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BIOACCESSIBILITY OF METALLIC IONS AND PHENOLIC COMPOUNDS IN TEAS FROM *MICONIA ALBICANS*

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Abstract: The bioaccessibility of Al, Ca, Cr, Cu, Mg, Zn, phenolics and flavonoids during in vitro gastrointestinal digestion of Canela de Velho (Miconia albicans) tea was investigated in 10 samples. Determinations were made by atomic and molecular absorption spectrometry. Metal concentrations in Canela de Velho tea ranged from 343 ± 18 to 1163 ± 36 μg g-1 for Al, 102 ± 23 to 347 ± 13 μg g-1 for Ca, 0.093 ± 0.009 to 0.109 ± 0.003 μg g^{-1} for Cr, 0.30 ± 0.05 to 0.9 ± 0.2 μg g-1 for Cu, 264 ± 27 to 633 ± 53 μg g-1 for Mg and 1.3 ± 0.3 to 3.1 ± 0.4 μg g-1 for Zn, while the bioaccessibility percentages were from 62 ± 4 to 98 ± 3 % for Al, 26 ± 6 to 120 ± 3 % for Ca, 30.0 ± 1.0 to 62 ± 8% for Cr, 62 ± 2 to 90 ± 3% for Cu, 79 ± 8 to 108.2 ± 0.1% for Mg and 76 ± 5 to 114.0 ± 0.2% for Zn. Regarding organic compounds, the bioaccessibility values ranged from 70 ± 4 to 99 ± 11 % for phenolics and from 20 ± 4 to 92 ± 2 % for flavonoids.

Keywords: Cinnamon de Velho, cooking, metals, reducing action, bioaccessible fraction.

INTRODUCTION

The use of medicinal plants has been carried out for many years, and there are records of this practice around 3,000 BC (BRASIL et al., 2019). Medicinal plant teas are traditionally used because they have the ability to relieve the symptoms of some diseases, have lower costs and few side effects when compared to synthetic drugs (BALBINO & DIAS, 2010). Among a variety of plants with medicinal characteristics, Miconia albicans is popularly known in Brazil as Canela de Velho. Miconia albicans belongs to the Melastomataceae family, is a species native to Brazil. Found mainly in the Brazilian cerrado, the shrub can reach up to 3 m in height and has small pinkish fruits that turn green when ripe (ALLENSPACH & DIAS, 2012; OLIVEIRA, MOTA & AGNES, 2014; BRASIL, et al., 2019). Canela de Velho is easily found in stores in raw form, stems and dried leaves, and in by-products such as capsules, liquid extracts and ointments. It is popularly used to treat digestive disorders, back pain, arthritis, osteoarthritis, joint pain and inflammation. These activities can be explained by the presence of substances such as flavonoids, ursolic acid and oleanoic acid (OLIVEIRA, MOTA E AGNES, 2014; BRASIL, et al., 2019; IGLESIAS & FONSECA, 2022).

Metallic elements are also extracted during the tea preparation process and can be partially or fully bioaccessible and bioavailable to the body (POHL, et al., 2016). This is because in the digestion process the compounds undergo biotransformation processes. First they are released from their matrices, they are soluble in the gastrointestinal tract and then they are absorbed to carry out their action in the body. This action can be essential or toxic. Therefore, bioaccessibility refers to the amount of a nutrient or essential or non-essential compound, released from its matrix to the gastrointestinal tract and solubilized in body fluids during digestion (bioaccessible fraction), thus becoming potentially available for absorption in the small intestine (PEIXOTO, MAZONB & CADORE 2013; DE ANDRADE, et al., 2020).

Therefore, the present work aimed to determine the metallic elements (Al, Ca, Cr, Cu, Mg and Zn) and phenolic compounds in Canela de Velho teas using atomic absorption spectrometry and molecular absorption spectrophotometry, respectively, and to evaluate their bioaccessibility to the human organism, through gastrointestinal simulations.

METHODOLOGY

SAMPLING

The samples of Canela de Velho were acquired in the Brazilian market. In total, 10 samples were acquired (CV1 to CV10), and of

these, only the CV10 sample refers to a liquid extract of the plant (Table 1).

INSTRUMENTS AND REAGENTS

Determinations of the elements Al, Ca, Mg and Zn were performed using a Varian Flame Atomic Absorption Spectrometer (FAAS), model SpectraAA-220, equipped with Agilent hollow cathode lamps.

