da S. Alves et al. Nutrire (2017) 42:31
DOI 10.1186/s41110-017-0058-6

Nutrire

RESEARCH Open Access

Physicochemical characterization and antioxidant activity of decolorized *Moringa oleifera* Lam leaf flour

A. da S. Alves[1], E. M. B. Teixeira[1,2*], G. C. Oliveira[1], L. Arantes-Pereira[1], C. C. Oliveira[1] and L. L. Costa[1]

Abstract

Background: *Maringa oleifera* leaves are rich in antioxidant substances; however, when lyophilized leaves were used in flour form in meat products, they presented no antioxidant effect and even accelerated the oxidation process of the product. Thus, the objective of this study was to evaluate the effect of chlorophyll extraction on the physicochemical composition and antioxidant activity of Moringa leaves.

Methods: Moringa leaves were dried and ground in order to obtain uniform flour. A treatment using chlorophyll extraction (decolorized) was tested versus a control treatment (non-decolorized) for proximate composition, instrumental color, and antioxidant activity using ANOVA followed by Tukey's test.

Results: Higher crude fiber, ash, and protein contents were observed for decolorized flour (19.41 and 38.13%, 11.87 and 14.02%, and 28.81 and 31.33%, respectively) when compared to those for the control. Chlorophyll extraction significantly affected ($p < 0.05$) the instrumental color of the leaves flour. The half maximal effective concentration (EC50) of both decolorized and control flour was 3.74 and 4.30 mg/L, respectively. The equivalent of antioxidant per gram of non-decolorized leaves was higher than that observed for the decolorized leaves (0.36 and 0.32 g/g DPPH, respectively). The antioxidant activity (AA%) of the extract from non-decolorized leaves was higher in the concentrations of 5 and 2.5 mg/0.1 mL, while the decolorized leaves was higher in the extract concentration 5 and 2 mg/0.1 ml.

Conclusion: The decolorization process affected the chemical composition and color of *Moringa oleifera* leaves flours however did not improve its antioxidant activity.

Keywords: Antioxidant activity, Chlorophyll, Chemical characterization, *Moringa oleifera* Lam

Background

Considering the increasing demand for healthy foods with added technological value, the insertion of plant products containing bioactive compounds has been a promising alternative for the food industry and Moringa is a potential plant still underutilized.

Moringa *(Moringa oleifera* Lam)is a perennial species, belonging to the *Moringaceae* family from the Indian northwest. It is recognized for its food and nutritional value, with forage, medicinal, and seasoning properties, being used in culinary, fuel, and cosmetics industries and in water treatment for human consumption [1].

Maringa leaves are rich in antioxidant substances such as polyphenols, quercetin, and kaempferol [2, 3]. However, when lyophilized leaves were used in flour form in beef burgers, they presented no antioxidant effect and even accelerated the oxidation process of the product [4]. This behavior could be related to two factors: the photo-oxidation mechanism of fats promoted by UV radiation in the presence of photosensitizers, such as chlorophyll, found on Moringa leaves [5] and the presence ofβ-carotene that can act as a pro-oxidant agent [6]. In other words, the presence of pigments could affect the antioxidant activity of Moringa leaves flour. This study aimed to evaluate the impact of the decolorization on the antioxidant activity of Moringa leaves flour.

* Correspondence: estelamar@iftm.edu.br
[1]IFTM – Instituto Federal do Triângulo Mineiro, Campus Uberaba, Uberaba, Brazil
[2]Rua Panamá, 75 Fabrício 38067-320, Uberaba, MG, Brazil

Methods

The experiment was conducted in the Food Analysis Laboratory at Federal Institute of Education, Science and Technology Campus Triangulo Mineiro Uberaba, MG, using *Moringa oleifera* Lam leaves collected from trees planted in the fruit sector.

Preparation of leaves flour

Moringa leaves were randomly collected, cleaned with soap and water, and 200 ppm hypochlorite for 15 min in the Food Analysis laboratory. Excess water was removed by manual centrifugation, followed by drying in an oven with circulation and air renewal for approximately 28 h at 40 °C. Throughout the process, the material was homogenized to ensure uniform drying. Subsequently, the leaves were ground in an industrial blender (Vitalex LI-02) for about 1 min and passed through a mill (TecnalTE-648). This non-decolorized powder (control sample) was vacuum-packed and stored at 25 °C (Fig. 1).

Decolorization of leaves flour

Chlorophyll extraction was performed in triplicate as described by Sinnecker et al. [7], using 5 g of sample. The sample was homogenized with 30 mL of 95% ethanol and filtered under vacuum. The residue was immersed in 95% ethanol two more times to ensure complete decolorization and dried at 25 °C. This powder was named as decolorized flour.

