

# Use of Multivariate Analysis Techniques for Evaluation of Analytical Data—Determination of the Mineral Composition of Cabbage (*Brassica oleracea*)

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**Abstract** Cabbage (*Brassica oleracea*) is a vegetable food that is found in red and white varieties, and it has been consumed worldwide as raw or cooked. In this paper, the mineral composition of cabbage collected in 24 Brazilian cities was determined and the results were evaluated using multivariate analysis. The samples were digested using nitric acid and hydrogen peroxide and were analyzed using inductively coupled plasma optical emission spectrometry. The accuracy of the method was confirmed by analysis of a certified reference material of spinach leaves, furnished by National Institute of Standard and Technology. The study involved 55 samples, being 31 of the white specie and 24 of the red specie. The results expressed as milligrams of element per kilogram of sample demonstrated that the concentration ranges varied from 1,602.9 to 4,068.3 for potassium, 217.5 to 766.2 for phosphorous, 221.9 to 744.7 for calcium, 67.2 to 286.0 for magnesium, 0.81 to 4.40 for manganese, 1.91 to 8.60 for iron, 0.01 to 0.24 for molybdenum, 1.17 to 5.10 for zinc, 27.2 to 591.0 for sodium, and 0.35 to 5.79 for strontium. The principal component analysis and hierarchical cluster analysis evidenced that the mineral composition of the red specie is not different of the white specie.

**Keywords** Cabbage · Elements · Mineral composition · ICP OES · PCA · HCA

## Introduction

The analyses of fruits, leaves, and roots that are used as food for humans are often required. The determination of organic and inorganic nutrients in some cases may reveal the nutritional potential of new plants, as well as contribute to the formulation of food composition tables, which are used by nutritionists and doctors (Candeias et al. 2009; Borah et al. 2009). This way, many studies involving determination and evaluation of food composition have been performed with this objective (Uluozlu et al. 2007; Divrikli et al. 2003; Richert and Sneddon 2008). Aberoumand determined the content of potassium, sodium, calcium, iron, and zinc in *Portulaca oleracia L.* leaves and stem. The results indicated that leaves and stem of this vegetable can be used as nutritional supplement (Aberoumand 2009). Thriveni et al. performed a method employing differential pulse adsorptive stripping voltammetry for the determination of phenothrin in agricultural formulations, vegetables, and storage bags of wheat and rice (Thriveni et al. 2009). Sharma used 2,6-bis(1-hydroxy-2-naphthylazo)pyridine as spectrophotometric reagent for the determination of zinc in milk samples (Sharma and Singh 2009). Saleh et al. proposed a method using slurry sampling and electrothermal atomization atomic absorption spectrometry for the determination of zinc in fish feed and feces (Saleh et al. 2009). Salahinejad and Aflaki determined aluminum, arsenic, lead, chromium, cadmium, nickel, iron, zinc, copper, manganese, calcium, and magnesium in black tea leaves and their tea infusions. The extraction efficiency of each element into tea infusions was also evaluated (Salahinejad and Aflaki 2010).

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Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are multivariate analysis techniques (Sharaf et al. 1986; Correia and Ferreira 2007), which in the last years have often been employed for evaluation and characterization of analytical results (Nuzzi et al. 2008; do Nascimento et al. 2010; Korhonova et al. 2009). Feliciano et al. employed PCA for characterization of the phenolic content and antioxidant activity of moscatel dessert wines from the Setubal region in Portugal. This chemometric tool allowed the groupings of the samples in terms of both winemaking producer and vintage (Feliciano et al. 2009). Lima et al. used PCA and HCA for homogeneity evaluation during the preparation of a wheat flour laboratory reference material for inorganic analysis (Lima et al. 2010). Ferreira et al. determined the mineral composition of kale. PCA and HCA revealed different mineral compositions of the samples collected in the summer and winter. Samples collected in the winter have a higher concentration of micronutrients (iron, zinc, and manganese) and macronutrients (calcium and magnesium). The average contents (wet weight and mg per 100 g) for the winter and summer were 551 and 535 for calcium; 117 and 106 for magnesium; 2.13 and 1.48 for iron; 2.63 and 1.95 for zinc, and 2.05 and 1.34 for manganese, respectively (Fadigas et al. 2010). Gromboni et al. compared the sample preparation procedures: photo-Fenton reaction, microwave irradiation and direct determination for the quantification of barium, calcium, potassium, magnesium, manganese, sodium, and strontium in several fruit juices (passion fruit, pineapple, orange, and grape). The evaluation of the analytical results was performed using PCA and HCA (Gromboni et al. 2010). Pasiás et al. determined the content of vanadium, chromium, copper, cobalt, selenium, strontium, tin, antimony, barium, bismuth, lead, cadmium, arsenic, nickel, manganese, iron, magnesium and zinc in leaves, flowers, and infusion of *Salvia fruticosa*. Chemometric techniques were used to investigate the original site classification according to their elemental content, and it