The determinations of Cr and Cu were carried out using an Atomic Absorption Spectrometer with Electrothermal Atomization in a Graphite Furnace (GF-AAS), (Varian, AA 240Z, GTA 120), equipped with a cross-sectional Zeeman corrector, autosampler (PSD 120), pyrolytic graphite tubes (Varian) and data acquisition system (SpectraA). Argon was used as an inert and carrier gas. To carry out the analyses, standard stock solutions of Al, Ca, Cu, Cr, Mg and Zn of 1000 mg L^{-1} (Biotec) with a purity of 99.9% were used. The concentration ranges used for the analytical curves and the instrumental parameters of analysis are presented in Table 2.

A UV-Vis Molecular Absorption Spectrophotometer (Spectrum) was used to determine the total flavonoid and phenolic contents. The wavelengths used for the determination of flavonoids and total phenolics were 425 nm and 760 nm, respectively.

All solutions were prepared using ultrapure water obtained by the Milli-Q system (Merck Millipore). All flasks and glassware used were properly decontaminated in a 5% (v/v) nitric acid bath for at least 24 hours (BUTIK, et al., 2018).

MOISTURE

To determine the moisture content, 1.0 g of sample were weighed in a porcelain crucible, heated in a drying oven at 105 °C for 15 min. Afterwards, the samples were cooled in a desiccator and weighed again. The operation was repeated until constant weight was obtained (IAL, 2008).

DRY DIGESTION OF SAMPLES

The calcination of the samples by dry digestion was performed according to method 393/IV of the Physico-Chemical Methods for Food Analysis (IAL, 2008). The samples were previously ground and weighed (1.0 g) in porcelain capsules. Then heated on an electric plate (~100 °C / 10 min) and then carbonized in a Bunsen burner. Finally, the samples were taken to a muffle furnace for 4 h at 450 °C. Afterwards, 1.0 mL of HNO3 was added and taken again in a muffle furnace for 4 h. The ash was solubilized with 1.0 mL of HNO_3 10 %.

SAMPLE COOKING PREPARATION

2.0 g of the samples were used to prepare the teas with the addition of 100 mL of ultrapure water. The mixture was heated to boiling and kept at this condition for 5 min. After cooling, the samples were stored in decontaminated plastic bottles and kept in a refrigerator (DE ANDRADE, 2018).

DETERMINATION OF IN VITRO BIOACCESSIBILITY OF AL, CA, CR, CU, MG AND ZN

To evaluate the bioaccessibility of metals in tea, in vitro assays were performed, simulating human digestion, according to the method described by De Andrade et. al. (2017). 20.0 mL of Canela de Velho cooking was taken. Afterwards, 2.5 mL of gastric juice was added (20 g L^{-1} of pepsin in 0.10 mol L^{-1} of HCl) and incubated for 2 h, under agitation at 150 rpm, at 37 °C. After 2 h, the solutions were placed in an ice bath to stop enzymatic digestion. Then, the pH was adjusted to 7.0 with a solution of $NaHCO_3$ (1,5 mol L^{-1}). Subsequently, 2.5 mL of intestinal juice

Sample	Origin	Form of commercialization
CV1	Guarapuava – PR	Original factory packaging
CV2	Piraquara – PR	Original factory packaging
CV3	Maringá – PR	In bulk
CV4	Planalto – PR	Original factory packaging
CV5	Guarapuava – PR	Original factory packaging
CV6	Paraná	In bulk
CV7	São Paulo	In bulk
CV8	São Paulo	In bulk
CV9	Marechal Cândido Rondon – PR	Original factory packaging
CV10	Marataízes- ES	Liquid extract

Table 1: Origin and form of commercialization of Canela de Velho samples.

Equipment	Metal	Analytical Curve*	Flame	$T_{pyrolysis}$ (°C)	$T_{atomization}$ (°C)	I (mA)	λ (nm)
FAAS	Al**	0,5-3,0	Acetylene/Nitrous Oxide	-	-	10,0	309,3
	Ca	0,5-3,0	Acetylene/Nitrous Oxide	-	-	10,0	422,7
	Mg	0,2-0,8	Air / Acetylene	-	-	4,0	285,2
	Zn	0,1-0,4	Air / Acetylene	-	-	5,0	213,9
GFAAS	Cr	2,0-10,0	-	1000	2600	7,0	357,9
	Cu***	3,0-15,0	-	800	2300	4,0	324,8

* The concentration units for Al, Ca, Mn and Zn are in mg L^{-1}, and for Cr and Cu are in μg L^{-1}

** For Al, 10% v/v of KCl was used at a concentration of 3.35 mol L^{-1} for the construction of the analytical curve and analysis of the tea samples, due to the refractory character of the metal.

