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Determination and Evaluation Employing Multivariate Analysis of the Mineral Composition of Broccoli (*Brassica oleracea L.* var. *Italica*)

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Abstract Broccoli (*Brassica oleracea L.* var. *Italica*) is a vegetable food that belongs to the family *Cruciferaceae*, and it is a good source of vitamins and minerals. Among other characteristics, the kind of culture (conventional or organic), the climate of origin, and the way a vegetal will be consumed influence directly in the concentrations of minerals. The aim of this work was to determine mineral composition (Fe, Mn, Zn, K, Ca, Mg, and Na) of 16 broccoli samples collected in four cities of Bahia, Brazil, in summer and winter, from organic and conventional cultures. These elements were also determined in a lot of raw and cooked summer samples and then all results were evaluated using multivariate analysis. Broccoli samples were analyzed by inductively coupled plasma optical emission spectrometry after digestion with HNO_3 and H_2O_2. The results expressed as milligrams of element per 100 g of sample demonstrated that the concentration ranges for conventional and organic broccoli varied, respectively, from 0.13 to 0.90 and 0.14–1.18, for iron; from 0.15 to 1.79 and 0.04–1.40, for manganese; from 0.20 to 1.58 and 0.17–1.28, for zinc; from 317.65 to 484.45 and 321.58–521.78 for potassium; from 7.14 to 153.50 and 9.38–131.55, for calcium; from 21.09 to 47.15 and 29.51–61.23, for magnesium; from 3.66 to 21.21 and 1.89–27.09 for sodium. The accuracy of the method was confirmed by analysis of a certified reference material of spinach leaves (NIST 1570a). The statistical evaluation of the obtained results showed that broccoli samples were not differentiated by city of origin, station, or culture. Nevertheless, by comparing raw and cooked samples, it was noticed a tendency of separation in the principal component analysis and hierarchical cluster analysis.

Keywords Broccoli · Mineral composition · ICP OES · PCA · HCA

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Introduction

The nutritional value of vegetables as a source of essential minerals, vitamins, and dietary fiber is well recognized. Minerals play an important role in the development and good health of body. These nutrients act in tissues and body fluids like electrolytes to maintain the acid–base balance, osmotic pressure, and permeability of cell membranes, especially Ca, P, Na, and l. Functions like activators of enzymatic processes are related to Cu and Mn; Zn and Mn are associated to the structure of metalloenzymes. The deficiency of one or more mineral components can result in severe organic disorders such as osteoporosis, anemia, and goiter. These nutrients are present in various sources especially green leafy, tea leaves, flower vegetables, plant products among others (Kuhnlein 1990; Soylak et al. 2007; White and Broadley 2005a, b; Bhat et al. 2010).

Table 1 Concentrations of minerals in fresh and cooked broccoli samples (milligrams of analyte per 100 g of sample)

