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Artigo

Assessment of the Concentration of Trace Elements in Cassava Flour (*Manihot esculenta* Crantz) by Inductively Coupled Plasma Optical Emission Spectrometry

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Avaliação da Concentração de Elementos-traço em Farinha de Mandioca (*Manihot esculenta* Crantz) por Espectrometria de Emissão Óptica com Plasma Acoplado Indutivamente

Resumo: The concentrations of Ca, Cu, Fe, K, Mg, Mn, P and Zn in 10 commercially available cassava flours (*Manihot esculenta* Crantz, Euphorbiaceae) were determined using inductively coupled plasma optical emission spectrometry (ICP OES). The elements were found in descending order of concentration, as K > Ca > P > Mg > Fe > Cu > Zn > Mn. Based on the recommended daily intake for adults, the values showed that 10 g day^{-1} cassava flour provides 5.3% of the daily intake of Cu, 0,5-1.2% for Fe, 0.5% for Ca, 0.9-1.2% for Mg, 0.6-0,8% for Mn, 0.6% for P, 0.2% for K and 0.2-0.3% for Zn. Based on the RDIs (Recommended Daily Intake) of the elements studied, the results indicate that cassava flour contains several essential elements, and the levels found in the cassava flours presents no risk to the human health. With the results of the multivariate analysis, it was possible to locate information about clusters and dissimilarities between the samples and between the variables, as well as to provide useful information for future studies on quality control and genetic improvement of cassava with better nutritional values.

Palavras-chave: *Manihot esculenta* Crantz, Alimento, Elementos-traço, ICP OES, RDI.

Abstract

The concentrations of Ca, Cu, Fe, K, Mg, Mn, P and Zn in 10 commercially available cassava flours (*Manihot esculenta* Crantz, Euphorbiaceae) were determined using inductively coupled plasma optical emission spectrometry (ICP OES). The elements were found in descending order of concentration, as K > Ca > P > Mg > Fe > Cu > Zn > Mn. Based on the recommended daily intake for adults, the values showed that 10 g day^{-1} cassava flour provides 5.3% of the daily intake of Cu, 0,5-1.2% for Fe, 0.5% for Ca, 0.9-1.2% for Mg, 0.6-0,8% for Mn, 0.6% for P, 0.2% for K and 0.2-0.3% for Zn. Based on the RDIs (Recommended Daily Intake) of the elements studied, the results indicate that cassava flour contains several essential elements, and the levels found in the cassava flours presents no risk to the human health. With the results of the multivariate analysis, it was possible to locate information about clusters and dissimilarities between the samples and between the variables, as well as to provide useful information for future studies on quality control and genetic improvement of cassava with better nutritional values.

Keywords: *Manihot esculenta* Crantz; Food; Trace Elements; ICP OES; RDI.

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Assessment of the Concentration of Trace Elements in Cassava Flour (*Manihot esculenta* Crantz) by Inductively Coupled Plasma Optical Emission Spectrometry

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1. Introduction

Cassava (*Manihot esculenta* Crantz) is a tuberous root that belongs to the Euphorbiaceae family. Due its high adaptability to a variety of environmental conditions, cassava is considered an important nutritive source.[1] It is the third largest source of carbohydrates after rice and maize, being an important dietary component for populations worldwide.[2] According to the Food and Agricultural Organization of the United Nations (FAO)[3], cassava is planted in more than 80 countries, as Nigeria, Thailand, Brazil, Indonesia and the Democratic Republic of Congo, the largest producers.

Cassava can be consumed either *in natura* (i.e. boiled) or in a variety of industrially- or traditionally-processed forms, known under various names depending on the preferences and local customs.[4] Cassava flour is one of these products, as well as chips and starch. The root flour is gaining recognition as a suitable wheat flour substitute in biscuits and the fast food industries. Indeed, although cassava flour has a low nutritional value, it remains an important food in several parts of the world, as it is less expensive than wheat and can be used to produce a variety of food products.[5,6]

In Brazil, cassava flour consumption is 7.9 kg/capita/year, with highest consumption in the northern and northeast regions at 32.0 and 15.7 kg/per capita/year, respectively, compared with the south and southeast regions of the country that consume approximately 1.5 kg/per capita/year.[7] According to Sgarierie[8], the northern regions consume this food in the form of cassava bread, porridge and crumbs, whereas the northeastern population consumes cassava flour in the form of *pirão*, or as an accompaniment to beans, dry meat, coffee, and as part of typical regional dishes.

