Research, Society and Development, v. 11, n. 13, e331111335618, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i13.35618

Nutritional and bioactive compounds of *Amaranthus* spp. in Brazil

Compostos nutricionais e bioativos de *Amaranthus* spp. no Brasil

Compuestos nutricionales y bioactivos de *Amaranthus* spp. en Brasil

Received: 09/21/2022 | Revised: 09/28/2022 | Accepted: 09/30/2022 | Published: 10/08/2022

Krisnanda Kelly Castro de Souza
ORCID: https://orcid.org/0000-0001-6729-6991
Federal University of Lavras, Brazil
E-mail: krisnandasouza@gmail.com
Juliana Pace Salimena
ORCID: https://orcid.org/0000-0002-5374-0091
Federal University of Lavras, Brazil
E-mail: julianapsalimena@estudante.ufla.br
João Barcellos Xavier
ORCID: https://orcid.org/0000-0001-8058-7400
Federal University of Lavras, Brazil
E-mail: bxjoao@yahoo.com.br
Douglas Correa de Souza
ORCID: https://orcid.org/0000-0003-3956-1342
Federal University of Lavras, Brazil
E-mail: douglascorrea@ymail.com
Suzan Kelly Vilela Bertolucci
ORCID: https://orcid.org/0000-0001-8796-7043
Federal University of Lavras, Brazil
E-mail: suzan@ufla.br
Wanderley José Mantovani Bittencourt
ORCID: https://orcid.org/0000-0001-7418-1818
University Center of Lavras, Brazil
E-mail: wanderleyjose@unilavras.edu.br
Luciane Vilela Resende
ORCID: https://orcid.org/0000-0002-2014-4453
Federal University of Lavras, Brazil
E-mail: luciane.vilela@ufla.br

Abstract
The objective of this research was to evaluate the physicochemical characteristics of *Amaranthus* spp. commonly happening in Brazil, besides defining the efficiency of different extraction processes of the phenolic compounds and of the antioxidant activity. Five species of the genus *Amaranthus* (*A. spinosus*, *A. viridis*, *A. retroflexus*, *A. hybridus* var. *paniculatus* e *A. deflexus*) were evaluated in regard to their physicochemical characteristics such as vitamin C, soluble solids, pH, moisture, lipids and protein levels as well as the coloration of the leaves. Also, the phenolic compounds and the antioxidants were defined through four extraction processes (reflux; hydroalcoholic turbo-extraction; aqueous; and methanolic). The species *A. spinosus* has stood out for its vitamin C levels, pH and moisture, indicating the potential of the species when compared to the others. The leaf extracts of the five Amaranthus species present differences in the total phenol levels, flavonoids and hydro flavonoids when subject to different extraction methods. The antioxidant activities were favored by the reflux and hydroalcoholic turbo-extraction, with *A. viridis* standing out in the reflux methods and *A. spinosus* in the hydroalcoholic.
Keywords: Antioxidant activity; Extracting techniques; Food security; Non-conventional vegetable; Vitamin C.

Resumo
O objetivo deste trabalho foi avaliar as características físico-químicas de Amaranthus spp. comumente encontrados no Brasil, além de definir a eficiência de diferentes processos de extração dos compostos fenólicos e da atividade antioxidante. Cinco espécies do gênero *Amaranthus* (*A. spinosus*, *A. viridis*, *A. retroflexus*, *A. hybridus* var. *paniculatus* e *A. deflexus*) foram avaliadas quanto às suas características físico-químicas como vitamina C, sólidos solúveis, pH, umidade, níveis de lipídios e proteínas, bem como a coloração das folhas. Além disso, os compostos fenólicos e os antioxidantes foram definidos por meio de quatro processos de extração (refluxo; turboextração hidroalcóolica; aquoso; e metanólico). A espécie *A. spinosus* tem se destacado pelos teores de vitamina C, pH e umidade, indicando o potencial da espécie quando comparada às demais. Os extratos de folhas das cinco espécies de *Amaranthus* apresentam diferenças nos teores de fenóis totais, flavonoides e hidro flavonoides quando submetidos a

diferentes métodos de extração. As atividades antioxidantes foram favorecidas pelo refluxo e turbo extração hidroalcóolica, com destaque para *A. viridis* nos métodos de refluxo e *A. spinosus* no hidroalcóolico.
Palavras-chave: Atividade antioxidante; Técnicas de extração; Alimentação segura; Hortaliças não convencionais; vitamina C.

Resumen
El objetivo de este trabajo fue evaluar las características fisicoquímicas de *Amaranthus* spp. comúnmente encontrado en Brasil, además de definir la eficiencia de diferentes procesos de extracción de compuestos fenólicos y actividad antioxidante. Se evaluaron cinco especies del género *Amaranthus* (*A. spinosus*, *A. viridis*, *A. retroflexus*, *A. hybridus* var. *paniculatus* y *A. deflexus*) por sus características fisicoquímicas como vitamina C, sólidos solubles, pH, humedad, de lípidos y proteínas, así como el color de las hojas. Además, se definieron compuestos fenólicos y antioxidantes mediante cuatro procesos de extracción (reflujo; turboextracción hidroalcohólica, acuosa y metanólica). La especie *A. spinosus* ha sido destacada por los niveles de vitamina C, pH y humedad, indicando el potencial de la especie en comparación con las demás. Los extractos de hojas de las cinco especies de *Amaranthus* muestran diferencias en los niveles de fenoles totales, flavonoides e hidroflavonoides cuando se someten a diferentes métodos de extracción. Las actividades antioxidantes se vieron favorecidas por reflujo hidroalcohólico y turboextracción, con énfasis en *A. viridis* en los métodos de reflujo y *A. spinosus* en el hidroalcohólico.
Palabras clave: Actividad antioxidante; Técnicas de extracción; Comida segura; Vegetales no convencionales; Vitamina C.

