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Multivariate analysis of the mineral content of raw and cooked okra (*Abelmoschus esculentus* L.)☆

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ABSTRACT

Okra (*Abelmoschus esculentus L.*) is a plant native to Africa. It was introduced to Brazil with the slave trade and spread to all regions, including the state of Bahia, northeastern Brazil. In this work, the mineral content of raw and cooked okra marketed in the state of Bahia, of both conventional and organic cultivars was determined. The results were evaluated using multivariate analysis. The samples were digested in a heating block using nitric acid and hydrogen peroxide, and analysed using inductively coupled plasma optical emission spectrometry (ICP OES). The accuracy of the method was confirmed using a standard reference material, tomato leaves (NIST 1573a). Average mineral concentrations in raw and cooked okra (in mg/100 g), were: 366 to 325 (Ca); 0.102 to 0.052 (Cu); 267 to 97.7 (K); 45.3 to 18.3 (Mg); 18.3 to 7.00 (Na); 44.5 to 25.8 (P); and 0.233 to 0.094 (Zn). Raw and cooked samples tended to separate in principal component analysis (PCA) and hierarchical cluster analysis (HCA). The elements that contributed most for the variability between raw and cooked samples are: K, P, Mg, Cu, Na and Zn, with minor contributions from Ca.

1. Introduction

Minerals are inorganic elements, some of which are essential nutrients. The major minerals (Ca, K, Na and Mg) and essential trace elements (Fe, Cu, Zn and Mn) play very important roles in human metabolism [1]. Deficiencies of these minerals can lead to metabolic disorders and organ damage, leading to acute and chronic disease and ultimately death [2]. Thus, they must be obtained from food.

Natural plant foods such as fruits and vegetables are the main sources of minerals. Many studies [1,3–5] have characterised the composition of these foods, to formulate diets for the prevention and treatment of disease, and also for the purposes of nutritional education. Okra (*Abelmoschus esculentus* L.) is a plant native to Africa that has long been a part of the diet in various parts of the world [6]. It was introduced to Brazil with the slave trade and spread to all regions in the country, including the northeast and southeast. In the state of Bahia, northeastern Brazil, okra is the main ingredient of the popular dish "caruru".

Nutritionally, okra plays an important role in the human diet because it contains carbohydrates, protein, fibre, minerals and vitamins, including vitamin C [6,7]. It is beneficial to the digestive system and contributes to healthy intestinal functioning due to its high polysaccharide and micro-element content [8,9]. Due to its antioxidant and anti-inflammatory properties, okra can be used to treat asthma [7]. The consumption of okra may also assist in the control of blood sugar. Sabitha et al. [7] demonstrated the antidiabetic activity of aqueous extracts of okra peel and seed through inhibition of the enzymes α-glucosidase and α-amylase. Additionally, okra has a cholesterol-lowering effect [10] and may therefore be an ally in the treatment not only of diabetes but also of excessive cholesterol.

A recent study of okra from northeastern Brazil showed significant levels of protein (22.14%) and lipids (14.01%) and high amounts of unsaturated lipids (66.32%), especially oleic (20.38%) and linoleic acids (44.48%) [11]. However, chemical investigations of Brazilian okra are still in their infancy, especially with regard to regional variations. Nutrient levels in foods are variable. The major sources of variability in nutrient composition are soil, climatic conditions (geographical origin), seasonal variations, physiological state and maturity, and the use of agricultural chemicals. Another important factor is how food is prepared. Many researchers have expressed growing concern about the effects of cooking methods on the nutritional value of food [12–15].

Exploratory analytical techniques such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) are used for the statistical evaluation of data, making it possible to correlate several variables and to identify information relevant to characterisation of samples. Identification of more representative variables or results can reveal a lack of homogeneity in the data or similarities and differences between data sets. These techniques are also easy to interpret, so they are frequently used for data processing in several areas. PCA employs a linear combination of variables, generating graphs in which the

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Table 1
Instrumental parameters used in the ICP OES.