Sample	Method	Fe	Mn	Zn	K	Ca	Mg	Na
FWC 11	Raw	0.60	0.44	0.68	337.02	152.78	46.03	17.94
FWC 12	Raw	0.67	0.33	0.57	333.10	150.88	45.90	18.63
FWC 13	Raw	0.67	0.43	0.72	339.19	153.50	47.15	19.89
FWC 21	Raw	0.81	1.80	1.57	376.16	93.15	35.34	19.26
FWC 22	Raw	0.78	1.71	1.59	382.18	92.89	35.59	19.91
FWC 23	Raw	0.70	1.63	1.44	367.31	85.13	31.96	16.64
JWC 11	Raw	0.66	0.35	0.74	347.99	116.45	46.91	14.60
JWC 12	Raw	0.63	0.33	0.73	368.82	111.78	45.64	16.50
JWC 13	Raw	0.87	0.38	0.74	341.64	113.22	44.87	15.13
JWC 21	Raw	0.70	1.07	1.49	319.52	74.62	27.66	18.40
JWC 22	Raw	0.83	1.03	1.52	333.63	77.83	29.15	18.05
JWC 23	Raw	0.82	1.09	1.50	317.65	85.67	29.76	19.39
MWO 11	Raw	0.93	0.33	0.71	511.93	118.86	58.87	14.06
MWO 12	Raw	0.87	0.32	0.67	491.06	114.41	55.78	16.21
MWO 13	Raw	0.83	0.33	0.72	521.78	123.36	61.23	14.90
MWO 21	Raw	0.89	0.35	0.70	426.55	112.66	47.56	10.07
MWO 22	Raw	0.89	0.37	0.71	425.07	125.96	49.70	11.26
MWO 23	Raw	0.89	0.33	0.72	415.72	104.16	43.30	9.96
BWO 11	Raw	0.70	1.31	1.22	329.80	111.75	37.27	23.02
BWO 12	Raw	0.75	1.40	1.28	345.34	122.70	40.42	23.80
BWO 13	Raw	0.67	1.38	1.26	321.60	131.56	40.84	27.09
BWO 21	Raw	0.79	0.46	1.19	397.93	76.30	36.15	17.33
BWO 22	Raw	0.74	0.43	1.20	389.60	69.84	36.43	17.38
BWO 23	Raw	0.76	0.47	1.25	381.65	73.82	38.95	17.06
JSC 11	Raw	0.18	0.16	0.20	341.91	11.18	38.46	4.10
JSC 12	Raw	0.18	0.17	0.21	339.45	11.63	40.80	4.24
JSC 13	Raw	0.19	0.17	0.21	328.58	12.09	40.38	4.38
JSC 21	Raw	0.90	0.48	0.82	428.30	55.40	41.04	14.41
JSC 22	Raw	0.87	0.49	0.82	477.45	57.98	44.88	14.72
JSC 23	Raw	0.84	0.45	0.85	484.45	51.88	45.45	14.29
FSC 11	Raw	0.13	0.27	0.23	338.69	7.35	21.39	3.77
FSC 12	Raw	0.14	0.26	0.23	337.08	7.15	21.10	3.66
FSC 13	Raw	0.13	0.27	0.24	344.89	7.27	21.62	3.79
FSC 21	Raw	0.88	0.42	0.94	396.79	49.03	40.94	20.23
FSC 22	Raw	0.77	0.40	0.92	377.58	49.53	40.56	19.81
FSC 23	Raw	0.81	0.45	0.93	411.71	50.03	45.25	21.22
BSO 11	Raw	0.15	0.05	0.17	377.35	10.06	29.52	1.93
BSO 12	Raw	0.16	0.05	0.18	396.77	9.38	30.09	1.90
BSO 13	Raw	0.17	0.05	0.18	374.70	9.45	30.34	1.91
BSO 21	Raw	0.89	0.26	0.87	335.13	74.33	53.02	11.40
BSO 22	Raw	0.95	0.26	0.90	341.48	71.80	49.15	11.43
BSO 23	Raw	1.01	0.27	0.94	360.41	74.95	53.38	11.66
MSO 11	Raw	1.16	0.37	0.98	412.97	55.35	41.06	7.14
MSO 12	Raw	1.13	0.36	0.98	427.58	51.99	42.40	7.01
MSO 13	Raw	1.18	0.38	0.97	428.04	58.80	43.80	7.29
MSO 21	Raw	1.08	0.43	1.13	505.11	63.57	56.10	7.49
MSO 22	Raw	1.01	0.46	0.93	504.79	72.28	56.87	7.68
MSO 23	Raw	1.10	0.45	0.95	504.77	69.80	56.21	7.45
JSC 21	Cooked	0.67	0.35	0.50	129.76	37.67	17.48	7.06

Table 1 (continued)