1. Introduction

The genus *Amaranthus* includes around 70 species scattered across the globe, which are useful as food cultures and are cultivated both for leaves and seeds consumption (Silva et al., 2018; Zubillaga et al., 2020). In the past, some of these species were used in the feeding of rural populations, and, recently, its importance is restricted to the fact they are invasive plants. Sometimes, they can be found as food for small indigenous and quilombola populations, thus little known by the rest of the population being considered, in Brazil, as non-conventional vegetables (Ossani et al., 2020; Silva et al., 2018; Xavier et al., 2019).

Scientific studies aiming at the analysis of the nutritional potential of these species have proved that their leaves and grains present high levels of proteins, essential amino acids, carotenoids and minerals (Molina et al., 2018; Sarker & Oba, 2019; Silva et al., 2018; Sreelatha et al., 2012). The flour made out of these vegetables is an option for celiac people since it is gluten-free (Xavier et al., 2019). The use of their leaves has also been recommended for the treatment of various chronic degenerative disorders such as cancer, diabetes and heart diseases because of their antioxidant properties (Queiroz et al., 2009; Ramkisson et al., 2020).

In Brazil, there is a predominance of the species *A. viridis*, *A. spinosus*, *A. deflexus*, *A. hybridus*, *A. retroflexus* and *A. lividus* scattered all across the national territory (Francischini et al., 2019). However, in order to define, properly, the nature and/or the amount of compounds beneficial to the health in any vegetable species, it is necessary to examine the influence of the extraction process in the samples with different types of solvent and extraction methods (Azwanida, 2015) since the efficiency in the extraction of a specific compound can be related to the polarity of the solvent.

In view of the above, this work aimed at evaluating the physicochemical characteristics of Amaranthus spp. commonly found in Brazil, besides defining the efficiency of different extraction methods of the phenolic compounds and the antioxidant activity.

2. Methodology

The samples were collected from fresh leaves, in the middle portion, of the varieties of the genus Amaranthus from the germplasm collection of non-conventional vegetables of the Universidade Federal de Lavras (UFLA).

For the laboratory analysis, the experiment was done in completely randomized design replicated four times and five

species of the genus *Amaranthus* (*A. spinosus*, *A. viridis*, *A. retroflexus*, *A. hybridus* var. *paniculatus* and *A. deflexus*) were evaluated. Then, the levels of vitamin C, total soluble solids, pH, moisture, ethereal extract and crude protein were quantified according to AOAC (2019), and the coloration was evaluated by the coordinates L*, a* and b* (McGuire, 1992).

Fresh leaves in the middle portion of five varieties of the genus *Amaranthus* (*A. spinosus*, *A. viridis*, *A. retroflexus* [var. 1], *A. retroflexus* [var.2] and *A. hybridus* var. *paniculatus*) were used for the analyzes of phenolic compounds in four extraction processes (reflux; hydroalcoholic turbo-extraction (70%); aqueous turbo-extraction; and methanolic turbo-extraction), thus a factorial 5x4 in completely randomized design with three replications.

The quantification of total phenols was defined by the colorimetric method described by Slinkard and Singleton (1977), and the results were expressed in equivalent milligrams in gallic acid per g of fresh weight of leaf (mg EAG g-1). The quantification of flavonoids (flavones and total flavonols) was defined by the method of Ahn et al. (2007), and the results were expressed in equivalent milligrams in quercetin per gram of flesh leaf (mg EQ g-1). The quantification of dihydroflavonoids was carried out in accordance with Popova et al. (2004), and the results were expressed in equivalent milligrams in naringenin per g of fresh leaf (mg EN g-1). The total antioxidant capacity (TAC) was set in accordance with Prieto et al. (1999) and the results were expressed in equivalent milligrams in ascorbic acid per g of fresh leaf (mg EAG g-1). The DPPH radical scavenging was done in accordance with the Brand-Williams et al. (1995) protocol. The ABTS radical elimination was done in accordance with Oyaizu (1986), and the results were expressed as C_{50} mg mL-1 of the fresh leaf.

The results were analyzed with observations of the means and standard deviation, and the results were submitted to variance analysis. The means were compared through the Scott Knott test with help of the SISVAR® software (Ferreira, 2011). The multivariate technique of multidimensional scaling (MDS) was used to analyze the variables regarding the levels of phenolic compounds and the antioxidant potential of the species, using the statistical analysis system R Core Team (Team, 2013).

3. Results

The levels of vitamin C observed in the five species varied in the range of 109.20 to 174.18 mg $100g^{-1}$ (Table I), with the species *A. spinosus* and *A. hybridus* var. *paniculatus* not differing statistically and having the highest values (mean of 173.11 ± 1.50 mg 100g-1) and *A. deflexus* having the lowest levels (109.20 mg 100g-1).

The total soluble solids levels found in the five species varied in the range of 0.67 to 9.00 (Table I), with *A. viridis* e *A. hibrydus* presenting the lowest levels. Meanwhile, *A. spinosus* and *A. deflexus* did not differ statistically, occupying the average position for this characteristic (6.67 and 6.00 %, respectively). In the meantime, in *A. retroflexus* it was found the highest levels (9.00 %).

The pH values found in the five species varied in the range of 6.49 to 7.23, with *A. spinosus* having the highest value (7.23) in comparison with the others as it can be observed in Table 1. Although there were significant differences among some species, it can be said that the leaves of these vegetables contain low acidity.