*** For Cu, the analytical curve and samples were prepared in the presence of 0.1 mol L^{-1} of HNO_3.

Table 2 - Concentration ranges and atomization conditions for the metals in the cooking of Canela de Velho.

was added to the solution(0,15 g L^{-1} of pancreatin,1,50 g L^{-1} of bile salts and 0,10 mol L^{-1} of $NaHCO_3$), and incubated again under the same conditions as before. Finally, the samples were centrifuged for 20 min, with a rotation of 5000 rpm, at 4 °C.

The analytical curves for the quantification of metals were evaluated with an external standard (PE) and in an enzymatic medium (ME, gastric juice and intestinal juice) to verify possible interferences during the determination of the concentrations of metal ions in the bioaccessibility samples (Table 2).

The method was validated through measurements of limits of detection (LD), limits of quantification (LQ), repeatability and intermediate precision (ICH, 1996; RIBANI, et al., 2004; AOAC, 2012).

QUANTIFICATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS

To quantify the total levels of phenolic compounds, the Folin-Ciocalteau colorimetric method was used using an analytical curve (40.0 to 340 mg L^{-1}) of gallic acid in methanol (SCHMIDT, et al., 2014; SOARES, et al., 2015).

The quantification of flavonoid contents was performed by complexation with aluminum chloride at 5% m v^{-1} in methanol (SCHMIDT, et al., 2014; SOARES, et al., 2015). The analytical curve of quercetin (10.0 to 125.0 mg L^{-1}) was performed in methanol.

STATISTICAL ANALYSIS

In this work, all statistical analysis applied was performed at the 95% confidence level using the statistical software Action Free, version 2.7. From the data obtained, a Principal Component Analysis (PCA) was performed using the Statistica 8.0 softwares.

RESULTS AND DISCUSSION

The analytical curves of PE and ME showed similarities, as the slopes of the curves were similar, indicating that the PE curve can be used to determine the bioaccessible fractions, making the method faster and simpler (Table 3).

The results of linear regression, F test, lack of fit test and the coefficient of determination (R^2) were significant for the concentration range evaluated, since the observed values of F_{reg} were bigger than the $F_{critical}$ ($p < 0,05$) at the 95% confidence level. At the same confidence level, the linear model for the analytical curves did not show a lack of fit, as the observed values of F_{faj} were lower than the values of $F_{critical}$ ($p > 0,05$). The curves showed values of coefficient of determination (R^2) that ranged from 96.2 to 99.9, that is, for the correlation coefficients (r) the values ranged from 0.98 to 0.99, indicating a linear relationship between the absorbance signal and the concentrations of the studied metals (ARAÚJO, 2009).

Confidence intervals for the slopes of each curve were also evaluated (Table 4), at a confidence level of 95%, according to the equation b1 ± t(n-2) *EP, where b1 is the slope, t of the t-distribution with n-2 degrees of freedom and EP is the standard error of the slope (NETO; SCARMINIO; BRUNS, 2010). The results of the confidence intervals indicated that the slopes of the analytical curves in PE and in ME are considered similar, as their confidence intervals overlap. Therefore, the analytical curves using PE were chosen as a form of calibration.

The limits of detection (LD) and quantification (LQ) were considered adequate for the method, as they were very close to or below the first point of the analytical curve for each metal (Table 5).

The RSD values found for the evaluated metals ranged from 2.7% to 14.2% for intermediate precision, while for repeatability