Sample	Method	Fe	Mn	Zn	K	Ca	Mg	Na
JSC 22	Cooked	0.72	0.36	0.50	129.22	37.86	17.93	7.05
JSC 23	Cooked	0.68	0.33	0.49	125.52	41.59	18.75	7.17
FSC 21	Cooked	0.69	0.32	0.60	132.94	44.94	17.43	5.90
FSC 22	Cooked	0.80	0.33	0.62	130.22	44.40	17.29	6.03
FSC 23	Cooked	0.71	0.37	0.62	130.55	47.38	16.24	5.73
BSO 21	Cooked	0.83	0.18	0.54	154.36	61.70	23.04	2.06
BSO 22	Cooked	0.87	0.19	0.55	156.21	64.90	23.12	2.04
BSO 23	Cooked	0.99	0.19	0.57	162.96	64.74	24.81	2.52
MSO 11	Cooked	0.73	0.21	0.42	91.15	49.31	7.57	4.33
MSO 12	Cooked	0.79	0.23	0.42	86.69	48.61	5.99	4.13
MSO 13	Cooked	0.73	0.21	0.43	83.95	50.36	6.01	4.13
MSO 21	Cooked	0.83	0.33	0.53	105.83	53.67	17.45	4.53
MSO 22	Cooked	0.83	0.35	0.54	103.59	53.15	16.49	4.69
MSO 23	Cooked	0.96	0.36	0.54	102.53	54.33	16.21	4.91

Cities: *F* Feira de Santana; *J* Jacuípe; *M* Morro do Chapéu; *B* Berimbau; Stations: *W* winter; *S* Summer; Culture: *C* Conventional; *O* Organic

According to the American Food Standards Agency and the US Department of Agriculture, broccoli is a good candidate as source of essential minerals for human consumption (White and Broadley 2005a, b). It contains high fiber, low calorie, and also important antioxidants like phytochemicals and isothiocyanates (Podsedek 2007). Currently, cultivation of vegetables, including broccoli, has been achieved by conventional methods and also by organic cultures (Saba and Messina 2003). These organic cultures can result in changes in nutritional composition, although there are few data about the influence of cultivation in nutrient levels. Concerning minerals, there are no data about possible changes related to the application of different cultivation techniques.

Unlike vitamins, minerals are not destroyed by light, heat, and oxygen but only removed from the food by leaching or physical separation (Miller and Rice-Evans 1996). Besides the kind of culture, the way the vegetable will be consumed, among other characteristics, must be considered once they can affect directly nutritional contents, including minerals. Some vegetables are consumed *in natura* (fresh) but cooking by boiling is the most common method involved in vegetables consuming. This is one of the factors responsible for chemical and physical changes and it may alter their health-promoting compounds such as glucosinolates, phenolic compounds, and minerals thus reducing the nutritional value of vegetables (Gliszczynska-Swiglo et al. 2006). Changes in mineral composition of vegetables submitted to cooking process depend on the element (Koplik et al. 2004), the kind of heating, the container used for cooking, etc. (Schnepf and Driskell 1994). However, a study revealed that in different microwave conditions, it was observed high retention level of different minerals (60–100 %) (López-Berenguer et al. 2007).

The application of chemometric tools to the characterization, determination of geographic origin, and quality control of food products has recently become a very interesting research area. Some authors have used these tools in order to classify food plants based on their profile of antioxidant compounds (Cárnara et al. 1995; Forina et al. 2002; Downey et al. 2003; Alonso-Salces et al. 2006; Woodcock et al. 2007). Multivariate techniques are powerful tools which permit an exploitation of the results of chemical analysis in order to verify the existence of similarities between the samples (Correia and Ferreira 2007). Among these techniques, the principal component analysis (PCA) and hierarchical cluster analysis (HCA) have been applied successfully in evaluation and characterization of analytical data of food samples (Anunciação et al. 2011; dos Santos et al. 2011). Thus, the aim of this work was to evaluate the mineral composition (calcium, magnesium, sodium, potassium manganese, iron, and zinc) of broccoli samples obtained from different cities, cultures, and stations. Moreover, a lot of summer samples were analyzed to evaluate possible losses by comparing fresh and cooked broccoli.