The moisture level present in the five *Amaranthus* species varied in the range of 74.25% to 82.74%. *A. deflexus* had the highest mean for this characteristic: 82.74%. The lipids levels observed in the *Amaranthus* species did not show significant difference. In relation to the protein levels obtained from the species, it was not possible to observe significant differences among them. The values varied in the range of 4.05% in *A. retroflexus* to 4.84% in *A. spinosus* and *A. deflexus* (Table 1).

Table 1. Nutritional composition in five species of *Amaranthus* spp.

Species	Vitamin C (mg 100g^{-1})	Soluble Solid (%)	pH	Umit (%)	Lipid (%)	Proteins (%)
A. spinosus	174,18 a	6,67 b	7,23 a	81,06 a	0,96 a	4,84 a
A. viridis	136,55 b	0,67 c	6,88 c	77,79 c	0,80 a	4,09 a
A. deflexus	109,20 b	6,00 b	6,71 c	82,74 a	1,19 a	4,84 a
A. retroflexus	156,92 b	9,00 a	6,95 b	80,45 b	0,95 a	4,05 a
A. hybridus	172,05 a	0,67 c	6,49 d	74,25 d	0,97 a	4,42 a
CV (%)	12,21	5,77	1,16	0,80	26,93	8,68

Means followed by the same letter in the column do not differ from each other by the Scott-Knott Test. Source: Authors.

When it comes to the color of these species, few differences were observed. The species *A. deflexus* had the highest value (47.56) which suggests darker-colored leaves, and *A. hybridus* var. *paniculatus* the lowest (33.84) which suggests higher brightness, while the other species occupied a middle position for such characteristic (Table 2).

The Chroma values are related to the color intensity if the vegetable has a more or less vibrant color. For this characteristic, A. *hybridus* var. *paniculatus* stood out with the value of 21.14, and the other species did not differ statistically and had values varying in the range of 11.90 (*A. spinosus*) to 16.60 (*A. deflexus*), indicating the *A. hybridus* var. *paniculatus* has the most vibrant color among the species (Table 2).

Table 2. Coloring by coordinates L*, a* and b* in five species of *Amaranthus.*

Espécies	L	a*	b*	Chroma	º hue
A. deflexus	47,56 a	-8,27 b	14,33 a	16,60 b	119,11 a
A. viridis	35,27 b	-5,19 b	14,13 a	14,75 b	109,11 b
A. retroflexus	44,93 a	-9,27 b	14,00 a	16,08 b	121,58 a
A. hybridus	33,84 b	19,72 a	12,41 a	21,14 a	33,66 c
A. spinosus	44,34 a	-6,24 b	11,03 a	11,90 b	123,16 a
CV (%)	10,28	11,12	11,12	12,57	4,68

Means followed by the same letter in the column do not differ from each other by the Scott-Knott Test. Source: Authors.

For the values of the Hue angle, which indicates the tonality of the leaves, *A. hybridus* var. *paniculatus* had the lowest value of 33.66 and differed statistically from the others. There were no significant differences among *A. deflexus* (119.11), *A. retroflexus* (121.58) and *A. spinosus* (123.16) since these three species have close tonality in the leaves.

The total phenol levels, flavonoids and dihydroflavonoids of the leaf extracts of the Amaranthus species were significantly affected by the extraction methods as shown in Table 3. It is possible to observe that, no matter the extraction method, the species had significant differences among them.

When analyzing the extract method/solvent, it is possible to observe that the hydroalcoholic turbo-extraction allowed higher extraction of phenols for the species *A. spinosus* (19.69 mg EAG g-1), *A. retroflexus* var. 1 (16.07 mg EAG g-1), *A. retroflexus* var. 2 (15.45 mg EAG g-1) and *A. hybridus* (13.30 mg EAG g-1). The reflux method extracted higher levels of total phenols in *A. viridis* (12.38 mg EAG g-1) while the hydroalcoholic (HT), aqueous (AT) and methanolic (MT) turbo-extractions did not show differences in the contents extracted.

In the flavonoid quantification, the hydroalcoholic turbo-extraction was, again, the method with the higher extraction rate in most of the species such as *A. spinosus* (20.37 mg EQ g-1), *A. retroflexus* var. 2 (19.67 mg EQ g-1), *A. retroflexus* var. 1 (19.43 mg EQ g-1) and *A. viridis* (17.81 mg EQ g-1). The only exception was *A. hybridus* var. *paniculatus* which obtained

high results through the methanolic turbo-extraction. Results of *Amaranthus retroflexus* var. 1 and 2 and *A. spinosus* did not differ between themselves in the hydroalcoholic turbo-extraction.

It was possible to observe in the quantification of dihydroflavonoids that the hydroalcoholic turbo-extraction was, also, a better extractor for most of the species: *A. spinosus* (4.05 mg EM g-1), *A. retroflexus* var. 2 (2.54 mg EM g-1), *A. hybridus* (2.32 mg EM g-1) in which the result did not differ statistically from the aqueous turbo-extraction (2.30 mg EM g-1) and *A. viridis* (2.35 mg EM g-1) in which it did not differ from the reflux (3.26 mg EM g-1). Only *A. retroflexus* var. 1 obtained better results through the methanolic turbo-extraction (3.05 mg EM g-1) and the aqueous turbo-extraction (2.77 mg EM g-1).