Metal	Calibration	Line Equation	$F_{regression}$	p	F_{faj}	p	R^2
Al	PE	Abs: 0,000072 + 0,00489xC	563,91	0,000	0,22	0,809	98,9
	ME	Abs: -0,000658 + 0,00439xC	1725,33	0,000	1,66	0,298	99,7
Ca	PE	Abs: -0,00722 + 0,131xC	8244,90	0,000	1,33	0,361	99,9
	ME	Abs: 0,0138 + 0,146xC	869,24	0,000	0,26	0,780	99,3
Cr	PE	Abs: 0,00345 + 0,0118xC	201,71	0,000	0,00	0,999	96,2
	ME	Abs: 0,00196 + 0,0125xC	1643,47	0,000	0,58	0,653	99,5
Cu	PE	Abs: 0,00133 + 0,00413xC	1086,48	0,000	0,32	0,809	98,8
	ME	Abs: 0,00451 + 0,00423xC	2143,53	0,000	0,31	0,815	99,4
Mg	PE	Abs: -0,0118 + 0,442xC	1434,73	0,000	5,81	0,066	99,6
	ME	Abs: 0,0278 + 0,398xC	1787,72	0,000	0,53	0,624	99,7
Zn	PE	Abs: 0,00825 + 0,286xC	3694,62	0,000	1,65	0,300	99,8
	ME	Abs: 0,00988 + 0,317xC	500,36	0,000	1,55	0,318	98,8

Cr (2,0 – 10,0 ug L^{-1}) $F_{critical\ (0,05;1,8)} = 5,32$; $F_{critic\ faj\ (0,05;\ 3,5)} = 5,41$

Cu (3,0 – 15,0 ug L^{-1}) $F_{critical\ (0,05;\ 1,\ 13)} = 4,75$; $F_{critic\ faj\ (0,05;\ 3,\ 10)} = 3,71$

Al (0,5 – 3,0 mg L^{-1}); Ca (0,5 – 3,0 mg L^{-1}); Mg (0,2 – 0,8 mg L^{-1}); Zn (0,1 – 0,4 mg L^{-1}) $F_{critical\ (0,05;1,6)} = 5,99$; $F_{critic\ faj\ (0,05;\ 2,4)} = 6,94$

Table 3: Linear regression results for the analytical curves of PE and ME for Al, Ca, Cr, Cu, Mg and Zn.

Metal	External Standard (PE)		Enzyme Medium (ME)	
	Coefficient angular	Confidence interval	Angular coefficient	Confidence interval
Al	0,00489 ± 0,00051	0,00439 a 0,00539	0,00439 ± 0,00025	0,00413 a 0,00465
Ca	0,131 ± 0,004	0,128 a 0,135	0,146 ± 0,012	0,134 a 0,158
Cr	0,0118 ± 0,0019	0,0099 a 0,0137	0,0125 ± 0,0007	0,0118 a 0,0132
Cu	0,00413 ± 0,00027	0,00386 a 0,00440	0,00423 ± 0,00020	0,00403 a 0,00443
Mg	0,442 ± 0,029	0,414 a 0,471	0,398 ± 0,023	0,375 a 0,421
Zn	0,286 ± 0,012	0,275 a 0,298	0,317 ± 0,035	0,282 a 0,352

Table 4: Confidence interval for the angular coefficients of the analytical curves evaluated for Al, Ca, Cr, Cu, Mg and Zn.

Metal	Total Concentration		Bioaccessible Concentration		*R*	*PI*	RSD% recommended
	LD	LQ	LD	LQ	RSD %		
Al	4,97	16,58	2,49	8,29	3,4	2,7	≤ 3,7
Ca	0,18	0,60	5,57	18,57	3,9	4,3	≤ 5,3
Cr	0,02	0,08	0,04	0,10	10,5	14,2	≤ 21
Cu	0,02	0,06	0,08	0,27	8,2	9,6	≤ 11
Mg	0,09	0,29	0,83	2,78	0,9	4,7	≤ 5,3
Zn	0,30	0,99	0,54	1,81	3,5	9,4	≤ 11

A: repeatability; PI: intermediate precision.

Table 5: LD and LQ values (µg g-1) for the total and bioaccessible determination procedure and precision results for determination of Al, Ca, Cr, Cu, Mg and Zn in Canela de Velho tea.

Sample	Al	Ca	Cr	Cu	Mg	Zn
	µg g^{-1}					
CV1	2353 ± 358	4250 ± 212	0,481 ± 0,001	2,6 ± 0,8	1373 ± 20	16,7 ± 0,3
CV2	3010 ± 498	3795 ± 316	0,32 ± 0,05	2,6 ± 0,2	1787 ± 117	18 ± 1
CV3	2674 ± 281	3563 ± 178	0,68 ± 0,07	3,0 ± 0,4	1847 ± 162	22 ± 1
CV4	3024 ± 127	3608 ± 233	0,47 ± 0,06	3,3 ± 0,2	2095 ± 65	26,4 ± 0,7
CV5	2178 ± 8	3054 ± 156	1,7 ± 0,1	3,4 ± 0,3	2151 ± 122	25 ± 1
CV6	2542 ± 88	5089 ± 209	1,5 ± 0,2	3,8 ± 0,2	2352 ± 68	30,0 ± 0,4
CV7	1282 ± 207	2632 ± 23	0,45 ± 0,04	2,3 ± 0,4	1297 ± 10	20 ± 1
CV8	2470 ± 201	2652 ± 284	0,59 ± 0,05	3,6 ± 0,3	2051 ± 165	21 ± 1
CV9	6025 ± 235	3973 ± 225	0,22 ± 0,05	2,2 ± 0,5	1867 ± 81	10,3 ± 0,9