Physicochemical characterization

The flours were characterized for moisture, ash, crude protein, total lipids, crude fiber, total carbohydrates, energy value, and color, in triplicate.

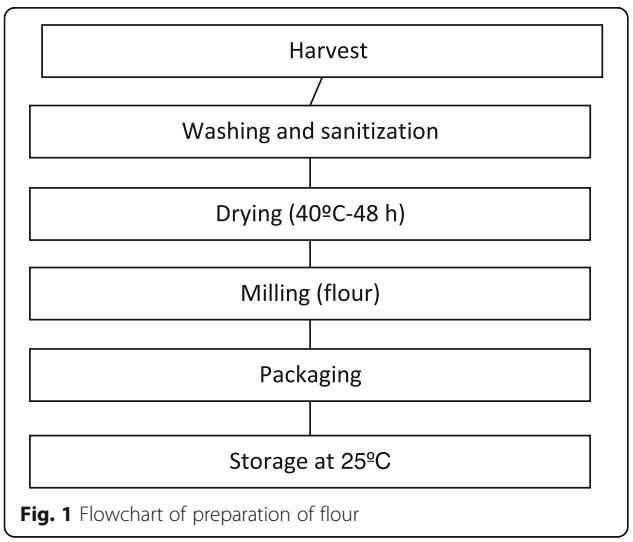

Fig. 1 Flowchart of preparation of flour

Table 1 Chemical composition and energy value of *Moringa oleifera* Lam flours in 100 g of dry matter

Determination	Control	Decolorized
Moisture	$6.60^{a} \pm 0.35$	$4.28^{b} \pm 0.25$
Ash	$11.87^{c} \pm 0.04$	$14.02^{a} \pm 0.01$
Lipids	$8.97^{a} \pm 0.18$	$2.83^{b} \pm 0.05$
Crude protein	$24.14^{c} \pm 0.54$	$31.33^{a} \pm 0.13$
Total fiber	$19.41^{c} \pm 0.32$	$38.13^{a} \pm 0.09$
Total carbohydrates[1]	$28.81^{a} \pm 1.11$	$9.08^{c} \pm 0.09$
Energy value[2]	$295.23^{a} \pm 1.74$	$187.41^{c} \pm 0.31$

Means followed by the same letter ± standard deviation do not differ by Tukey's test $p \leq 0.05$
[1]Total carbohydrates calculated by difference
[2]Calculated from Atwater Conversion factor: 4 kcal (proteins and carbohydrates) and lipids 9 kcal. Different small letters on the line indicate significant difference ($p < 0.05$)

Determination of moisture

The moisture content was determined by drying method at 105 °C until constant weight, according to A.O.A.C. method 31.1.02. [8].

Determination of crude fiber

Crude fiber content was determined by digestion of the samples, according to the A.O.A.C. method [8].

Determination of ash

Ash content was determined by incineration in a muffle at 550 °C according to the A.O.A.C method 31.1.04 [8].

Determination of protein

Total nitrogen was determined by the Kjeldahl method, according to A.O.A.C. method 31.1.08 [8], and the conversion factor of 6.25 was used to calculate the protein content.

Determination of lipids

Total lipids were determined by the Soxhlet extraction method using ethyl ether, according to the A.O.A.C. method 31.4.08 [8].

Determination of carbohydrates

Carbohydrates were calculated by the difference between 100 and the sum of moisture, ash, lipids, fiber, and protein contents, according to TACO [9].

Table 2 Color parameters of *Moringa oleifera* Lam flours

Flour	Parameter		
	L	*a*	*b*
Control	$56.70^{b} \pm 0.63$	$-10.55^{a} \pm 0.35$	$26.90^{a} \pm 1.11$
Decolorized	$73.64^{a} \pm 1.84$	$-1.04^{b} \pm 0.06$	$19.1^{b} \pm 0.66$

Different small letters on the column indicate significant difference ($p < 0.05$)

Table 3 EC values (mg/L) and equivalent in g leaves/g DPPH of *Moringa oleifera* Lam flours

Flour	EC 50 (mg/L)	g Flour/g DPPH
Control	4.30 ± 0.56	0.36 ± 0.03
Decolorized	3.74 ± 0.88	0.32 ± 0.02

Energy value

Energy value was calculated through the Atwater conversion factors: 4 kcal/g (protein), 4 kcal/g (carbohydrates), and 9 kcal/g (lipids), as reported by Osborne &Voogt [10].