For the phenolic compounds, the multivariate technique of multidimensional scaling confirms that the species were more influenced by the hydroalcoholic turbo-extraction methods, being different form the other methods (Figure 1). This can be observed through the resulted presented in Table 3 where this method shows high values when compared to the others with 19.69 mg EAG g-1 in total phenols, 20.37 mg EQ g-1 in total flavonoids and 4.05 mg EM g-1 in dihydroflavonoids, unfolding as a more efficient method in the extraction of the phenolic compounds in these species.

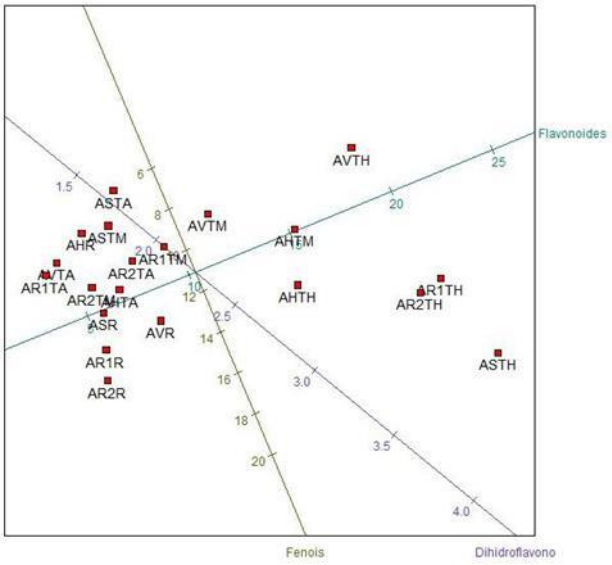
Table3. Contents of phenolic compounds present in the leaves of *Amaranthus retroflexus* var. 1 (AR1), *A. retroflexus* var. 2 (AR2), *A. spinosus* (AS), *A. hybridus* var. *paniculatus* (AH), *A. viridis* (AV) submitted to reflux extraction (R), hydroalcoholic (TH), aqueous (TA) and methanolic (TM) turbo-extraction.

		Processo de extração			
	Espécies	R	TH	TA	TM
Fenóis (mg EAG g^{-1})	AR2	14,13 aB	15,45 bA	9,50 bC	9,93 bC
	AS	11,17 cB	19,69 aA	6,35 dD	7,64 dC
	AH	7,45 dD	13,30 cA	10,46 aC	11,24 aB
	AV	12,38 bA	8,59 dB	8,29 cB	8,81 cB
	AR1	12,81 bB	16,07 bA	8,61 cD	9,36 cC
Flavonóides (mg EQ g^{-1})	AR2	4,77 bC	19,67 aA	7,80 aB	5,79 eC
	AS	5,88 bC	20,37 aA	8,31 aB	7,46 dB
	AH	6,18 bC	13,40 cB	6,68 bC	15,23 aA
	AV	7,85 aC	17,81 bA	4,59 cD	11,83 bB
	AR1	5,22 bC	19,43 aA	3,99 cC	9,30 cB
Dihidro (mg EN g^{-1})	AR2	1,40 bB	2,54 bA	1,59 bB	1,11 bB
	AS	1,38 bB	4,05 aA	1,30 bB	1,06 bB
	AH	1,39 bB	2,32 bA	2,30 aA	1,30 bB
	AV	3,26 aA	2,35 bA	1,36 bB	1,60 bB
	AR1	1,52 bB	2,20 bB	2,77 aA	3,05 aA

Lowercase letters in the column; capital letters in the line do not differ from each other by the Scott-Knott test. Source: Authors.

For some species, there were similarities among the results from the reflux, aqueous turbo-extraction and methanolic methods, separating these samples in a group apart. The sample *A. hybridus* var. *paniculatus* in the methanolic turbo-extraction (AHMT) stood out from the group above since it had higher flavonoid levels when compared to the other samples of this group (Figure 1).

Figure 1. Biplot with predictive axes considering the variables phenols, flavonoids and dihydroflavonoids of the samples treated in the different extractive methods.



Source: Authors.

When comparing the four species using EMD, it was verified that *A. spinosus*, in the hydroalcoholic turbo-extraction, has higher levels of phenolic compound than the other species studied. The extracts of *A. viridis* showed total phenol levels in the range of 8.29 to 12.38 mg EAG g-1 and flavonoid in the range of 4.59 to 17.81 mg EQ g-1.

The hydroalcoholic turbo-extraction was a method that obtained better results in the quantification of phenolic compounds for the species studied when compared to the other methods/solvents, especially for the *A. retroflexus* var. 2 and *A. spinosus* species.

The results of the DPPH, ABTS, CAT and chelating antioxidant tests were significantly affected by the extracting method as shown in Table 4.In the DPPH trial, *A. retroflexus* var. 2 and *A. spinosus* obtained higher results of radical scavenging through the hydroalcoholic turbo-extraction (IC_{50} 0,80 and 0,67 mg mL-1 respectively).In total phenols and flavonoids, these two species also obtained higher results in this same extracting method, especially in flavonoids, suggesting that this method/solvent directly interfered in the quantity/quality of the flavonoids that act in the DPPH radical scavenging. Even though it is not the only method/solvent responsible for the best activity in the other species, it is possible to notice that, in the hydroalcoholic turbo-extraction, all the species showed 50% of inhibition of the DPPH radical, and that the results did not differ among themselves.