Essential macroelements and microelements, such as Ca, Cu, Fe, K, Mg, Mn, P and Zn play an important role in the human body. Ca, for example, is responsible for several metabolic functions, such as blood coagulation muscle contraction regulation, hormone secretion, and neurotransmission.[9-11] Mg is a macroelement that participates in energy metabolism and is involved in protein synthesis.[11,12] Cu has a crucial role in cell physiology, acting as a cofactor in several enzymatic reactions. It is also required for mitochondrial respiration and Fe absorption.[13,14] Fe has participation as a cofactor of several enzymes in the Krebs cycle is particularly important.[9,15]

Determination of the total levels of inorganic constituents, such as Ca, Fe, K and P in food, is crucial to assess the possible impact of the food on human health. Various elements in cassava and flour samples from various countries have been determined using flame atomic absorption spectrometry (FAAS),[16-19] graphite furnace atomic absorption spectrometry (GF AAS),[20,21] inductively coupled plasma-optical emission spectrometry (ICP OES)[22-25] and inductively coupled plasma mass spectrometry (ICP-MS)[26-29]. However, the studies are scarce in the literature that determined the levels of inorganic constituents in cassava flour. Emurotu et al.[18] determined nine elements (K, Ca, Mg, Fe, Cu, Mn, Pb, and Cd) in cassava flour sold in Nigeria. On the other hand, Ofori et al.[30] evaluated the levels of Cu, Fe, As, Hg, Pb and Zn in high quality cassava flour marketed in Ghana.

Chemometric tools like PCA[31], are useful to extract information about differences and similarities between samples and also the relation with their variables (in this case, mineral composition), being applied in this work for purposes of nutritional quality control of cassava flour and also for future studies about genetic improvement.

This study aimed to assess the concentrations of eight essential metals (Ca, Cu, Fe, K, Mg, Mn, P and Zn) in 10 cassava flour samples commercially available in State Pará (Brazil), to better understand their nutritional value and confirm their safe consumption for humans.

2. Materials and methods

2.1. Instrumentation

All measurements were performed with an iCAP 6500 inductively coupled plasma optical emission spectrometer (Thermo Fisher Scientific, Cambridge, England) with dual configuration (axial and radial). The plasma operating conditions and parameters of the sample introduction system are shown in Table 1.

Table 1. Operating conditions of the ICP OES

Parameter	Operating condition
RF applied power (kW)	1.15
Argon plasma flow rate (L min^{-1})	12
Argon auxiliary flow rate (L min^{-1})	0.5
Argon nebulizer flow rate (L min^{-1})	0.5
Nebulizer	Concentric
Number of replicates	3
Viewing mode	Duo (axial and radial)
Analytical emission line (nm)	Ca I (317.9)[b]
	Cu I (224.7)[a]
	Fe I (259.9)[b]
	K II (769.8)[b]
	Mg I (280.2)[b]
	Mn I (257.6)[b]
	P II (185.9)[a]
	Zn II (213.3)[a]

[a] Axial; [b] Radial.

A Liotop model L101 lyophiliser (São Carlos, SP, Brazil) was used to dry the samples. A SPEX SamplePrep model 6770 cryogenic mill (Metuchen, NJ, USA) was used to grind the samples. Cassava flour samples were microwave assisted acid digested using a microwave oven cavity (Start E, Milestone, Sorisole, Italy).

2.2. Reagents and reference solutions

All solutions were prepared with ultrapure water (resistivity 18.2 MΩ cm^{-1}) from a Synergy-UV purification system (Millipore, Bedford, MA, USA) in polypropylene flasks. All materials used were previously decontaminated in 10% v v^{-1} HNO_3 for 24 h and rinsed with deionised water before use.

HNO_3 (Sigma-Aldrich, Germany), previously purified using a sub-boiling distillation system (Berghof, model BSP 929-IR, Germany) and 30% w w^{-1} H_2O_2 (Impex, Brazil) was used to digest the samples.