Table 6: Mean contents and standard deviation of metals present in Canela de Velho samples by dry digestion.

they ranged from 0.9% to 10.5%, values lower than those recommended by the AOAC (2012) and by Inmetro (2016).

Table 6 presents the total concentrations of metals present in the samples from Canela de Velho. Al concentrations were high in the samples, followed by Ca and Mg and lower for Zn, Cu and Cr, in that order.

Plants have the ability to accumulate metals during their growth and development from soil, water and the atmosphere. Factors such as climatic conditions, place of origin, soil and harvest time can change the levels of compounds present in plants (LEAL, et al., 2013). The CV samples were acquired from different manufacturers and cultivation sites, which may interfere in the relationship between the quantification of the elements and the plants.

It is important to know the composition of medicinal plants, as they are used for therapeutic purposes. But, depending on the compounds present and their concentration in these plants, there may be the appearance of a toxic potential associated with these components, which can harm the organism of living beings. For this work, cooking was used as a procedure for preparing the tea samples, due to greater stability in the preparation, when compared to infusions (DE ANDRADE, 2018).

Table 7 shows the moisture data, the average concentrations of metals and their bioaccessible fractions.

The moisture values of the CV samples were lower for the samples sold in plastic packaging and higher for those sold in bulk. The moisture measured in the dry CV samples were below the value established by Anvisa (BRASIL, 1998), which is 12% for mixed tea.

For Al, the samples showed a concentration that ranged from 343 ± 18 to 1163 ± 36 μg g^{-1}, being the samples CV7 and CV2, respectively. The CV7 sample was purchased in bulk and has its place of origin in São Paulo and the CV2 sample was purchased in the original factory packaging and has its place of origin in Piraquara - PR. This difference in the metal concentration values may be related to the place where the plant was planted, and the type of soil in the region (generally the state of Paraná has soils that are rich in aluminum). Studies carried out with CV fruits (PASTA, et al, 2019) showed an Al concentration of 2600,00 ± 0,03 μg g^{-1}. However, studies that used the leaves of the plant were not found so far.

The bioaccessible fractions for Al ranged from 62 ± 4 to 98 ± 3%. Al is an element that is normally complexed to phenolic compounds (melanoidins), therefore, this can influence the availability of the metal during the absorption process by the body (DE CAMPOS, et al., 2014).

The Ca concentration ranged from 102 ± 23 to 347 ± 13 μg g-1 for the samples. The study performed by collaborators (2019) indicated a concentration of 11400 μg g^{-1} in the fruit of the CV.

For Ca, it can be observed that the bioaccessible fractions ranged from 26 ± 6 to 122 ± 1% in the samples. Thus, the possibility of intestinal absorption of Ca from CV teas, with the exception of the CV9 sample (26 ± 6%), can be considered total, indicating that Ca is extracted in enzymatic digestion.

The element Cr can be quantified only in samples CV1 and CV8, with concentrations of 0.109 ± 0.003 and 0.093 ± 0.009 μg g-1, respectively. The other samples had values below the LQ. It was possible to detect the bioaccessible fractions of the metal in these samples, being 30.0 ± 1.0 for CV1 and 62 ± 8% for CV8.

The concentrations of Cu in the samples ranged from 0.30 ± 0.05 to 0.9 ± 0.2 μg g^{-1}. And bioaccessible fractions were obtained that ranged from 62 ± 2 to 90 ± 3%.