In the ABTS, it is not possible to evaluate the antioxidant capacity of any compound with redox potential under the Trolox standard, and, in this test, all the species showed capacity to inhibit 50% of the ABTS radical (Table 4). The reflux system provided better results in all the species. Only in *A. spinosus*, A. hybridus and *A. retroflexus* var. 1 the results in reflux did no differ statistically from the ones in the hydroalcoholic turbo-extraction (IC_{50} 1.30; 2.28 and 1.61 mg mL-1 respectively). The ABTS trial has action mechanisms similar to DPPH; however, the species showed better results in different extraction methods/solvents, suggesting that the type of oxidant acting in this radical were different.

In total antioxidant capacity, through the reduction of ammonium molybdate, there was not a single method responsible for the best results in the species (Table 4). *A. spinosus* showed results above the other species' through the aqueous turbo-extraction (2.73 mg EAA g-1), supporting the results of total phenols where this same species showed high results when compared to the others. Next, we have this same species in reflux (1.55 mg EAA g-1), *A. retroflexus* var. 2 in the hydroalcoholic turbo-extraction with 1.22 mg EAA g-1. Finally, in the methanolic turbo-extraction, the results among all the

species did not differ significantly, showing that, for this trial, the relation between method (turbo-extraction) and solvent (methanol) acted similarly in the results of the Amaranthus species.

Moreover, in the chelating power test, the results of the species were not determined by a single extracting method, but by various. When analyzing the species, it is possible to notice that *A. spinosus* showed better results when compared to the other species, obtained a IC_{50} 3.39 mg mL-1 through the reflux method.

The results showed that the solvent composed of ethanol 70% was responsible for a large part of the results in the Amaranthus species as well as the obtainment of extracts through a dynamic method, the turbo-extraction, where the material is ground in contact with the liquid.

Table 4. Antioxidant activities present in the leaves of *Amaranthus retroflexus* var. 1 (AR1), *A. retroflexus* var. 2 (AR2), *A. spinosus* (AS), *A. hybridus* var. *paniculatus* (AH), *A. viridis* (AV) submitted to reflux extraction (R), hydroalcoholic (TH), aqueous (TA) and methanolic (TM) turbo-extraction.

		Processo de extração			
	Espécies	R	TH	TA	TM
DPPH IC_{50} mg mL^{-1}	AR2	1,93 bB	0,80 aA	6,07 dD	2,67 bC
	AS	5,87 cD	0,67 aA	3,99 cC	1,46 aB
	AH	1,20 aA	1,23 aA	12,45 eB	1,46 aA
	AV	0,67 aB	0,99 aB	0,00 aA	2,30 bC
	AR1	1,34 aA	1,02 aA	1,62 bA	1,19 aA
ABTS IC_{50} mg mL^{-1}	AR2	1,48 cA	1,73 bB	2,81 bC	3,19 bD
	AS	1,26 bA	1,30 aA	1,79 aB	3,00 bC
	AH	2,37 dA	2,28 cA	4,00 dC	2,54 aB
	AV	1,06 aA	1,66 bB	3,66 cC	3,62 cC
	AR1	1,48 cA	1,61 bA	1,95 aB	2,65 aC
CAT mg EAG g^{-1}	AR2	0,92 bA	1,22 aA	0,42 bB	0,41 aB
	AS	1,55 aB	0,93 bC	2,73 aA	0,20 aD
	AH	0,38 cB	0,68 bA	0,19 bB	0,52 aA
	AV	0,96 bA	0,86 bA	0,23 bB	0,45 aB
	AR1	0,38 cB	0,98 bA	0,41 bB	0,59 aB
Quelante IC_{50} mg mL^{-1}	AR2	0,00 aA	6,56 aB	0,00 aA	7,42 aC
	AS	3,39 bA	6,64 aC	4,64 bB	13,66 dD
	AH	0,00 aA	8,12 cB	20,36 cD	9,28 bC
	AV	0,00 aA	10,31 dC	0,00 aA	7,44 aB
	AR1	19,53 cC	7,35 bA	36,72 dD	11,36 cB

Lowercase letters in the column; capital letters in the line do not differ from each other by the Scott-Knott test. Source: Authors.

The species *A. spinosus* stood out among the others because it showed better values in the antioxidant activities in DPPH, CAT and chelating power. These activities are related to the high levels of phenolic compounds presented in Table III.

For the multivariate technique of multidimensional scaling in the tests of DPPH and ABTS radicals' elimination, the samples behaved similarly in the biplot graph space, proving the similarity and coherence of the DPPH and ABTS trials (Figures 2A and 2B).

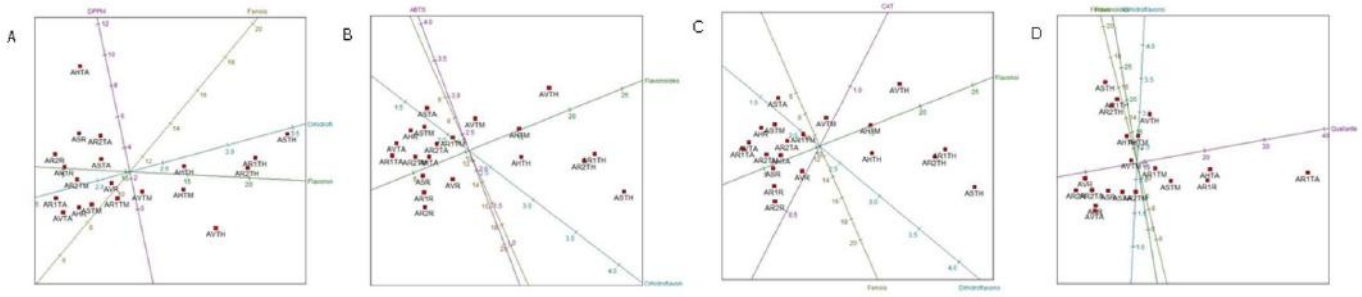
The predictive axis represented by the CAT variable (Figure 2C) did not go through changes when comparing to the other results of the biplot, showing, always, the same sample separation patterns, indication with that, also, an answer pattern from the solvents in front of the the various antioxidant tests.