was proved that the initial cultivation sites were characterized by only five elements (antimony, vanadium, zinc, cadmium, and chromium). The application of factor analysis revealed significant correlation between certain elements, denoting their common sources (Pasiás et al. 2010).

Cabbage (*Brassica oleracea*) is a vegetable food, consumed worldwide as raw or cooked and, it is found in the red and white varieties. This paper had as objective the determination and evaluation of the mineral composition of cabbage. The nutrients calcium, phosphorous, potassium, magnesium, sodium, iron, manganese, molybdenum, strontium, and zinc were determined employing inductively coupled plasma optical emission spectroscopy (ICP OES). Samples of 24 Brazilian cities were collected. The results were evaluated using the multivariate analysis techniques PCA and HCA.

## Experimental

### Instrumentation

A Varian model Vista PRO inductively coupled plasma optical emission spectrometer (Mulgrave, Australia) with axial viewing and a charge-coupled device detector was used for multi-element determination. A Sturman–Master chamber and a V-Groove nebulizer were also used. The metal determinations were carried out under the followings instrumental conditions: power (1.3 kW), plasma gas flow (15.0 Lmin<sup>-1</sup>), auxiliary gas flow (1.5 Lmin<sup>-1</sup>), and nebulizer gas flow (0.8 Lmin<sup>-1</sup>). The elements and the analytical wavelengths used for quantification were: Ca II (422.673), P I (177.434), K (766.491), Mg II (285.213), Na I (589.592), Fe II (238.204), Mn II (257.610), Mo II (277.539), Sr II (421.552), and Zn II (334.502).

### Chemicals and Reagents

All chemical reagents used in the experiment were of analytical grade. Ultra pure water (18.2 MΩ cm<sup>-1</sup>) from a Milli-Q system (Millipore, MA, USA) was used to prepare all solutions. Glass apparatus used were maintained in nitric acid solution (10% v/v) during 12 h for decontamination prior to use.

Stock standard solutions for all elements (Merck, Darmstadt, Germany) with a concentration of 1,000 mgL<sup>-1</sup> were used for preparing working standard solutions by appropriate dilution of these solutions with 1% (v/v) nitric acid.

### Sample Collection and Storage

The samples were collected in the following Brazilian cities: Salvador, Madre de Deus, Simões Filho, Jequié,

**Table 1** Evaluation of the accuracy of the analytical method used for quantification

Element	Achieved value	Certified value
K (%)	2.887±0.583	2.903±0.052
Na (%)	1.777±0.367	1.818±0.043
Ca (%)	1.448±0.277	1.527±0.041
P (%)	0.496±0.126	0.518±0.011
Mg (%)	0.86±0.144	0.89 <sup>a</sup>
Zn (mg kg <sup>-1</sup> )	80±12	82±3
Mn (mg kg <sup>-1</sup> )	67.3±5.2	75.9±1.9
Sr (mg kg <sup>-1</sup> )	53.22±1.77	55.6±0.8