Sample	Moisture (%)	Al (µg g^{-1})	Al %	Ca (µg g^{-1})	Ca %	Cr (µg g^{-1})	Cr %	Cu (µg g^{-1})	Cu %	Mg (µg g^{-1})	Mg %	Zn (µg g^{-1})	Zn %
CV1	6,7 ± 0,1	804 ± 43	62 ± 4	147 ± 11	104,9 ± 0,1	0,109 ± 0,003	30,0 ± 1,0	0,69 ± 0,07	90 ± 3	405 ± 34	108,2 ± 0,1	2,8 ± 0,4	102 ± 4
CV2	9,5 ± 0,3	1163 ± 36	84 ± 7	232 ± 26	118 ± 1	< LQ	ND	0,30 ± 0,05	70 ± 2	327 ± 14	96 ± 3	1,3 ± 0,3	ND
CV3	10,1 ± 0,2	882 ± 23	62 ± 7	102 ± 23	122 ± 1	< LQ	ND	0,47 ± 0,05	78 ± 3	338 ± 33	103 ± 2	< LQ	ND
CV4	8,8 ± 0,8	445 ± 6	80 ± 4	218 ± 16	99 ± 5	< LQ	ND	0,70 ± 0,02	89 ± 4	371 ± 23	103 ± 1	2,3 ± 0,2	101 ± 2
CV5	7,5 ± 0,3	744 ± 15	70 ± 7	271 ± 11	120 ± 3	< LQ	ND	0,7 ± 0,1	90 ± 2	633 ± 53	98 ± 2	2,8 ± 0,5	76 ± 5
CV6	7,5 ± 0,3	629 ± 13	74 ± 9	335 ± 43	110 ± 5	< LQ	ND	0,63 ± 0,01	71 ± 5	464 ± 32	99 ± 5	3,1 ± 0,4	101 ± 7
CV7	7,7 ± 0,1	343 ± 18	82 ± 10	230 ± 32	108 ± 4	< LQ	ND	0,7 ± 0,1	62 ± 2	264 ± 27	111 ± 8	1,3 ± 0,4	114,0 ± 0,2
CV8	8,12 ± 0,01	493 ± 41	98 ± 3	347 ± 13	107 ± 3	0,093 ± 0,009	62 ± 8	0,9 ± 0,2	74 ± 3	577 ± 29	103 ± 2	1,7 ± 0,1	109 ± 5
CV9	8,1 ± 0,5	635 ± 32	67 ± 6	238 ± 46	26 ± 6	< LQ	ND	0,6 ± 0,1	78 ± 4	429 ± 81	79 ± 8	ND	ND
CV10*	*	4294 ± 130	132 ± 17	10700 ± 495	82 ± 13	6,6 ± 0,1	127 ± 5	37 ± 1	77 ± 2	7893 ± 654	93 ± 7	4 ± 1	ND

CV10* sample of Canela de Velho ready-made extract. Values are expressed in ug L-1 and has no moisture value.

< LQ concentrations less than the limit of quantification.

ND nothing detected.

Table 7: Mean values of humidity, concentration of metals and bioaccessible fractions determined by AAS in Canela de Velho teas.

For Mg, the concentrations obtained ranged from 264 ± 27 to 633 ± 53 μg g^{-1} for the samples. And the bioaccessible fractions ranged from 79 ± 8 to 111 ± 8%. It can be observed that the bioaccessible fractions were close to 100%, indicating the possibility of Mg being extracted in the enzymatic digestion. These data help to prove the effectiveness of one of the uses of the old man's cinnamon: treatment of pain caused by arthritis and osteoarthritis. CV tea, extracts and ointments are widely used, especially by the elderly, for this purpose. Mg is a very important mineral in collagen replacement and pain relief caused by bone problems. In this sense, a high bioaccessibility of this metal, through the consumption of the old man's cinnamon, shows that Mg can be metabolized by the body and thus, act in various processes of our body.

The concentrations found for Zn in the samples varied from 1,3 ± 0,3 a 3,1 ± 0,4 μg g^{-1}, Zn was not detected for sample CV9 and for sample CV3 the value was below the LQ. And the bioaccessible fractions of Zn ranged from 76 ± 5 to 114.0 ± 0.2%, with the exception of samples CV2, CV3 and CV9 in which they were not detected. Therefore, the bioaccessible fractions of Zn for CV indicate that the metal can be absorbed by the intestine.

The low values for the bioaccessible fraction of Zn in some samples may be related to the fact that Zn easily complexes with some compounds. Depending on the concentration of organic compounds present in the VC, this complexation may occur and the Zn ends up not being available in the enzymatic digestion of the tea. For sample CV3 the concentration of Zn was below the LQ and was not detected in the bioaccessible fraction, and sample CV9 was not detected Zn.