In the chelating power trial, A. *spinosus*, in the reflux method, presented the best result with IC_{50} 3.39 mg mL-1,

followed by the same species in the aqueous turbo-extraction with IC_{50} 4.64 mg mL-1, *A. retroflexus* var. 2 (IC_{50} 6.56 mg mL-1) and *A. spinosus* (IC_{50} 6.64 mg mL-1) in the hydroalcoholic turbo-extraction, and, finally, *A. retroflexus* var. 2 (IC50 7.42 mg mL-1) and *A. viridis* (IC50 7.44 mg mL-1) in the methanolic turbo-extraction. It is also important to point out that the CAT and chelating power trials presented the same answer pattern in the method-solvent relation once, in both trials, the reflux and the methanolic turbo-extraction were more selective to *A. spinosus*, with the hydroalcoholic turbo-extraction doing the same for *A. retroflexus* var. 2. Thus, it is possible to infer that the phenolic compounds present in these species were responsible for this event in both tests.

In the chelating power trial, it is also possible to notice that the insertion of this variable (Figure 2D) triggered a dispersal of the samples in the biplot. This happens because the samples *A. retroflexus* var.2 (in reflux and aqueous turbo-extraction), *A. hibridus* (in reflux) and *A. viridis* (in reflux and aqueous turbo-extraction) did not hit the C_{50} values. Only *A. spinosus* and *A. retroflexus* var.1 presented IC_{50} for all the solvents used, as seen in Table 3.

Figure 2. Biplot with predictive axes considering the variables (A) DPPH, phenols, flavonoids and dihydroflavonoids; (B) ABTS, phenols, flavonoids and dihydroflavonoids; (C) CAT, phenols, flavonoids and dihydroflavonoids; and (D) chelating, phenols, flavonoids and dihydroflavonoids from the samples treated in the different extractive methods



Source: Authors.

4. Discussion

Vitamin C has antioxidant potential and takes part in the synthesis of some amino acids and hormonal compounds of the nervous system besides increasing the bioavailability of iron since it keeps it in its reduced form (Fe^{2+}). The deficiency of this vitamin can lead to certain diseases such as scurvy. The need of vitamin C is in the range of 25 mg to 30 mg per 1000 kcal and must be obtained through the ingestion of vegetables and fresh fruits since humans do not possess the capacity of synthesizing it (Silva et al., 2018; Vannucchi & Rocha, 2012).

The vitamin C levels found in the Amaranthus species is, in general, higher than the ones observed by Moraes et al. (2010) in vegetables commonly consumed, with *A. spinosus* and *A. hybridus* var. *paniculatus* standing out. These authors quantified the vitamin C losses due to processing and, initially, they obtained the value of this vitamin in fresh vegetables with no treatment whatsoever. They found 16.52 mg 100g-1 in lettuce, 11.60 mg 100g-1 in chicory, 34.50 mg 100g-1 in cabbage, 19.07 mg 100g-1 in tomato, 2.21 mg 100g-1 in carrot and 152.00 mg 100g-1 in kale. With this information it is possible to say that the five Amaranthus species are potential sources of vitamin C.

The sweet flavor of the vegetables depends on the relative amount of sugar, being directly connected to the soluble solids' levels. It is import to point out that the higher the levels of soluble solids in the freshly harvested vegetables, the longer the period in which their quality can be preserved (Covre et al., 2020).

Food is classified as low acidity (pH > 4.50), acid (pH in the range of 4.00 to 4.50) and very acid (pH < 4.00). The information about acidity is important in the characterization of food and in the realization of after-harvest studies since it is

related to the proliferation of microorganisms, adding, therefore, to the final quality of the products (Alp & Bulantekin, 2021). There are some studies of inactivation of microorganisms by dipping or soaking a product in ascorbic acid, citric acid, lactic acid, and acetic acid. These solutions can help reduce the number of normal flora and pathogenic microorganisms and also reduce the enzyme activity that causes browning (Alp & Bulantekin, 2021).

Generally, the lipid content found in fruits is low, around 0.1 to 0.6%; with the exception of some fruits, such as, for example, avocado, which has 8.4% of lipids (Krumreich et al., 2015). The levels observed in the Amaranthus species was higher than the ones found in some vegetables such as *Lactuca sativa* var. crispa (0.2%), *Cichorium intybus intybus* (0.2%), broccoli (0.3%), spinach (0.2%), collard greens (0.5%), common sowthistle (0.7%), and equal to the arrow leaf elephant ear (0.9%) and *Solanum tuberosum* 'Doré'(0.9%) (TBCA, 2020). Although the values are high when compared to some vegetables, they are still low values and they are within what is usually observed in fruits and vegetables.

Fruits and vegetables have protein levels in the range of 1% to 2% and they play an important part in the conservation of living organisms as structural and metabolic functions (Silva et al., 2018).

The coloration and the brightness in the food are important attributes in their appearance. In the case of fruits and vegetables, the perceived freshness, a criterion related to sensory appeal, is one of the most important factors for buying this kind of food (Curvelo et al., 2019).