<sup>a</sup> Value reported

**Table 2** Determination of the mineral composition of cabbage samples (mg of analyte per Kg<sup>-1</sup>)

Sample	Element									
	P	Na	Mg	K	Ca	Mn	Fe	Zn	Sr	Mo
W-BAR	401.5	61.9	120.5	2,219.8	546.9	1.57	2.30	3.08	2.25	0.12
W-JEQ	227.5	56.8	117.2	1,696.7	238.3	1.19	2.84	1.79	0.35	0.08
W-REC	343.2	442.8	141.6	1,902.9	335.6	0.81	1.94	1.53	2.01	0.23
W-AJU	298.8	72.2	163.2	1,602.9	375.9	0.87	2.88	1.37	0.63	0.10
W-SMF1	303.2	50.2	111.6	1,749.9	314.1	1.62	1.96	2.07	1.46	0.03
W-SSA1	414.8	473.0	153.6	1,980.0	433.5	2.96	3.69	2.29	2.14	0.03
W-SMF2	353.7	45.9	136.4	1,767.7	560.9	1.63	2.15	2.53	2.63	0.02
W-SSA2	438.7	52.0	131.8	2,179.4	352.8	1.78	2.48	2.80	1.45	0.04
W-BEH	511.0	67.9	174.0	2,913.9	532.5	1.45	3.01	1.83	2.27	0.02
W-FLO	383.9	30.5	135.9	1,853.3	414.4	1.42	2.21	1.67	0.81	0.06
W-PAL	490.8	50.9	201.1	2,680.9	448.0	2.85	4.54	2.42	1.10	0.03
W-UBA	374.2	66.0	112.9	1,963.1	478.5	2.98	2.03	3.22	2.05	0.10
W-JAG1	502.4	54.5	161.5	2,325.8	373.3	1.91	3.31	3.48	0.99	0.06
W-ITI	405.7	64.0	118.6	2,144.0	422.5	1.46	2.26	2.00	1.66	0.03
W-SMT	572.4	71.5	208.1	2,998.8	585.2	2.72	4.22	3.09	2.11	0.08
W-LAV	755.3	61.4	199.5	3,421.6	690.0	4.40	5.65	5.10	5.79	0.04
W-CAM	524.7	46.9	206.3	2,883.1	654.6	1.19	1.91	2.19	0.60	0.03
W-SCR	476.9	27.2	156.7	2,508.4	413.0	2.63	3.21	3.02	0.81	0.05
W-SMA	598.8	64.6	266.8	2,809.9	471.4	1.09	1.99	1.67	1.20	0.04
W-VIT	419.3	40.7	130.1	1,852.2	367.0	2.28	2.35	1.48	0.99	0.08
W-SSA3	290.7	182.0	152.7	1,969.9	338.1	1.91	2.90	2.71	2.90	0.02
W-LFR	680.4	282.4	196.0	2,583.2	621.0	2.47	8.60	3.24	4.21	0.05
W-JAG2	724.4	376.5	248.7	3,030.1	559.3	2.63	2.67	1.42	0.60	0.06
W-JAG3	665.0	556.0	160.8	1,867.3	514.7	2.46	3.44	3.79	1.91	0.13
W-SSA4	665.1	85.0	214.1	2,901.6	479.3	0.86	2.98	2.65	2.16	0.11
W-ITA	498.2	39.6	143.9	3,080.6	604.8	3.33	2.91	3.48	1.83	0.05
W-ILH	286.3	74.7	175.9	3,184.2	521.3	1.44	3.07	2.61	0.81	0.06
W-MDE1	329.0	157.9	165.3	2,306.0	292.1	1.65	2.73	2.41	1.13	0.11
W-MDE2	315.0	121.2	162.5	2,460.0	321.0	1.68	2.00	2.11	1.65	0.07
W-SSA5	629.9	591.0	176.7	1,993.3	637.5	2.53	2.62	4.82	1.53	0.12
W-SSA6	302.3	70.8	135.3	2,195.3	486.0	2.16	3.63	1.92	1.12	0.06
R-BAR	383.0	41.6	129.0	2,618.4	438.0	1.92	2.46	1.80	2.87	0.02
R-JEQ	357.7	31.8	118.1	2,467.6	416.1	1.72	1.94	1.69	1.32	0.05
R-REC	279.4	34.1	98.3	2,036.7	289.8	0.95	1.99	1.17	1.29	0.04
R-AJU	254.5	27.7	67.2	1,793.2	333.1	1.62	1.91	1.59	0.41	0.14
R-SMF1	280.8	49.0	121.3	2,004.4	221.9	1.25	2.36	2.65	2.04	0.04
R-SSA1	348.7	40.9	134.4	2,454.9	529.6	0.85	2.16	2.00	0.97	0.09
R-SMF2	414.9	45.8	149.9	2,387.7	399.0	1.49	2.82	2.95	0.92	0.11
R-SSA2	571.8	37.3	129.8	2,229.6	432.3	2.73	2.37	3.18	3.81	0.03
R-BEH	467.7	37.9	139.3	2,467.6	374.5	2.59	3.25	2.66	0.66	0.06
R-FLO	515.1	36.4	185.1	2,584.7	531.4	1.61	2.33	1.68	2.45	0.11
R-JAG1	420.0	43.2	100.1	2,567.5	391.8	2.55	2.98	3.03	1.68	0.04
R-ITI	469.9	70.9	129.2	2,153.0	403.4	4.38	3.76	2.84	3.80	0.05
R-SMT	707.0	99.9	236.5	3,851.9	499.2	3.48	3.74	3.67	4.44	0.01
R-LAV	727.3	43.8	206.6	3,896.9	544.3	1.84	2.99	2.33	2.99	0.05
R-SCR	494.3	38.9	134.4	2,514.3	384.6	4.19	3.88	4.03	0.57	0.03
R-SMA	766.2	86.8	286.0	3,622.8	520.6	2.03	2.83	1.86	0.51	0.24