Experimental

Instrumentation

All determinations were performed with an inductively coupled plasma optical emission spectrometer, ICP OES Varian model Vista PRO (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 following instrumental conditions: power (1.3 kW), plasma gas flow (15.0 L min^{-1}), auxiliary gas flow (1.5 L min^{-1}), and nebulizer gas flow (0.8 L min^{-1}). The elements and the analytical wavelengths used for quantification were: Ca II (422.673), Fe II (238.204), K (766.491), Mg II (285.213), Mn II (257.610), Na I (589.592), and Zn II (334.502).

Reagents and Solutions

All chemical reagents used in the experiment were of analytical grade. Ultra pure water (18.2 MΩ cm^{-1}) from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all solutions. Commercially available standard metal solutions of 1,000 mg L^{-1} (Ca, Fe, Mn, Zn, Mg, K, and Na; Titrisol Merck, Damstadt, Germany) were used to prepare working solutions by appropriate dilution of these standards with 1 % (v v^{-1}) nitric acid. Solutions of concentrated nitric acid (Merck, Darmstadt, Germany) and hydrogen peroxide 30 % (v v^{-1}) (Merck, Darmstadt, Germany) were used for sample digestion. Glassware and centrifuge tubes were kept overnight in nitric acid solution 10 % (v v^{-1}) for decontamination and then rinsed with ultra pure water before using. Analytical curves were obtained within 5.0–50 mg L^{-1} for calcium, magnesium, sodium, and potassium and within 0.5–5.0 mg L^{-1} for manganese, zinc, and iron.

Sample Collection, Storage, and Preparation

Sixteen different bunches or heads of Broccoli (*Brassica oleracea L.* var. *Italica*) grown in conventional or organic agriculture were purchased from four cities of Bahia, Brazil: Conceição de Jacuipe (C), Feira de Santana (F), Berimbau (B), and Morro do Chapéu (M), during periods of summer and winter.

Table 2 Evaluation of the accuracy of the analytical method used for elements quantification of Broccoli samples

Elements	Obtained 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
Mg (%)	0.86±0.144	0.89[a]
Zn (mg kg^{-1})	80±12	82±3
Mn (mg kg^{-1})	67.3±5.2	75.9±1.9

[a] Value reported

Table 3 Evaluation of the precision of the method employing two broccoli samples

Elements	Sample JSC 1		Sample FSC 1	
	Concentration (mg per 100 g)	RSD (%)	Concentration (mg per 100 g)	RSD (%)
Fe	0.18	1.45	0.14	3.76
Mn	0.17	4.35	0.27	2.22
Zn	0.21	1.56	0.24	2.45
K	336.64	2.11	340.22	1.21
Ca	11.64	3.90	7.25	1.42
Mg	39.88	3.13	21.37	1.23
Na	4.24	3.26	3.74	1.81

Each sample, weighting about 0.7 kg was represented by two heads of broccoli. In the laboratory, these samples were enclosed separately in polyethylene bags and stored in refrigerator to prevent the proliferation of fungi and bacteria. Samples were washed with 3 % Extran solution (v v^{-1}) (Merck, Darmstadt, Germany), rinsed with deionized water and then the inflorescences were cut and homogenized with plastic knives. Before digesting, samples were separated in two groups: the first one was of raw broccoli (fresh) and the second group was of broccoli which would be cooked according to Franco and Chaloub's methodology (1992). For the cooking procedure, 100 g of broccoli was added to 200 mL of boiling water and cooked for 5 min. After this time, the broccoli was drained and cooled at room temperature, dried with paper cut into pieces for later digestion.