Sample CV10 is a concentrated extract of CV. For her, concentrations were detected in all metals studied. Regarding the bioaccessible fractions, with the exception of Zn, all metals were detected with high percentages.

However, no similar works were found in the literature (in relation to the mineral profile) with this plant, not having.

Based on the results obtained in the bioaccessible fractions of CV tea, with the exception of the CV1 Cr and CV9 Ca samples, in the other elements the percentage was greater than 60%, indicating a high potential for intestinal absorption of these metals, with the consumption of tea from this plant.

Regarding bioaccessibility, no work has been found, so far, that uses the CV plant.

After studies on the quantification of metals in cooking and in bioaccessible fractions, a study was carried out to quantify total phenolic compounds and flavonoids. Phenolic compounds have been widely studied, as studies indicate that their use causes several health benefits, such as in the form of antioxidants and in the prevention of inflammatory diseases (ACOSTA-ESTRADA, GUTIÉRREZ-URIBE & SERNA-SALDÍVAR, 2014).

To determine the flavonoid content in CV cooking and its respective bioaccessible fractions, an analytical curve was constructed with solutions of different concentrations of quercetin, and the following equation was obtained: $y = 0.0688x - 0.0304$ ($r^2 = 0,9965$).

To determine the phenolic content in CV cooking and its respective bioaccessible fractions, an analytical curve was constructed with solutions of different concentrations of gallic acid, and the following equation was obtained: $y = 0.0439x - 0.0326$ ($r^2 = 0,9867$).

The coefficients of determination for the curves were close to 0.99, indicating an adequate relationship between the evaluated data.

After the construction of the analytical curves, the readings of the absorbances in the

cookings and bioaccessible fractions of all the VC samples were carried out (Table 8). It can be verified that in the determination of total flavonoids the concentrations varied from 0.6 ± 0.1 to 1.42 ± 0.05 mg g-1, with bioaccessible fractions between 20 ± 4 to 92 ± 2%. A low bioaccessible fraction of these compounds indicates that they may be interacting with the enzymes of the gastrointestinal process, influencing the bioaccessibility of metals.

In the literature, it is described that plants tend to have higher concentrations of metals and organic compounds in their aerial parts, such as leaves (SOARES, et al., 2015). The CV samples, in addition to the leaves, also contained parts of the stem. It was observed in Table 7 that metals such as Cr and Zn had some bioaccessible fractions not detected. Perhaps, a possible interaction of flavonoids with these metals explains the reduction of bioaccessible fractions in both cases.

For the determination of total phenolics, the contents ranged from 17 ± 3 to 62 ± 1 mg g-1 with bioaccessible fractions between 70 ± 4 to 99 ± 3%. In this case, the bioaccessibility for phenolics was almost total, which may represent the low interaction of these compounds with the process enzymes, and even with the evaluated metals.

For the CV10 sample, the concentration of total flavonoids was 149 ± 8 mg L^{-1} and for the total phenolics it was 588 ± 17 mg L-1. The bioaccessible fractions for flavonoids and phenolics were close, being 90 ± 1% and 99 ± 11%, respectively. The bioaccessibility of the organic compounds found in this sample can be considered total, indicating that they are extracted in enzymatic digestion.

The literature presents a study containing the quantification of phenolics and flavonoids in Canela de Velho, indicating total phenolic concentrations of 551,3 ± 3,7 mg g^{-1} and flavonoid of 367.2 ± 10.5 mg g-1 (LIMA, et al., 2020). However, the sample preparation method by these authors was through ethanolic extraction.

A possible correlation between the variables metals, the bioaccessible fraction and reducing activity of the Canela de Velho samples was evaluated through principal component analysis (PCA) (Figure 1A and B).

Sample	Total Flavonoids (mg g^{-1})	Bioaccessible fraction (%)	Total phenolics (mg g^{-1})	Bioaccessible fraction (%)
CV1	0,90 ± 0,06	40 ± 8	24 ± 4	94 ± 1
CV2	0,6 ± 0,2	54 ± 2	24 ± 5	81 ± 5
CV3	0,8 ± 0,2	57 ± 3	28 ± 3	94 ± 6
CV4	0,8 ± 0,1	72 ± 3	17 ± 3	83 ± 4
CV5	1,0 ± 0,1	50 ± 4	35 ± 5	70 ± 4
CV6	0,9 ± 0,1	42 ± 2	28 ± 3	76 ± 10
CV7	0,6 ± 0,1	67 ± 1	26 ± 5	79 ± 3
CV8	1,42 ± 0,05	20 ± 4	55 ± 5	82 ± 1
CV9	1,0 ± 0,3	92 ± 2	62 ± 1	99 ± 3
CV10*	149 ± 8	90 ± 1	588 ± 17	99 ± 11

CV10* sample of Canela de Velho ready-made extract. Values are expressed in mg L^{-1}.