**Table 2** (continued)

Sample	Element									
	P	Na	Mg	K	Ca	Mn	Fe	Zn	Sr	Mo
R-MAR	646.6	73.6	185.5	3,423.9	343.6	1.59	2.30	1.85	1.45	0.02
R-VIT	217.5	43.8	91.9	1,861.7	263.1	2.72	3.56	5.06	3.45	0.10
R-SSA3	723.1	73.7	166.7	3,154.2	567.5	2.36	3.55	3.68	2.44	0.09
R-LFR	616.9	68.3	202.1	3,774.3	603.6	3.07	4.77	4.24	2.78	0.11
R-CON	755.7	73.6	200.6	4,068.3	744.7	1.68	3.34	2.24	2.40	0.07
R-MDE1	234.6	54.4	215.2	2,527.6	410.4	1.88	2.39	2.25	1.09	0.06
R-MDE2	322.4	110.2	157.7	2,605.8	268.2	1.57	3.80	2.51	2.21	0.05
R-SSA5	345.2	78.3	176.1	3,107.9	544.6	1.27	3.20	2.15	1.74	0.08

SSA Salvador, MDE Madre de Deus, SMF Simões Filho, JEQ Jequié, BAR Barreiras, ITA Itabuna, ILH Ilhéus, MAR Maracás, VIT Vitória da Conquista, ITI Itiruçu, UBA Ubaitaba, REC Recife, AJU Aracaju, BEH Belo Horizonte, LAV Lavras, FLO Florianópolis, PAL Porto Alegre, SMA Santa Maria, SMT São Mateus, VIT Vitória, SCR São Carlos, CAM Campinas, JAG Jaguaquara, LFR Lauro de Freitas

Barreiras, Itabuna, Ilhéus, Maracás, Vitória da Conquista, Itiruçu, Ubaitaba, Recife, Aracaju, Belo Horizonte, Lavras, Florianópolis, Porto Alegre, Santa Maria, São Mateus, Vitória, São Carlos, Campinas, Jaguaquara, and Lauro de Freitas. A total of 55 were analyzed, being 24 of red and 31 white species.

In the laboratory, these samples were kept under refrigeration to prevent the proliferation of fungi and bacteria. Grating used in the partitioning of sample was plastic to avoid metal contamination. After grating, samples were heated at 60 °C for 1 h in order to remove superficial water maintaining its integrity. Then, they were homogenized by mechanical agitation (manual) using plastic pallets. After using samples were discarded.

#### Sample Digestion

About 2.0 g of each sample were accurately weighed into glass vessels. A volume of 3.0 mL of concentrated nitric

acid (Merck, Darmstadt, Germany) and 3.0 mL of 30% (v/v) hydrogen peroxide (Merck, Darmstadt, Germany) were added. The hot plate temperature was adjusted to 140 °C and the samples were digested for 3 h. Later, the digested samples were transferred to centrifuge tubes of 15 mL and then the volume was completed to 12 mL with ultrapure water.