Color measurements

The leaves' color was determined by MINOLTA Chroma Meter model CR-3000, L * a * b * CIELAB. The color parameters measured against the white plate were L = lightness (0 = black to 100 = white); a* = range from green to red (− 120 to + 120) = b* ranging from blue to yellow (− 120 to + 120).

Antioxidant activity by the DPPH scavenging activity

DPPH scavenging activity was determined according to the methodology described by Brand-Williams and Berset [11]. DPPH (1,1-diphenyl-2-picrilidrazil) is a stable free radical, which accepts an electron or a hydrogen radical to become a stable diamagnetic molecule, being reduced in the presence of antioxidants. The antioxidant activity was calculated in percentage, as follows: absorbance values at DPPH concentrations of 5, 2.5, 1.7, 1.27, and 1 mg/0.1 mL within 42 min. To evaluate the antioxidant activity, the EEP (ethanolic extracts of two samples—control and ethanol—-with the stable DPPH radical in an ethanol solution) was used. The DPPH radical has a characteristic absorption at 515 nm, which disappears after reduction by hydrogen pulled from an antioxidant compound.

The reaction mixture consisted of 0.5 mL sample, 3 mL absolute ethanol, and 0.3 mL of 0.3 mMDPPH radical in ethanol. The reduction of DPPH radical was measured by absorbance readings at 515 nm after 57 min of reaction. Antioxidant activity was expressed according to Eq. 1, as reported by Mensor et al. [12] described below:

$$\%AA = \{[AbsControl\text{-}(AbsSample - AbsBlank)] \times 100\}/AbsControl$$

where Aa = absorbance of the sample; Ab = absorbance of the blank; Ac = absorbance of control. Thus, the blank of different extracts concentrations was obtained for each sample. The blank of the sample was determined using 3.3 mL ethanol and 0.5 mL sample in each concentration, and the absorbance was read at 515 nm after 57 min of reaction. A tube containing 3 mL absolute ethanol, 0.5 mL of 70% ethanol, and 0.3 mL of 0.5 mM DPPH was used as a negative control.

Statistical analysis

Chemical determinations were submitted to a completely randomized design with two treatments and three repetitions, for a total of six plots. The effects of treatments were subjected to analysis of variance (ANOVA), and the means were analyzed by Tukey's test ($p \leq 0.05$). The results were statistically analyzed using the software SISVAR v.5.1 [3].

Results

The results for the chemical composition of decolorized *Moringa oleifera* flour are shown in Table 1. The control flour showed 24.14% protein content. The decolorized flour, in turn, showed higher value than the control sample, which was also observed for total crude fiber.

As shown in Table 2, significant differences were observed for the color parameters L*, b*, and a*. The decolorized flour had higher ($p < 0.05$) L* value and lower a* and b* values.

The decolorized and control flours showed EC50 values of 4.30 and 3.74 mg/L, respectively. The equivalent in a gram of control flour was 0.36 g/g of DPPH, which was greater than the value of 0.32 g/g found in the decolorized flour (Table 3).

The higher antioxidant activities (AA%) were observed on the concentrations 5 and 2.5 mg at 42 min for both flours. The control flour presented AA% values of 88.15

Table 4 Antioxidant activity (%) of *Moringa oleifera* Lam leaf extract at time 0, 7, 14, 21, 28, 35, and 42 min for the control sample

TIME	Concentration 5 mg	Concentration 2.5 mg	Concentration 1.7 mg	Concentration 1.27 mg	Concentration 1 mg
0 min	38.08 ± 1.02	37.92 ± 0.78	26.41 ± 0.30	19.44 ± .036	15.39 ± 0.24
7 min	64.39 ± 1.06	62.27 ± 0.56	44.87 ± 0.13	34.95 ± 0.82	28.78 ± 1.22
14 min	71.82 ± 2.18	70.03 ± 0.70	49.83 ± 0.70	39.25 ± 1.01	31.75 ± 0.14
21 min	77.48 ± 1.52	76.01 ± 1.09	53.34 ± 0.66	42.08 ± 0.72	34.09 ± 0.80
28 min	80.42 ± 0.88	79.11 ± .082	55.30 ± 1,70	43.39 ± 1.61	34.74 ± 1.06
35 min	82.87 ± 1.09	81.56 ± 1.44	56.77 ± 1.33	44.69 ± 1.41	35.39 ± 1,01
42 min	85.15 ± 1.72	84.01 ± 1.39	58.40 ± 1.60	45.51 ± 1.49	36.05 ± 1.55

As shown in Table 5, it was found that the extract without chlorophyll showed a high AA% values of 89.07 and 79.64%