Sample Digestion

Samples were digested according to the adapted literature (Anunciação et al. 2011). Briefly, about 2 g of each sample was weighted into a glass vessel in triplicates. Then, 5 mL of

Table 4 Loadings of variables for the first three principal components and their total variance for all samples

Variables	PC 1	PC 2	PC 3
Fe	−0.536925	−0.128866	0.790656
Mn	−0.681420	−0.556413	−0.380296
Zn	−0.863261	−0.360218	−0.020321
K	−0.600502	0.700738	−0.248792
Ca	−0.772057	−0.021470	0.238117
Mg	−0.680001	0.707521	0.039725
Na	−0.876789	−0.143740	−0.238703
% Total Variance	52.65	20.98	13.53
% Cumulative Variance	52.65	73.63	87.17

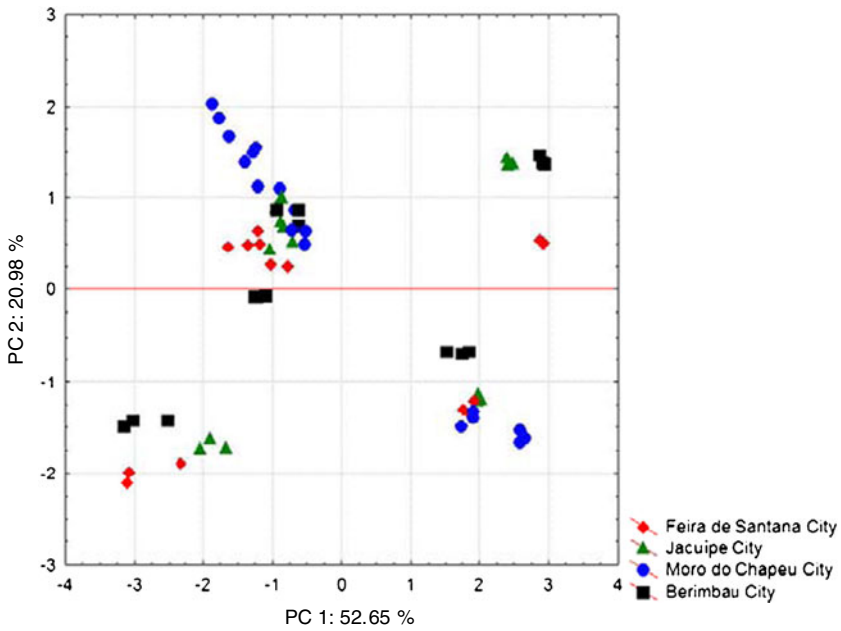

Fig. 1 Scores plot of PC1 × PC2 for all samples

concentrated nitric acid and 3 mL of 30 % (v v^{-1}) hydrogen peroxide were added to the glass vessel. The temperature of the heating block, model TE-040/25 (TECNAL, São Paulo, Brazil), was adjusted to 140 °C, and the samples were digested for 3 h. Finally, the content was quantitatively transferred to centrifuge tubes of 15 mL, and then the volume was completed with ultrapure water.

Validation

The accuracy of the method used for determination of the elements was evaluated by analysis of a certified reference material of spinach leaves purchased from the National Institute of Standard and Technology (NIST 1570a) (Gaithersburg, MD, USA). This material was digested under the same conditions of broccoli samples, and the results are presented in Table 1.

Results and Discussion

Determination of Chemical Elements in Broccoli Samples

The concentrations of the elements Ca, Fe, K, Mg, Mn, Na, and Zn were determined in 16 broccoli samples obtained from different cities, cultures, stations, and treatments employing ICP OES. The results of triplicates expressed as mg of element per 100 g of sample on a wet basis are presented in Table 1. The predominant element in all samples was potassium, and these data are in accordance with the literature which reports the high concentrations of this element in food plants (Brody 1999).