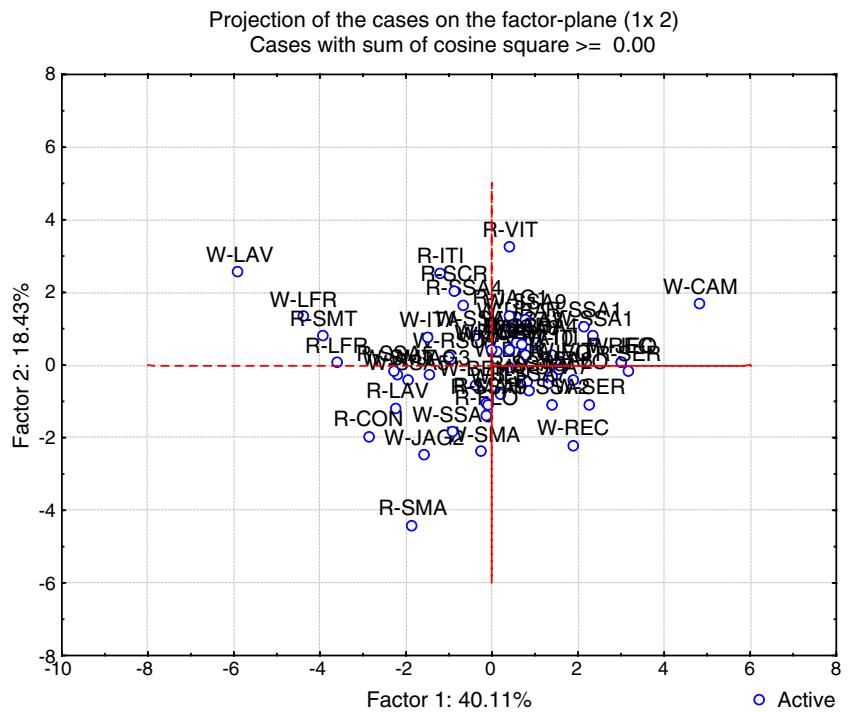
#### Accuracy of the Analytical Method Used for Quantification

The accuracy of the method used for determination of the elements was confirmed by analysis of a certified reference material purchased from National Institute of Standard and Technology (Gaithersburg, MD, USA), spinach leaves National Institute of Standard and Technology 1570a. Digestion of this material was performed with the same decomposition procedure used for the cabbage samples. The results were in agreement with the certified values, as can be seen in Table 1.

**Table 3** Loadings of the variables for the first four principle components

Variable	PC1	PC2	PC3	PC4
Phosphorous	-0.8566	-0.3033	-0.0274	-0.0455
Sodium	-0.1999	-0.1579	0.8290	-0.4498
Magnesium	-0.6468	-0.5899	-0.1056	-0.1862
Potassium	-0.7471	-0.3670	-0.4154	0.1413
Calcium	-0.7913	-0.2668	-0.0337	0.0529
Manganese	-0.6334	0.5381	0.0889	0.1278
Iron	-0.6995	0.3024	0.0870	-0.1635
Zinc	-0.5868	0.5446	0.3059	0.3204
Strontium	-0.6322	0.4447	-0.0443	-0.0753
Molybdenum	0.0094	-0.5447	0.5914	0.5308
Total variance (%)	40.11	18.43	13.33	6.95
Cumulative variance (%)	40.11	58.54	71.87	78.82

**Fig. 1** Plot of the first principal component (PC1) versus the second principal component (PC2) for the 55 cabbage samples