Monoelement stock solutions of 1000 mg L^{-1} Ca, Cu, Fe, K, Mg, Mn, P and Zn (Sigma, USA) were diluted and used to prepare multielement analytical calibration solutions.

Calibration solutions for Ca, Cu, Fe, K, Mg, Mn, P, and Zn were prepared with analyte concentrations ranging from 2.0 to 10.0 mg L^{-1} in 5.0 % v v^{-1} HNO_3.

2.3. Samples

Ten cassava flour samples were acquired from producers in various cities of Pará State, Brazil (Table 2). After collection, the samples were packed and transported to the laboratory in polyethylene bags.

Table 2. Flour samples and its location

Sample	Location
F1	Colares, PA
F2	Colares, PA
F3	Vigia, PA
F4	Vigia, PA
F5	Vigia, PA
F6	Moju, PA
F7	Bragança, PA
F8	Bragança, PA
F9	Cametá, PA
F10	São Domingos do Capim, PA

2.4. Sample preparation

Cassava flour samples were first lyophilised for 24 h then ground in a cryogenic mill. An aliquot (250 mg) of the cassava flour samples (*n* = 3) was digested with 4 mL of 7 mol L^{-1} HNO_3 and 4 mL of 30% w w^{-1} H_2O_2. A one-step heating program was used, with (1) 10 min to reach 200 °C and (2) 15 min at 200 °C. The maximum microwave power used was 800 W. After, the vessels were removed from the microwave rotor and cooled down to room temperature. Then, the vessels were opened and the digests were quantitatively transferred to volumetric flasks and diluted to 14 mL with ultrapure water. Blank experiments were similarly treated.

2.5. Statistical analysis

Samples were analysed in triplicate (*n* = 3). The data were reported as mean ± standard deviation (SD). Data were analysed using Origin 6.0 for Windows (Microcal Software, Northampton, MA, USA). The instrumental relative standard deviations (RSDs) ranged from 5–10%, depending on the element. To better extract information on the data, multivariate analysis was applied to the table 4 (mineral composition of eight elements) of the flour samples. It was used the scores and loadings graphs[32], using full cross validation method. Statistica[33] version 10.0 was used.

3. Results and discussion

3.1. Figure of merit

The limits of detection (LODs) and limits of quantification (LOQs) by ICP OES were calculated using the background equivalent concentrations (BECs), the signal-to-background ratios (SBRs) and the RSDs for 10 measurements of the analytical blank.[34] LODs and LOQs values were, respectively, calculated as 3-and 10-times the BEC, multiplied by the RSDs, divided by 100. The analytical parameters are summarised in Table 3.

Table 3. Figures of merit by ICP OES

Elements	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	R^2
Ca	0.4	1.3	0.9989
Cu	0.1	0.3	0.9993
Fe	0.2	1	0.9987
K	18	54	0.9996
Mg	0.01	0.04	0.9997
Mn	0.05	0.2	0.9985
P	0.5	1.6	0.9991
Zn	0.01	0.03	0.9999

LOD: limit of detection; LOQ: limit of quantification; R^2: Correlation coefficient.

The LOD's and LOQ's obtained show that the calibration used was able to reproduce results in low concentrations with levels of reliable precision and accuracy. The values of the linear coefficients (R^2) show a good linearity in the relation analytical response *versus* concentration, with values between 0.9985 to 0.9999.

3.2. Accuracy

The accuracy of the method was evaluated by recovery studies. These were performed by adding 3, 5, 7 and 9 mg L^{-1} of the analytes to the samples before microwave digestion. The recoveries ranged from 84-111%, indicating the excellent efficiency of the sample preparation procedure.

The accuracy of the ICP OES measurements was also verified by the recovery test, where a spiking experiment was carried out for all the evaluated analytes. In this instance, 3, 5, 7 and 9 mg L^{-1} of the analytes were added to the digested samples. The determined values were in agreement with the added concentrations and the recoveries ranged from 95–112%.