The Amaranthus species show nutritional potential and unfold as superior to fruits and vegetables consumed by the Brazilian population. However, there are still studies to be carried out related to these vegetables. The consume of these species is an excellent option as a source of nutritional compounds, especially for populations with lower purchasing power. Studies about the anti-nutritional factor of these species, such as protein inhibitors, calcium oxalate and others, are still incipient. Sreelatha et al. (2012)showed the presence of flavonoids, tannins and saponins in *A. paniculatus* which have chemoprotective effects, and can be used as antioxidant and antitumor agents.

Ahmed et al. (2013) found, for the leaves of this same species, appreciable phenol levels in the range of 2.81 to 3.61 g EAG 100g-1 and total flavonoids in the range of 5.42 to 18.4 g EQ 100g-1, in agreement with the results of this work.

According to (Boroski et al., 2015), during the extraction process, a series of factors are relevant to the results of phenolic compounds such as the extraction time, the extracting method and the solvent used. Also, he says that the use of mechanical agitation in the solid-liquid process helps in the cell rupture, fomenting higher obtainment of the phenolic compounds.

Yet, according to Boroski et al. (2015), the use of organic solvents, such as the mixes of ethanol and water or methanol and water, allows better results in the levels of antioxidant compounds, in accordance to the results of this work. In recent studies, others species of *Amaranthus* genus also showed different levels of phenolic compounds in relation to the species and to the extraction methods. In quantification test of total phenols, Routray et al. (2013) reported, for *A. gigantea*, 0.1 mg g-1 (methanol), 0.02 mg g-1 (ethanol), 0.42 mg g-1 (petroleum ether), and, in *A. tricolor*, 1.29 mg g-1 (methanol), 1 mg g-1 (ethanol) e 1.12 mg g-1 (petroleum ether), showing that this genus is rich in phenolic compounds and, possibly, beneficial to human health.

In DPPH tests, Zeashan et al. (2009), using 50% ethanolic extracts of *A. spinosus*, found IC_{50} 29 μg mL-1 and 336 ± 14.3 mg g-1 of total phenols equivalent in gallic acid. Routray et al. (2013), using methanolic extracts in 0.01%. obtained 7.01% ± 0.11 for *A. viridis* and 91.80% ± 0.195 for *A. tricolor*. Meanwhile, in ethanolic extracts in the same concentration, results of 73.39 % ± 0.11 in *A. viridis* and 77.01% ± 0.11 in *A. tricolor* were found. These results are in accordance with the current study, showing that *Amaranthus* spp. has the capacity to inhibit more than 50% of the DPPH radical in methanolic and ethanolic extracts.

Queiroz et al. (2009) noticed that most of the compounds that have antioxidant activity in *Amaranthus* species are more soluble in polar solvents such as the ethanol than in apolar solvents.

The vitamin C levels pointed out in the quantification might have also played a role in the antioxidant results of this species once the vitamin C is a powerful antioxidant. This species is also rich in other antioxidants frequently used in the food and pharmaceutical industries such as vitamin E, β-cyanines, β-xanthines, chlorophyll and carotenoids (Sarker & Oba, 2019).

According to Prieto et al. (1999), the evaluation through the phosphomolybdenum method measures the total antioxidant capacity and holds the advantage of evaluating lipophilic and hydrophilic components. Furthermore, this method allows to evaluate a complex mixture of compounds such as extracts and fraction obtained from plants (Merino et al., 2015).

The phenolic compounds and antioxidant activity levels of the Amaranthus species accentuate the importance of these vegetables in human diet once, when its natural physiological antioxidant capacity is exceeded, the natural antioxidant mechanisms can be reinforced by the consume of plants rich in antioxidant compounds such as the species used in this study.

The *Amaranthus spinosus* stood out for its vitamin C, pH and moisture levels, indication the potential of the species when compared to the others. For the lipid and protein levels, the five species studies were superior to the fruits and vegetables usually consumed by the Brazilian population. Meanwhile, when it comes to the characterization by the color, the species showed few differences, with *A. hybridus* var. *paniculatus* because of its vibrant color, with reddish leaves and intense tonality.

5. Conclusion

The leaf extracts of the five Amaranthus species presented differences in the total phenol, flavonoids and dihydroflavonoids levels when submitted to different extracting methods. The hydroalcoholic turbo-extraction method is the most efficient in the extraction of most of these compounds. The antioxidant activity, for most of these species, was favored by the reflux and hydroalcoholic turbo-extraction methods, with the *A. viridis* (reflux) and *A. spinosus* (hydroalcoholic turbo-extraction) standing out in most of the tests. For future research, we suggest that our studies involving different nutritional characteristics, chemical composition and methodologies involving antioxidant activities should be studied due to the great potential of these species.

Acknowledgments

The authors thank the Federal University of Lavras, Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brasil (CNPQ), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial and support assist.

References

Ahmed, S. A., Hanif, S., & Iftkhar, T. (2013). Phytochemical profiling with antioxidant and antimicrobial screening of Amaranthus viridis L. leaf and seed extracts. *Open Journal of Medical Microbiology*, *2013*.

Ahn, M.-R., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F., & Nakayama, T. (2007). Antioxidant activity and constituents of propolis collected in various areas of China. *Food Chemistry*, *101*(4), 1383-1392.

Alp, D., & Bulantekin, Ö. (2021). The microbiological quality of various foods dried by applying different drying methods: a review. *European Food Research and Technology*, *247*(6), 1333-1343.

Azwanida, N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants*, *4*(196), 2167-0412.
Boroski, M., Visentainer, J., Cottica, S. M., & Morais, D. d. (2015). *Antioxidantes: princípios e métodos analíticos* (Appris, Ed. 1 ed., Vol. 1).

Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.