**Results and Discussion**

Determination of Chemical Elements in Cabbage Samples

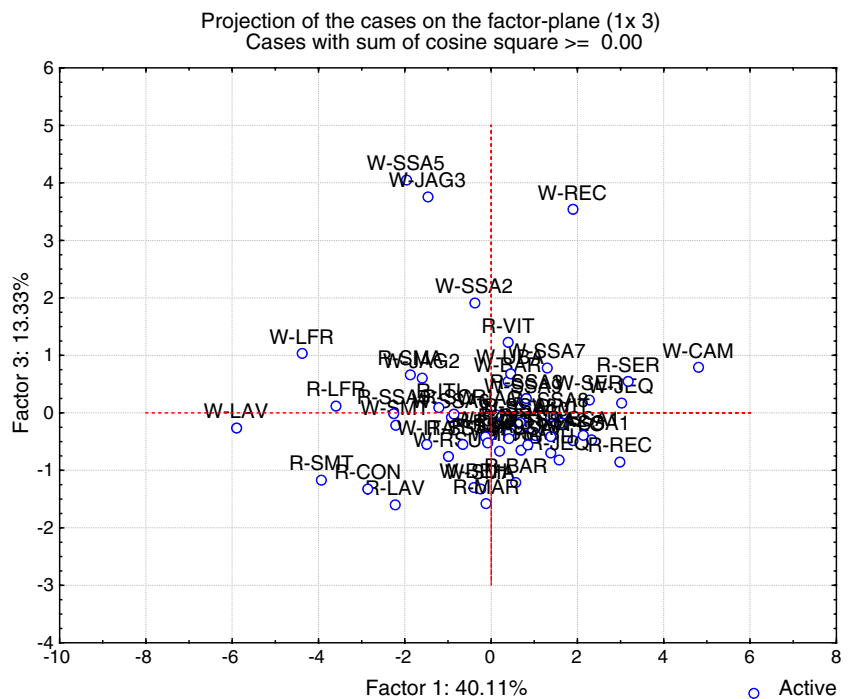
The determination of the elements: phosphorus, sodium, magnesium, potassium, calcium, manganese, iron, zinc, strontium, and molybdenum were performed in 55 samples, being 31 of the white specie and 24 of the red specie. The

results expressed as average of triplicates (mg of element per kg of sample) are shown in Table 2.

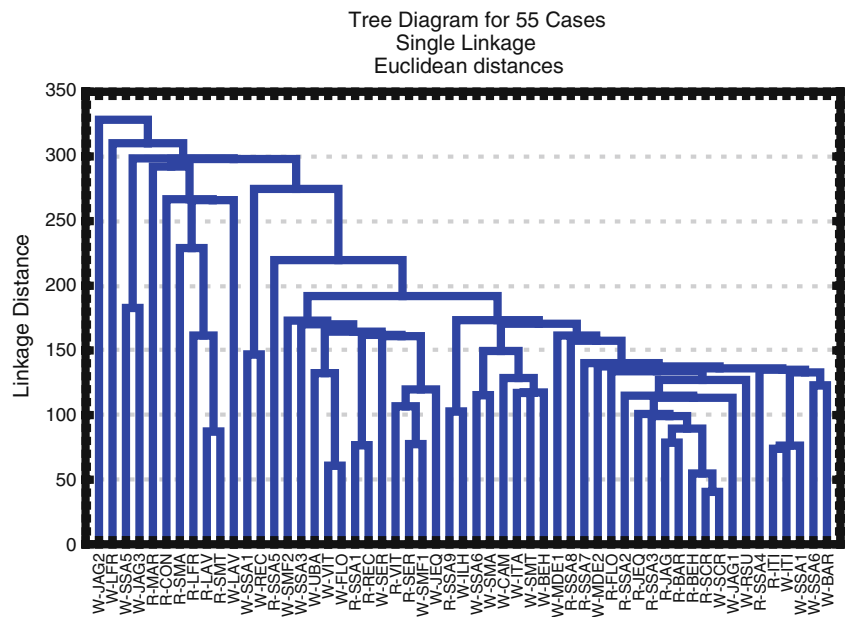
Data Evaluation Employing Principal Component Analysis

The results of the determination of the ten elements in the 55 samples analyzed showed in Table 2 were evaluated using the PCA technique. The data matrix was established

**Fig. 2** Plot of the first principal component (PC1) versus the third principal component (PC3) for the 55 cabbage samples



**Fig. 3** Dendrogram for cabbage samples showing single linkage with Euclidean distances



using the elements as columns and the cabbage samples as rows. This evaluation was performed on auto-scaled data due to the different orders of magnitude of the element concentrations. The loadings of original variables on the first four principal components and the variances explained by each component are given in Table 3.