3.3. Analysis of cassava flour samples

The mean contents of the elements determined in the cassava flour samples are presented in Table 4. In descending order, the mean concentration of the elements in the studied cassava flour samples was K > Ca > P > Mg > Fe > Cu > Zn > Mn. Generally, the concentrations of the elements studied were higher than those reported in the literature.[18,24,32]

In this study, the Ca levels ranged from 322.1–706.7 mg kg^{-1}. The mean concentration of the samples was 521.1 mg kg^{-1}. The lowest and the highest Ca levels were 322.1 mg kg^{-1} in sample F2 and 706.7 mg kg^{-1} in sample F10, respectively. Aregahegn et al.[35] reported that the average concentrations of Ca in *Diospyros abyssinica* flour samples from Ethiopia ranged from 172–448 mg kg^{-1}. The Ca concentrations of four cassava flour samples from Nigeria ranged from 1.18–1.53 mg kg^{-1}.[18]

Table 4. Concentration of inorganic elements (mg kg^{-1}) in cassava flour (mean ± S.D.; *n* = 3)

Sample	Ca	Cu	Fe	K	Mg	Mn	P	Zn
F1	516.1 ± 8.7	9.17 ± 0.27	12.15 ± 1.13	690.1 ± 31.1	279.6 ± 2.2	2.21 ± 0.01	539.6 ± 27.5	1.15 ± 0.06
F2	322.1 ± 2.2	9.92 ± 0.33	2.95 ± 0.22	796.1 ± 21.5	577.0 ± 19.8	1.25 ± 0.08	739.9 ± 50.9	1.05 ± 0.16
F3	557.7 ± 10.8	8.84 ± 0.36	11.41 ± 0.12	1036.1 ± 7.2	356.5 ± 2.1	0.97 ± 0.05	535.3 ± 20.1	< 0.01[a]
F4	672.5 ± 9.2	15.48 ± 4.52	7.73 ± 0.46	798.7 ± 38.5	439.2 ± 11.4	1.63 ± 0.04	596.3 ± 55.9	1.21 ± 0.11
F5	479.3 ± 11.1	0.71 ± 0,11	10.48 ± 0.65	562.1 ± 12.4	609.8 ± 13.8	1.43 ± 0.04	448.6 ± 25.7	5.48 ± 0.08
F6	517.9 ± 10.9	1.20 ± 0.38	4.64 ± 0.57	1951.1 ± 183.5	403.4 ± 25.6	2.17 ± 0.16	293.8 ± 13.4	5.05 ± 0.37
F7	551.2 ± 16.3	0.71 ± 0.14	7.22 ± 0.55	209.7 ± 26.6	141.4 ± 5.8	0.91 ± 0.03	254.9 ± 38.6	1.45 ± 0.21
F8	457.4 ± 9.1	1.25 ± 0.17	8.57 ± 0.28	519.4 ± 24.3	196.3 ± 7.5	1.22 ± 0.03	341.7 ± 1.1	1.41 ± 0.03
F9	429.5 ± 53.3	0.33 ± 0.04	15.45 ± 2.12	915.5 ± 175.4	309.4 ± 40.5	1.15 ± 0.17	431.2 ± 67.8	1.31 ± 0.01
F10	706.7 ± 46.6	0.36 ± 0.08	16.82 ± 1.21	543.2 ± 39.1	271.2 ± 18.9	0.97 ± 0.05	372.4 ± 70.2	2.50 ± 0.08
Mean	521.1	4.79	9.74	802.2	358.4	1.39	455.4	2.29
Min. – Max.	322.1 – 706.7	0.33 – 15.48	2.95 – 16.82	209.7 – 1951.1	141.4 – 609.8	0.91 – 2.21	254.9 – 739.9	<0.01 – 5.48
CV (%)	3.4	15.4	7.4	6.8	4.4	5.1	8.5	6.5

[a] Below the limit of detection (LOD).

The Cu content in the studied cassava flours ranged from 0.33 (sample F9) to 15.48 mg kg^{-1} (sample F4). The mean Cu concentration in the samples was 4.79 mg kg^{-1} with a CV of 15.4%. In general, the values were higher than those reported in previous studies. The Cu levels in 21 corn flour samples from Turkey[28] and 50 wheat flour samples from Spain[24] ranged from 0.08–2.00 and 2.27–2.87 mg kg^{-1}, respectively.