Validation of Method

The accuracy of the method employed for the determination of Ca, Fe, K, Mg, Mn, Na, and Zn was confirmed by

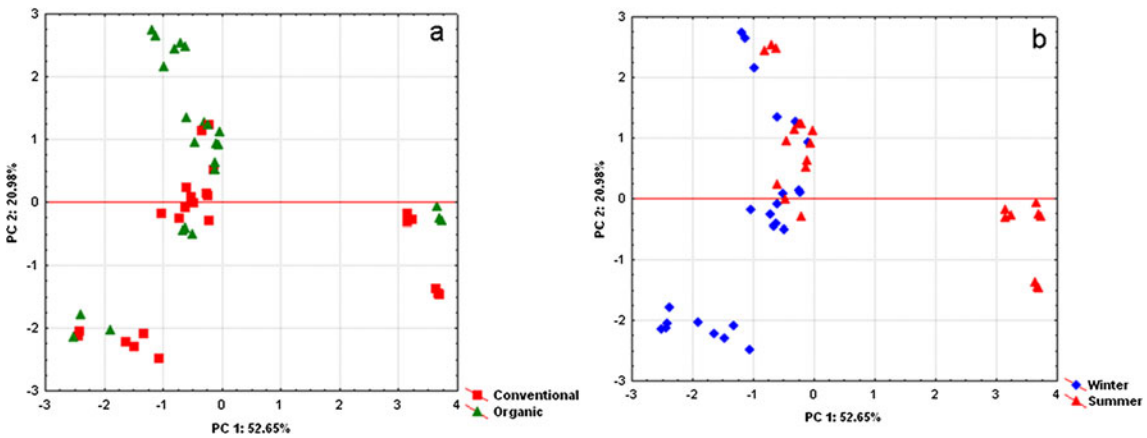

Fig. 2 Scores plot of PC1 × PC2 for conventional and organic samples (**a**); winter and summer samples (**b**)

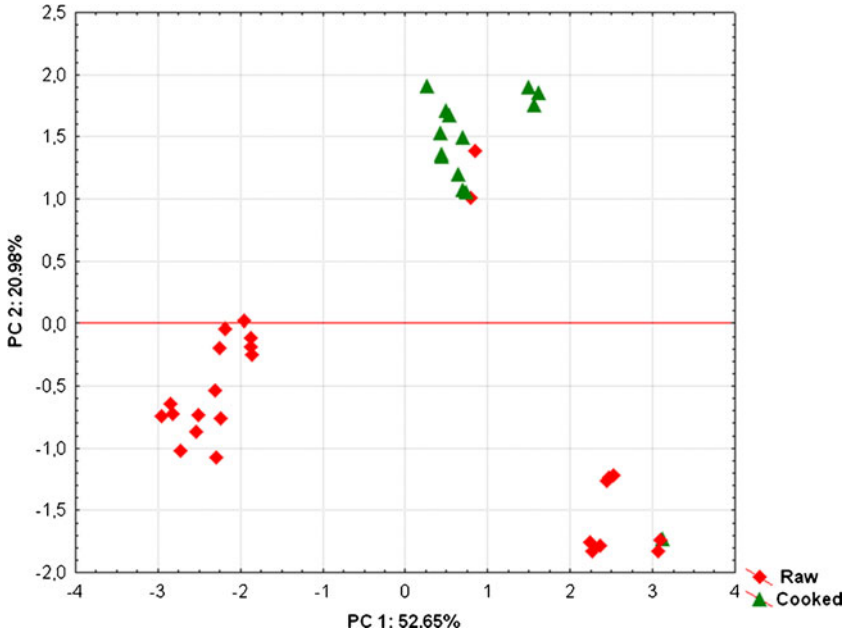

Fig. 3 Score plots of PC1 × PC2 for raw and cooked samples

analysis of a certified reference material (CRM) of spinach leaves, purchased from NIST 1570a (Gaithersburg, MD, USA). This CRM was submitted to the same procedure used for sample digestion, and the results are presented in Table 2.

The precision of the proposed method was evaluated making use of two samples (Table 3). The relative standard deviation, shown in Table 3, varied from 1.21 to 4.35 %, and according to this data, the method showed good precision.