Table 8: Content of total flavonoids and phenolics in cooking and in bioaccessible fractions.

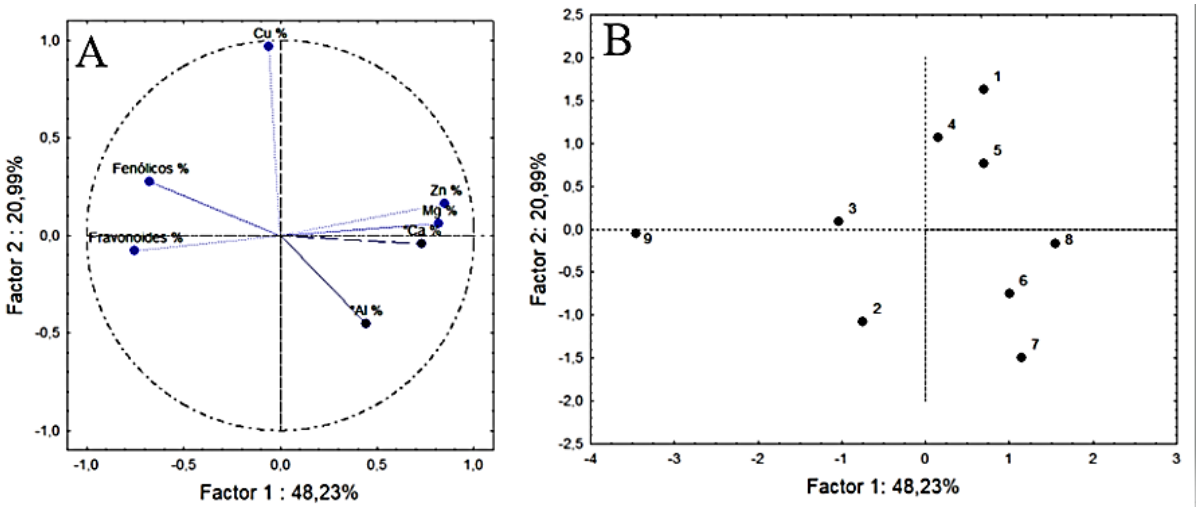

Figure 1: **(A)** Correlation graph between the variables studied in the CV samples. **(B)** Graph of CV samples grouped by similarities between metals and reducing activity.

The first component was responsible for explaining 48.23% of the data variability and the second component for 20.99%, totaling 69.22% of the data evaluated. It can be seen from the first component that samples CV2, CV3 and CV9 are inversely proportional to metals, with the exception of Cu, that is, the greater the fraction of bioaccessible organic compounds, the smaller the fraction of metals. By the second component, it is observed that flavonoids correlated with Al for samples CV2, CV6, CV7, CV8 and CV9, while samples CV1, CV3, CV4 and CV5 showed correlation with phenolics, Cu, Mg, Ca and Zn. The bioaccessible fractions of Cr were not used in PCA, because in most samples no bioaccessible fractions of Cr were detected. The distribution of Canela de Velho samples among the different quadrants of the PCA indicates that the place of cultivation can influence the amounts of components present in them.

CONCLUSIONS

It was verified that only Al, Ca, Cu, and Mg presented total and bioaccessible concentrations quantified in all samples evaluated from Canela de Velho. Cr, in turn, was detected in only three samples, indicating a possible relationship between their concentration and the place of cultivation. For Zn, a quantifiable concentration of the metal was obtained in 8 samples, however, in the bioaccessibility assay, the bioaccessible fraction of the metal was detected only in 6 samples.

Phenolic compounds showed high bioaccessibility, close to 90%, while flavonoids presented percentages close to 50%. This lower bioaccessibility of flavonoids in Canela de Velho may be related to the reduced bioaccessibility of metals such as Cr and Zn in the plant, indicating an interaction of these metals with flavonoids.

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