Table 5 Antioxidant activity (%) of *Moringa oleifera* Lam leaf extract at time 0, 7, 14, 21, 28, 35, and 42 min for the sample decolorized with ethanol

TIME	CConcentration 5 mg	Concentration 2.5 mg	Concentration 1.7 mg	Concentration 1.27 mg	Concentration 1 mg
0 min	43.63 ± 1.07	38.37 ± 1.27	24.84 ± 2.16	18.63 ± 1.37	21.49 ± 1.11
7 min	63.62 ± 0.98	56.53 ± 1.04	36.37 ± 1.63	30.07 ± 2.28	32.28 ± 1.12
14 min	75.23 ± 0.70	66.51 ± 2.49	41.74 ± 1,26	36.13 ± 1.87	36.91 ± 0.60
21 min	80.89 ± 1.20	71.18 ± 1.02	44.22 ± 0.88	38.98 ± 1.02	39.13 ± 0.67
28 min	84.60 ± 1.64	75.15 ± 1.85	46.03 ± 0.97	41.31 ± 1.42	40.54 ± 1.46
35 min	86.87 ± 1.13	76.92 ± 1.18	47.20 ± 1.80	43.89 ± 1.61	41.17 ± 2.03
42 min	89.07 ± 1.03	79.64 ± 0.36	48.20 ± 0.81	47.60 ± 0.40	42.20 ± 1.80

and 84.01% (Table 4) while the decolorized flour showed AA% values of 89.07 and 79.64% (Table 5) for the concentrations of 5 and 2.5 mg, respectively.

Discussion

The control flour protein content was similar to the values reported by Gopalan [14] (27.2%) and Moyo et al. [15] (30.3%).

According to Sgarbieri [16], the extraction rates are dependent on temperature, solvent type, and pH, due to the influence of these factors on protein solubility. In addition, leaf protein extraction depends largely on the degree of cell disintegration to release proteins from different cellular compartments. The cell disruption occurs in three ways: impact, cutting, and application of differential pressure or by a combination of these principles depending on the equipment used for this purpose [17]. Ethanol has high diffusion capability through semi-permeable membranes, once it is very low in hygroscopicity and water miscibility, and recognized for its effectiveness as protein carrier [18], which provides flexibility in the process.

The fiber contents of the control (19.41%) and decolorized flour (38.13%) were greater than the values of 11.4% obtained by Moyo et al. [15] and 7.48% obtained by Brito; Teixeira [19]. Carbohydrates of decolorized flour with ethanol (9.08%) were similar to those reported by Passos et al. 2012 (12.54%). The high carbohydrates level is indicative of potentially energy plant (Moura et al. 2009).

Gopalakrishnan et al. [20] and Moura et al. (2009) studied the chemical composition of *Moringa* leaves without decolorization and found 75 and 77.30% moisture, 2.3 and 2.0% ash, 1.7 and 6.0% lipids, 6.7 and 6.4% protein, and 13.4 and 8.3% carbohydrates, respectively. It is worth noting that the values obtained by Gopalakrishnan et al. [20] were similar to those observed in this study for the decolorized sample.

The high L*value indicates that both flours have clear color. The negative a* values showed higher tendency to greenish and the control flour has more intense green color than the decolorized flour, while b* values suggest more prone to yellowing.

Lima [21] reported that the antioxidant potential of *Moringa* leaves may be due to its low EC50 values. Thus, decolorized flour had similar antioxidant activity to the control flour (Table 3).When these results were compared to other leaves and fruits studied by Vieira et al. [22] such as ora-pro-nobis (3.22 mg/mL), guabiroba (3.81 mg/mL), and acerola fruit (9.32 mg/mL), it was observed to lower EC50 and increase the antioxidant potential of *Moringa* leaves flours.

The antioxidant activities results suggest that decolorized *Moringa oleifera* flour has a similar antioxidant potential when compared to the control flour.

Conclusion

The decolorization process affected chemical composition and color of *Moringa oleifera* leaves flours however did not improve its antioxidant activity.

Acknowledgements
Not applicable.

Funding
The design of this study, as well as the collection, analysis, and interpretation of data, and the writing of this manuscript were funded by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) through the scholarship granted to the student André da Silva Alves.

