 5), which differ from each other by the location of the double bond. Great variation was found for saturated fatty acids, from C14: 0 to C22:0. The amount of unsaturated fatty acids was similar to that reported in the literature, with the composition comparable to that described by Wiltshire et al. (2022).

The high saponification index of the seed oil (Table 3) from BA and RN is related to the higher amount of fatty acids with low molecular weight (Hailu; Gobosho; Teseme, 2023; Hamid; Hamid, 2015), such as N-tridecyl acid, myristic acid, and palmitoleic acid (Table 5). The 'moringa' seed oil collected in BA had the lowest fatty acid content. The differences in the fatty acid composition of *M. oleifera* oil may be related to the agro-climatic characteristics of the cultivation areas (Leone et al., 2016; Özcan, 2020). Also, processing conditions and moisture content can affect oil yield during extraction (Faisal et al., 2020; Fakayode; Ajav, 2016). 'Moringa' seed oil analyzed in this study is rich in unsaturated fatty acids and low in polyunsaturated fatty acids, which provides excellent oxidative stability in comparison to other oils also rich in oleic acid (Bhutada et al., 2016; Gharsallah et al., 2022; Nebolisa et al., 2023).

Table 4: Classes of substances found in *M. oleifera* oil.

Substances	Retention times	Percentage
Bahia		
Hydrocarbon	4.38	3.67
Not identified	4.34	5.94
Hydrocarbon	4.75	12.54
Hydrocarbon	6.36	18.48
Unsaturated acids	31.14	59.36
Rio Grande do Norte		
Unsaturated acids	31.19	88.53
saturated acids	31.52	6.57
Not identified	41.1	4.9
Paraíba		
Hydrocarbon	4.37	15.36
Not identified	4.43	8.81
Unsaturated acids	31.6	75.83
Ceará		
Not identified	4.385	4.29
Unsaturated acids	31.164	70.90
Not identified	31.234	5.90
Not identified	31.871	18.21

According to Normative Instruction No. 49 of the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA, 2006) and No. 87 of the Brazilian Health Regulatory Agency (Agência Nacional de Vigilância Sanitária, 2021), the optimal levels of acidity and saponification index are 0.20-0.60 mg KOH g^{-1} and ≥180 mg KOH g^{-1}, respectively, for the use of oil in the food industry. The saponification index of 'moringa' oil from BA and RN seeds is within this norm, and other parameters must be adjusted during the oil refining. Regarding the use of 'moringa' oil in the biofuel industry, the acidity index determines the transesterification process of vegetable oil because biodiesel yield is substantially reduced when the acidity index exceeds 1 mg KOH g^{-1} (Marques et al., 2019; Salaheldeen et al., 2014). Thus, only the oil extracted from the RN seeds could be converted into biodiesel without refining.

Table 5: Fatty acids (FA) in *M. oleifera* oil.

Unsaturated FA	Structural formula	Common name
Bahia (BA)		
7-hexadecenoic acid	$C_{17}H_{32}O_2$	Palmitoleic acid
6-octadecenoic acid	$C_{19}H_{36}O_2$	Petroselinic acid
9-octadecenoic acid	$C_{19}H_{36}O_2$	Oleic acid
1,7-octadecadienoic acid	$C_{19}H_{34}O_2$	Linolenic acid
Docosanoic acid	$C_{23}H_{46}O_2$	Behenic acid
Paraíba (PB)		
11-octadecenoic acid	$C_{19}H_{36}O_2$	Cis-vaccenic acid
7-hexadecenoic acid	$C_{17}H_{32}O_2$	Palmitoleic acid
9-octadecenoic acid	$C_{19}H_{36}O_2$	Oleic acid
6-octadecenoic acid	$C_{19}H_{36}O_2$	Petroselinic acid
cyclopropane pentanoic	$C_{20}H_{38}O_2$	-
Ceará (CE)		
Cyclopropane pentanoic acid	$C_{20}H_{38}O_2$	-
11-octadecenoic acid	$C_{19}H_{36}O_2$	Cis-vaccenic acid
3-docenoic acid	$C_{24}H_{46}O_2$	-
Cyclopropane pentanoic acid	$C_{23}H_{44}O_2$	-
9-octadecenoic acid	$C_{19}H_{36}O_2$	Oleic acid
Rio Grande do Norte (RN)		
9-octadecenoic acid	$C_{19}H_{36}O_2$	Oleic acid
7-hexadecenoic acid	$C_{17}H_{32}O_2$	Palmitoleic acid
11-octadecenoic acid	$C_{19}H_{36}O_2$	Cis-vaccenic acid
6-octadecenoic acid	$C_{19}H_{36}O_2$	Petroselinic acid
10-octadecenoic acid	$C_{19}H_{36}O_2$	-
Hyeneicosanoic acid	$C_{22}H_{44}O_2$	N-henoicosóico acid
Tridecanoic acid	$C_{15}H_{30}O_2$	N-tridecyl acid
Tetradecanoic acid	$C_{16}H_{32}O_2$	Myristic acid
Docosanoic acid	$C_{23}H_{46}O_2$	Behenic acid