The lowest Fe concentration in this study was 2.95 mg kg^{-1} in sample F2, while the highest concentration was 16.82 mg kg^{-1} in sample F10. The mean Fe concentration in the analysed cassava flours was 9.74 mg kg^{-1} (with a CV of 7.4%). In cassava flour from Nigeria, Fe values ranged from 0.66–1.27 mg kg^{-1} [18] and in wheat flour from Spain, 8.17–8.63 mg kg^{-1} Fe was detected.[24]

In the present study, the lowest and the highest K levels were 209.7 mg kg^{-1} in sample F7 and 1951.1 mg kg^{-1} in sample F6. The mean K concentration was 802.2 mg kg^{-1} with a CV of 6.8%. In previous studies, K concentrations ranged from 3590–4930 mg kg^{-1} [36], 761.1–933.9 mg kg^{-1} [24] and 4.8–6.7 mg kg^{-1} [18] for various flours.

The Mg levels in this study ranged from 141.4–609.8 mg kg^{-1}. The mean Mg concentration was 358.4 mg kg^{-1} with a CV of 4.4%. The values were within the range obtained by Tejera et al.[24], Nardi et al.[26] and Tang et al.[34] but much greater than those reported by Emurotu et al.[18]

The lowest and the highest Mn levels obtained were 0.91 mg kg^{-1} for sample F7 and 2.21 mg kg^{-1} for sample F1. In various flours from India, Mn values ranged from 18.6–49.4 mg kg^{-1} [38], and in flours from Italy, Mn varied from 11.8–18.7 mg kg^{-1} [39]. In this study, the mean Mn concentration was 1.39 mg kg^{-1} with a CV of 5.1%, which is lower than the 4.3 mg kg^{-1} reported by Tejera et al.[24]

The lowest P concentration in this study was 254.9 mg kg^{-1} in sample F7, while the highest concentration was 739.9 mg kg^{-1} in sample F2. The mean P concentration was 455.4 mg kg^{-1} with a CV of 8.5%. Previous P concentrations reported in the literature varied from 810–7150 mg kg^{-1} in 54 flour samples consumed in Brazil[22] and from 1014.6–1339.9 mg kg^{-1} in wheat flour from Brazil.[23]

The lowest and the highest Zn levels were 1.05 mg kg^{-1} in sample F2 and 5.48 mg kg^{-1} in sample F5, respectively. The mean Zn concentration was 2.29 mg kg^{-1} with a CV of 6.5%. In previous studies, Zn concentrations ranged from 6.4–8.8 mg kg^{-1} [23] and 6.15–6.31 mg kg^{-1} [24] in various flours. Martins et al.[25] reported a mean Zn concentration of 11.60 mg kg^{-1} in wheat flour from Brazil, which was higher than that found in the current study.

3.4. Recommended Dietary Allowances (RDAs) of the studied elements in cassava flour

The RDAs values were based in normative values established by the National Research Council for adults between 19-30 year[40]. Considering a cassava flour consumption of 10 g day^{-1}, the respective RDIs (reference daily intake) were calculated and are showed in Table 5.

Based on the RDIs (Recommended Daily Intake) of the elements studied, the results indicate that cassava flour contains several essential elements, and the levels found in the cassava flours presents no risk to the human health.

Table 5. Recommended Dietary Allowances (RDAs) of the studied elements in cassava flour

Element	RDAs (mg day^{-1})	RDIs (%)
Ca	1000	0.5
Cu	0.9	5.3
Fe	8 (M)	0.5
	18 (F)	1.2
K	4700	0.2
Mg	400 (M)	0.9
	310 (F)	1.2
Mn	2.3 (M)	0.6
	1.8 (F)	0.8
P	700	0.6
Zn	11 (M)	0.2
	8 (F)	0.3

M: for males; F: for females.

3.5. Multivariate Analysis

Scores Graph

They were verified following clusters (Fig.1): Group 1 formed by samples F5 and F6 (clockwise direction); Group 2 formed by samples F1, F2, F3, F4; and group 3 formed by groups F7, F8, F9, F10. It stands out the similarity between samples F7 and F10, as well as between samples F8 and F9. It was not possible to find clusters of these samples by geographic region, because in the Pará State there is no relation of chemical composition of the soil or of cassava with the geopolitical division of this State.