Statistical Analysis Applied to the Data

For further evaluation of the obtained results, the software Statistica 6.0 was used, and the PCA and HCA were employed. From a data matrix (63×7), obtained by triplicate of the data, in which the samples of broccoli were arranged in rows and the concentrations of analytes in columns; data were auto-scaled for further evaluation.The Table 4 presents the loadings of each variable on the first three principal components, the total and cumulative variances explained by each component. The first three components describe 87.17 % of the total variance. The first principal component (PC1) represents 52.65 % of the total variance and all the elements in this PC are correlated. The elements calcium, sodium, manganese, and zinc are the dominant variables on this PC. The second principal component (PC2) represents 20.98 % of the total variance, and the elements potassium and magnesium are the dominant variables on this PC. On the PC3, the dominant variable is iron.

The score plots obtained from principal component analysis are shown in Figs. 1, 2, 3. Figure 1 was obtained making use of a matrix 63×7 that represents all results, and according to this figure, no separation was identified when samples were evaluated on a basis of cities of origin.

Figure 2(a and b) represents the score plots for the raw samples from a data matrix 48×7 in order to evaluate the results on a basis of the kind of culture (Fig. 2a) and the stations of year (Fig. 2b). Based on the kind of culture, any group was identified; nevertheless, when samples were evaluated on a basis of the period of year (station), the score plots showed a tendency of separation between summer and winter samples related to the PC1. This tendency is in accordance with the higher loadings of the elements, specially Na, Ca, Zn, and Mn on negative values of PC1 and the higher concentrations of these analytes in winter samples as compared with summer ones. It can also be justified because in the cities where samples were collected, the pluviosity in the summer is higher than in the winter, and because of this,

Table 5 Averages and ranges of concentrations of minerals in raw and cooked broccoli samples (milligrams of analyte per 100 g of sample)

Analyte	Raw		Cooked	
	Average	Range	Average	Range
Fe	0.72	0.13–1.18	0.79	0.67–0.99
Mn	0.53	0.05–1.80	0.29	0.18–0.37
Zn	0.85	0.17–1.59	0.53	0.42–0.62
K	389.55	317.65–521.78	121.70	83.95–162.96
Ca	74.70	7.15–153.50	50.31	37.67–64.90
Mg	41.59	21.10–61.23	16.39	5.99–24.81
Na	13.11	1.90–27.09	4.82	2.04–7.17