Canonical analysis of variables

A canonical analysis of variables and confidence ellipses for mean scores of the first two canonical variables were used to verify the contribution of each variable to the difference among the seeds collected in each location (Figure 1). The mass of 100 almonds (MA), the mass of 100 seeds (MS), almond diameter (AD), and seed diameter

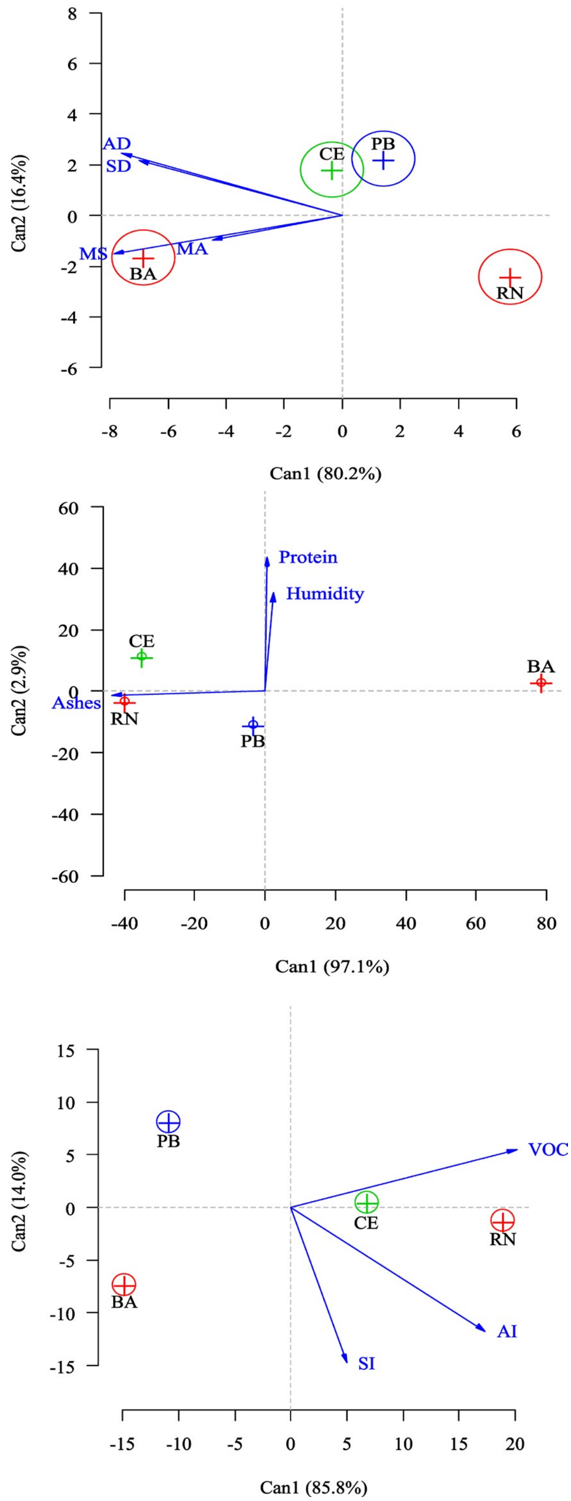

Figure 1: Canonical variables analysis and confidence ellipses for the first two canonical variables of moringa seeds collected in four states in Northeastern Brazil.

(SD) have close relation with the seeds collected in the state of BA and distant relation with seeds of RN. The content of ashes was more important for seeds collected in the states of RN and CE. Vegetable oil content (VOC), acidity index (AI), and saponification index (SI) were more important for seeds collected in the state of RN.

CONCLUSIONS

M. oleifera seeds from RN were the smallest in size but had higher contents of mineral salts (ashes), oil, and monounsaturated fatty acids, which provides less degradation of this oil. The seeds of *M. oleifera* from CE have the highest protein content. Thus, 'moringa' cultivation can be a viable alternative in these regions, as their seed oil can be used in different industries.

AUTHOR CONTRIBUTION

Conceptual idea: Chaves, J.T.L.; Souto, J.S.; Methodology design: Chaves, J.T.L.; Souto, P.C.; Souto, J.S.; Data collection: Chaves, J.T.L.; Silva, T.I.; Data analysis and interpretation: Chaves, J.T.L.; Silva, T.I.; Bicalho, E.M., and Writing and editing: Chaves, J.T.L.; Silva, T.I.; Bicalho, E.M.; De Paula, A.C.C.F.F.; Souto, P.C.; Souto, J.S.; Guidance and supervision: Souto, J.S.

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