Covre, E. A., Borba, K. R., Ferreira, M. D., Spoto, M. H. F., Sala, F. C., & Verruma-Bernardi, M. R. (2020). Physical-chemical and sensory characteristics of Brunela lettuce. *Revista Agrarian*, *13*(48), 265-272.

Curvelo, I. C. G., de Morais Watanabe, E. A., & Alfinito, S. (2019). Purchase intention of organic food under the influence of attributes, consumer trust and perceived value. *Revista de Gestão*.

Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciência e agrotecnologia*, *35*, 1039-1042.

Francischini, A., Constantin, J., Oliveira, R., Takano, H., & Mendes, R. (2019). Multiple-and cross-resistance of Amaranthus retroflexus to acetolactate synthase (ALS) and photosystem II (PSII) inhibiting herbicides in preemergence. *Planta Daninha*, *37*.

Krumreich, F. D., Corrêa, A. P. A., Silva, S. D. S. D., & Zambiazi, R. C. (2015). Composição físico-química e de compostos bioativos em frutos de Bromelia antiacantha Bertol. *Revista Brasileira de Fruticultura*, *37*, 450-456.

McGuire, R. G. (1992). Reporting of objective color measurements. *HortScience*, *27*(12), 1254-1255.

Merino, F., Oliveira, V., Paula, C., Cansian, F., Souza, A., Zuchetto, M., Hirota, B., Duarte, A., Kulik, J., & Miguel, M. (2015). Análise fitoquímica, potencial antioxidante e toxicidade do extrato bruto etanólico e das frações da espécie Senecio westermaniiDusén frente à Artemia salina. *Revista brasileira de plantas medicinais*, *17*, 1031-1040.

Molina, E., González-Redondo, P., Moreno-Rojas, R., Montero-Quintero, K., & Sánchez-Urdaneta, A. (2018). Effect of the inclusion of Amaranthus dubius in diets on carcass characteristics and meat quality of fattening rabbits. *Journal of Applied Animal Research*, *46*(1), 218-223.

Moraes, F. A., Cota, A. M., Campos, F. M., & Pinheiro-Sant'Ana, H. M. (2010). Vitamin C loss in vegetables during storage, preparation and distribution in restaurants. *Ciência & Saúde Coletiva*, *15*(1), 51.

Ossani, P. C., de Souza, D. C., Rossoni, D. F., & Resende, L. V. (2020). Machine learning in classification and identification of nonconventional vegetables. *Journal of Food Science*, *85*(12), 4194-4200.

Oyaizu, M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese journal of nutrition and dietetics*, *44*(6), 307-315.

Popova, M., Bankova, V., Butovska, D., Petkov, V., Nikolova-Damyanova, B., Sabatini, A. G., Marcazzan, G. L., & Bogdanov, S. (2004). Validated methods for the quantification of biologically active constituents of poplar-type propolis. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *15*(4), 235-240.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, *269*(2), 337-341.

Queiroz, Y. S. d., Soares, R. A. M., Capriles, V. D., Torres, E. A. F. d. S., & Áreas, J. A. G. (2009). Efeito do processamento na atividade antioxidante do grão de amaranto (Amaranthus cruentus L. BRS-Alegria). *Archivos Latinoamericanos de Nutrición*, *59*(4), 419-424.

Ramkisson, S., Dwarka, D., Venter, S., & Mellem, J. J. (2020). In vitro anticancer and antioxidant potential of Amaranthus cruentus protein and its hydrolysates. *Food Science and Technology*, *40*, 634-639.

Routray, R., Kar, M., & Sahu, R. K. (2013). Evaluation of antioxidant potential in selected leafy vegetables of Odisha, India. *Int. J. Pharm. Pharm. Sci*, *5*(1), 232-235.

Sarker, U., & Oba, S. (2019). Protein, dietary fiber, minerals, antioxidant pigments and phytochemicals, and antioxidant activity in selected red morph Amaranthus leafy vegetable. *PLoS One*, *14*(12), e0222517.

Silva, L. F. L. E., SOUZA, D. C., Resende, L. V., Nassur, R. D. C. M., Samartini, C. Q., & Gonçalves, W. M. (2018). Nutritional evaluation of non-conventional vegetables in Brazil. *Anais da Academia Brasileira de Ciências*, *90*, 1775-1787.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture*, *28*(1), 49-55.

Sreelatha, S., Dinesh, E., & Uma, C. (2012). Antioxidant properties of Rajgira (Amaranthus paniculatus) leaves and potential synergy in chemoprevention. *Asian Pacific Journal of Cancer Prevention*, *13*(6), 2775-2780.

Team, R. C. (2013). R: A language and environment for statistical computing.

Vannucchi, H., & Rocha, M. d. M. (2012). Funções Plenamente Reconhecidas de Nutrientes: Ácido ascórbico (Vitamina C). *São Paulo: Brasil International Life Sciences Institute do Brasil*.

Xavier, J. B., Andrade, D. B. d., Souza, D. C. d., Guimarães, G. C., Resende, L. V., & Guimarães, R. M. (2019). Morphological, chemical and physiological characterization of Amaranthus spp. Seeds. *Journal of Seed Science*, *41*, 478-487.

Zeashan, H., Amresh, G., Singh, S., & Rao, C. V. (2009). Hepatoprotective and antioxidant activity of Amaranthus spinosus against CCl4 induced toxicity. *Journal of ethnopharmacology*, *125*(2), 364-366.

Zubillaga, M. F., Camina, R., Orioli, G. A., Failla, M., & Barrio, D. A. (2020). Amaranth in southernmost latitudes: plant density under irrigation in Patagonia, Argentina. *Revista Ceres*, *67*, 93-99.