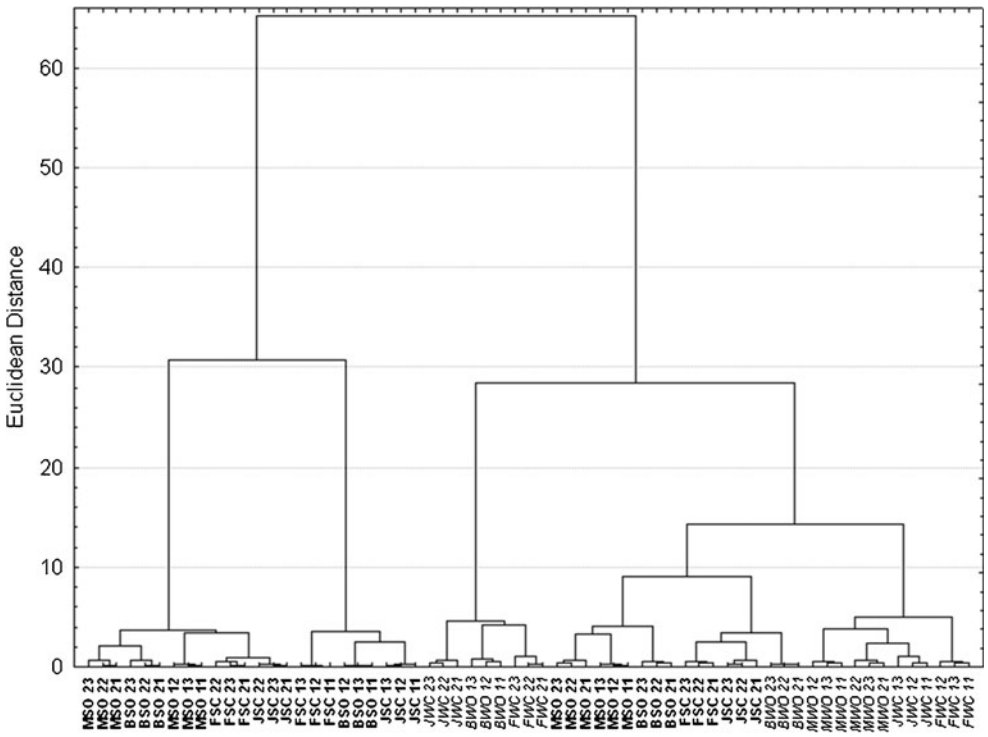

Fig. 4 Dendrogram for broccoli samples showing single linkage with Euclidean distances. Cooked samples (*bold*) and raw samples (*italic*)

minerals can be leached from soil and so there is a decrease of minerals available to plants.

For further investigation, five samples were separated in two lots in order to evaluate possible losses of minerals concentrations when samples were cooked in water (boiled). According to the scores plot of PC1 versus PC2 for the raw and cooked samples, shown in Fig. 3, it can be seen that most of broccoli samples were separated in two groups on a basis of the PC2. According to this figure and the information contained in Table 1, it is noticed that raw samples have higher concentrations for all minerals quantified which can be explained by the leaching of some mineral content from the broccoli by the water used for cooking. This information is corroborated by the information contained in Table 5 which presents the averages and ranges of concentrations for each mineral determined in raw and cooked broccoli samples.

The dendrogram obtained from hierarchical cluster analysis using the single linkage method with Euclidean distances for all samples is represented by Fig. 4. Some results obtained from PCA were also found in HCA such as the tendency of separation between summer and winter samples and raw and cooked ones.

Another information that is worth mentioning is a comparison between the concentrations of the elements determined in this work and other studies about food plants presented in Table 6. According to this table, kale is the vegetable with high concentrations of the determined elements followed by broccoli, watercress, and cabbage. Despite the differences in mineral concentrations, they are good source of minerals.

Conclusion

The method applied was satisfactory for the quantification of calcium, magnesium, sodium, potassium manganese, iron, and zinc by ICP OES in broccoli samples from different cities, cultures, and stations. These elements are correlated and probably absorbed by the vegetal in a similar way.

The PCA and HCA techniques demonstrated that there is no difference between broccoli samples from the studied

Table 6 Comparison between concentrations (milligrams of element per 100 g) of minerals in some vegetables

Sample	Fe	Mn	Zn	K	Ca	Mg	Na	Reference
Broccoli	0.7	0.9	1.1	347.0	109.0	38.8	17.9	This work
Watercress	0.9	0.8	0.8	–	250.0	37.1	–	de Souza et al. 2011
Kale	2.1	2.1	2.6	–	551.0	117.3	–	Fadigas et al. 2010
Cabbage	0.3	0.2	0.3	253.3	44.6	16.0	10.5	Anunciação et al. 2011

cities or from the different kinds of culture evaluated. So, according to the obtained results, it can be concluded that the soil of origin and kind of culture do not influence in the absorption of the determined analytes probably because their contents are similar.

A tendency of separation of samples from different stations can be associated to the pluviosity of the cities and the leaching of the determined elements.

The obtained results also showed that the determined elements had their levels decreased as the broccoli was cooked in relation to the raw one. These losses were probably because of leaching and may offer important information about the relationship between nutritional aspects of vegetables and the way they are ingested. These data can also help health professionals to establish adequate dietary for healthy and ill individuals.

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