Availability of data and materials
Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Authors' contributions
ASA contributed in the experimental analysis and collecting data. EMBT as the supervisor of the research contributed in all the process from the data collection to the writing step. GCO contributed in the decolorization process of the samples. LAP contributed in the experimental design planning, data interpreting, and English writing and reviewing. CCO and LLC contributed in all the laboratory analysis collecting data. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Received: 19 April 2017 Accepted: 19 October 2017
Published online: 31 October 2017

References

1. Bezerra, A.M.E.; Momenté, V.G.; Medeiros Filho, S.; Germinação de sementes e desenvolvimento de plântulas de moringa (Moringa oleifera Lam.) em função do peso da semente e do tipo de substrato. Hortic Brasil. v.22, n.2, p. 295–299, 2004.
2. Iqbal S.; Bhanger MI. Effect of season and production location on antioxidant activity of Moringa Oleifera leaves grown in Pakistan. J Food Compos Anal, v. 19, n. 6–7, p. 544–551, set. 2006.
3. Lako, J. et al. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods.Food Chem, v. 101, p. 1727–1741, 2007.
4. Teixeira, E. M. B. et al. Caracterização de hambúrguer elaborado com farinha de folhas de Moringa (Moringa oleífera Lam.). Revista da Sociedade Brasileira de Alimentação e Nutrição, v. 38, n. 3, p. 220–232, 2013.
5. Ramalho, V. C.; Jorge, N. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. Química Nova, v. 29, n. 4, jul. 2006.
6. Uenojo, M.; Maróstica Junior, M. R.; Pastore, G. M. Carotenóides: propriedades, aplicações e biotransformação para formação de compostos de aroma. Química Nova, v. 30, n. 3, p. 616–622, jun. 2007.
7. Sinnecker, P., Braga, N., Maccione, E.L.A., Lanfer-Marquez, U.M. Mechanism of soybean (*Glycine max* L. Merrill) Degreening related to maturity stage and postharvest drying temperature. Postharvest Biol. Technol., v.38, pp. 269–279, 2015.
8. AOAC – Association Official Analytical Chemists - Horwitz, W Official methods of analysis of the Association of Official Analytical Chemists.17ed. Arlington: AOAC Inc., v.1 e v.2, 2000.
9. TACO. Tabela brasileira de composição de alimentos/ NEPA-UNICAMP. – Versão II. Campinas: NEPA-UNICAMP; 2006. 105p
10. Osborne DR, Voogt P. The analysis of nutrient in foods. London: Academic; 1978. 47/156–158
11. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology. 1995; 28(1):25–30.
12. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research. 2001;15(2):127–30.
13. Ferreira DF. Análise estatística por meio do *SISVAR* para Windows versão 4. 0. In: Reunião Anual da Região Brasileira da Sociedade Internacional de Biometria. UFSCar, 45, 200. São Carlos. Anais São Carlos: UFSCar; 2000. p. 255–8.
14. Gopalan, C. Micronutrient malnutrition in SAARC, Boletín del NFI.Índia, 1994.
15. Moyo B, PJ M, Hugo A, Muchenje V. Nutritional characterization of Moringa (Moringa Oleifera lam) leaves. Afr J Biotechnol. 2011;10(60):12925–33.
16. Sgarbieri VC. Propriedades físico-químicas e nutricionais de proteínas de feijão (Phaseolus vulgaris, l.) var. Rosinha G2. Campinas: Faculdade de Engenharia de Alimentos e Agrícola, Universidade Estadual de Campinas; 1979.
17. Sgarbieri VC. Proteínas em alimentos protéicos: propriedades, degradações, modificações. São Paulo: Editora-Livraria Varela; 1996.
18. Ronen S. Alternative work schedules: selecting implementing and evaluation. Illinois: Dow Jones – Irwin; 1984.
19. Teixeira E M B Caracterização química e nutricional da folha de Moringa (Moringa oleíferaLam.). f. 94. 2012. Tese (doutorado) – Universidade Estadual Paulista. "Júlio de Mesquita Filho". Faculdade de Ciências Farmacêuticas. Programa de Pós Graduação em Alimentos e Nutrição. Araraquara, 2012.
20. Gopalakrishnan, P.K. et al, Drumstick (*Moringa oleifera*) a multipurpose Indian vegetable, Econ. Bot., v.34, n.3, pp 276 – 283, 1980.
21. De Lima A. Caracterização química, avaliação da atividade antioxidante in vitro e in vivo e identificação dos compostos fenólicos presentes no pequi (Caryocar brasiliense Camb.) In: 186 f. Tese (Doutorado em Bromatologia) - Faculdade de Ciências Farmacêuticas. São Paulo: Universidade de São Paulo; 2008. p. 2008.
22. Vieira LM, Bezerra MSS, Mancini-Filho J, Lima A. Total phenolics and antioxidant capacity "in vitro" of tropical fruit pulps. Revista Brasileira de Fruticultura. 2011;33:888–97.