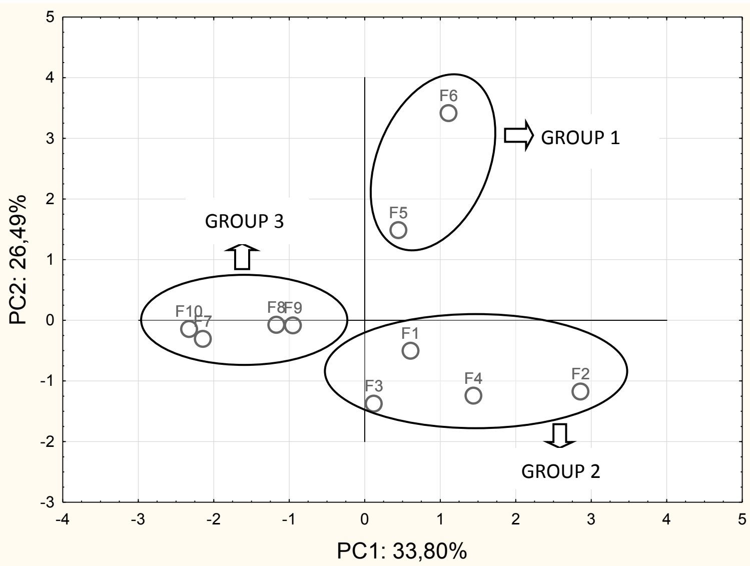

Figure 1. Scores Graph (PC1x PC2) of the 10 samples of cassava flour

Loadings Graph

It was observed a synergic relationship between the metals K and Mn, Cu and P, Ca and Fe.

Also, when the graph of the scores is related to the loadings, there is a positive correlation between the group 1 and the variables Zn (higher concentration in the sample F5: 5.48 mg kg^{-1} and F6: 5.05 mg kg^{-1}), Mn, K (F6: 1951.1 mg kg^{-1}) and Mg (F5: 609.8 mg kg^{-1}). As a good correlation between the samples of Group 2 and Cu (F4: 15.48 mg kg^{-1}) and P (F2: 739.4 mg kg^{-1}). They were also found high correlation between group 3 and the metals Fe (F10: 16.82 mg kg^{-1}) and Ca (higher concentration in the sample F10: 706.7 mg kg^{-1}).

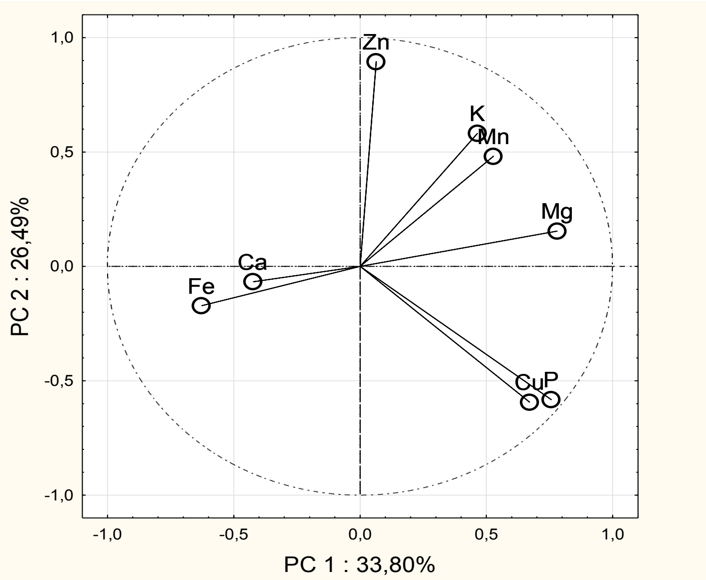

Figure 2. Gráfico de loadings (PC1 x PC2) for the 8 elements of mineral composition

4. Conclusion

In this study, the levels of essential elements in 10 cassava flour samples were determined. The mineral levels of the samples were relatively high. For the elements studied, the descending order of concentration found was K > Ca > P > Mg > Fe > Cu > Zn > Mn. In general, the concentrations of the elements studied such as Ca, Cu, Fe, K and Mg were higher than those reported in the literature. Based on the RDIs of the studied elements, the results indicate that cassava flour contains some minerals that are essential for the body and its consumption is not a risk to human health. With the results of the multivariate analysis, it was possible to locate information about clusters and dissimilarities between the samples and between the variables, as well as to provide useful information for future studies on quality control and genetic improvement of cassava with better nutritional values in this research group or for the world's scientific community.

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