The first four principal components were chosen for modeling the data because they describe almost 79% of the total variance. Phosphorus, calcium, potassium, iron, magnesium, manganese, strontium and zinc are the dominant variables for the first principal component (PC1) that represent 40% of the total variance. These eight elements contribute to the major variability presented in the samples and all are positively correlated. The second principal component (PC2) accounts for 18% of the total variance and magnesium, molybdenum, zinc and manganese are the dominant variables. The scores plot of the first two

components is shown in Fig. 1. In it, it can be seen that there is no separation between samples of red and white varieties. Samples with high concentrations for the elements phosphorus, calcium, potassium, iron, magnesium, manganese, strontium, and zinc have low scores on PC1, since their loadings are negative. Then, for example, the sample W-LAV, collected in Lavras city, has high concentrations of the elements phosphorous, calcium, manganese, zinc, strontium and iron. The sample W-LFR has highest concentration for iron. Obviously, samples with higher scores on PC1 have the lesser concentrations of these elements. Thus, the sample W-CAM, collected in Campinas city has low concentration for the elements manganese, iron and strontium. The elements magnesium and zinc are negatively correlated on PC2, thus, the sample R-VIT with high scores on PC2 has high concentration for zinc and low concentration for magnesium. This sample was collected in Vitoria city. Magnesium and molybdenum are positively correlated because both have negative loadings on PC2. Thus, the sample (R-SMA) collected in Santa Maria city, which has lower scores on PC2 has highest concentrations for magnesium and molybdenum.

**Table 4** Concentrations of chemical elements in cabbage samples (mg of element per kg of sample)

Element	Average	Concentration range
Phosphorous	459.5	217.5–766.2
Sodium	105.0	27.2–591.0
Magnesium	159.9	67.2–286.0
Potassium	2,532.6	1,602.9–4,068.3
Calcium	446.4	221.9–744.7
Manganese	2.06	0.81–4.40
Iron	3.00	1.91–8.60
Zinc	2.60	1.17–5.10
Strontium	1.84	0.35–5.79
Molybdenum	0.07	0.01–0.24

The third principal component (PC3) presents 13% of the total variance, being sodium and molybdenum the dominant variables, both with positive loadings. Figure 2 shows the scores plot of PC1×PC3. Considering this, the samples with higher scores on PC3 (W-SSA5, W-JAG3, and W-REC) have high concentrations for these two elements. These samples were collected in Salvador, Jaguaquara, and Recife cities, respectively. On the other hand, the samples R-LAV (Lavras city) and R-MAR (Maracas city) with lower scores on PC3 have respectively low concentration for sodium and molybdenum.

In order to evaluate preliminarily the influence of the seasonality on the mineral composition of cabbage, samples from the cities of Madre de Deus and Simoes Filho were collected in summer (SMF1 and MDE1) and winter (SMF2 and MDE2). The results as can be seen in Table 2 demonstrated that the seasonality does not influence the mineral composition of cabbage, considering these two cities.

#### Evaluation of Data Using Hierarchical Cluster Analysis

HCA was applied to the auto-scaled data using the single linkage method with Euclidean distances to calculate the sample interpoint distances and similarities. A hierarchical agglomerative procedure was employed to establish clusters, and Fig. 3 shows the dendrogram that was obtained.

Some results found by PCA were also achieved using HCA. For instance, the samples (W-JAG3 and W-SSA5) form a small group by HCA and by PCA. It was found that these samples have high concentrations of sodium and molybdenum. The two samples (R-SMT and R-LAV) that by PCA are close (Fig. 2) because these have high concentrations of phosphorous, magnesium, calcium, and potassium also form a small group by HCA and it can be found in Fig. 3. Another observation that can be seen is that the samples (R-ITI and W-ITI and also R-SCR and W-SCR), which are of red and white species form two groups, it confirming that the mineral composition of cabbage is independent of species.

#### Determination of the Mineral Composition of Red and White Cabbage

Considering that PCA and also HCA clearly demonstrated that the mineral composition is independent of white or red variety. The results for the average content and concentration ranges for the elements are shown in Table 4. These results show that cabbage can be used as nutritional supplement and contains levels of calcium, magnesium, phosphorous, zinc, manganese, and iron in agreement with those reported in the literature.

#### Conclusions

The method developed for the determination of minerals in white and red cabbage by ICP OES was satisfactory for the

quantification of calcium, phosphorous, potassium, magnesium, sodium, iron, manganese, molybdenum, strontium, and zinc.

Phosphorus, calcium, potassium, iron, magnesium, manganese, strontium and zinc are the elements that contribute to the major variability presented in the samples and all are positively correlated. It means that the assimilation processes of these elements by this vegetable probably are similar.

The PCA and HCA techniques demonstrated that there is no difference in the mineral composition between the red and white cabbage.

The results evidenced that cabbage can be used as nutritional supplement.

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