Parameters	Conditions	
RF generator (MHz)	40	
RF power (kW)	1.3	
Plasma gas rate (L min^{-1})	15.0	
Auxiliary gas rate (L min^{-1})	1.5	
Nebulizer pressure (kPa)	200	
Injector tube diameter (mm)	2.4	
Spray chamber	Cyclonic	
Nebulizer	Concentric. type K	
Lines (nm)	Ca (II) 422.673	Na (I) 589.592
	Cu (I) 327.395	K (II) 769.897
	Mg (II) 285.213	P (I) 213.618
	Zn (II) 213.857	

samples are represented in Cartesian coordinates, with the axes representing the PCs. Graphical representations of HCA are called dendrograms [16]. These techniques have recently been employed in the characterisation of foods such as broccoli [13], cabbage [17], wheat flour [18], kale [19] and beans [20].

In the present study, the mineral content of raw and cooked okra, both conventional and organic, was determined using inductively coupled plasma optical emission spectrometry (ICP OES). The results were evaluated using the multivariate analysis techniques PCA and HCA.

2. Materials and methods

2.1. Reagents and solutions

All reagents were of analytical grade. Ultrapure water (18.2 $M\Omega \cdot cm^{-1}$) from a Milli-Q system (Millipore, MA, USA) was used to prepare all solutions. Stock solutions of the elements K, P, Mg, Na, Ca, Cu and Zn (1.000 $mg \cdot L^{-1}$, Merck, Darmstadt, Germany) were used to prepare working standard solutions by dilution with 1% (v/v) nitric acid.

All laboratory glassware used was immersed in nitric acid solution (10% v/v) for 12 h for decontamination prior to use and then rinsed several times with deionised water.

For digestion, hydrogen peroxide (30% v/v, Merck, Darmstadt, Germany) and nitric acid (69–70% v/v,J.T. Baker) were used.

2.2. Instrumentation

Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) was performed using a Varian Vista PRO (Mulgrave, Australia) with axial viewing. A charge-coupled device detector was used for determination of elemental composition. A Sturman–Master chamber and a V-Groove nebuliser were also used. The operational parameters are given in Table 1.

2.3. Sample collection, storage and preparation

Samples of okra (*Abelmoschus esculentus* L.) were purchased from a supermarket in the northeast Brazilian state of Bahia. They were obtained from different cultivars: conventionally cultivated samples from the cities of Euclides da Cunha (EC) and Madre de Deus (MD) and organically cultivated from Conceição do Jacuípe (CJ), Morro do Chapeú (BS) and Salvador (SA). Three different batches were collected between December 2011 and January 2012. Each batch was analysed in triplicate: 15 raw samples and 15 cooked samples per batch. Therefore, a total of 90 samples were analysed for the three batches.

After collection, samples were stored separately in closed, refrigerated bags to prevent the proliferation of fungi and bacteria. Vegetables were washed in running water to remove sandy particles. They were then washed with 3% Extran solution (v/v) (Merck, Darmstadt, Germany), rinsed with deionised water and dried on paper towels, which were used for the removal of possible residues such as pesticides. Plastic knives were used for the cutting and homogenisation of okra samples to avoid metal contamination.

2.4. Sample digestion

Approximately 2.0 g of each sample was placed in two glass vials: the first for raw okra (fresh) and the second for cooked. For cooking, 8.0 mL of deionised water was added until the vegetable was covered. Samples were boiled for 15 min, and then, the cooking water was scorned and the vegetables were subjected to digestion.

For digestion (raw and cooked samples), 4 mL of concentrated nitric acid and 3.0 mL of hydrogen peroxide (30% v/v) were added to the glass vessels. The temperature of the heating block (Model TE-040/25, TECNAL, São Paulo, Brazil) was set to 140 °C, and the samples were digested for 4 h [21]. Later, the contents were quantitatively transferred to centrifuge tubes and topped up with ultrapure water to the 20 mL mark. All assays were conducted in triplicate.

2.5. Chemometric data processing

To identify the relationship between the samples studied and the mineral content, exploratory analysis using PCA and HCA were performed using *Stat Soft Statistic version 6*.

2.6. Validation of the analytical method used for quantification

The detection and quantification limits were determined as per IUPAC recommendations [22]. Precision was measured as the relative standard deviation (RSD in %). The accuracy of the method was confirmed by analysis of the standard reference material (SRM) tomato leaves, purchased from the National Institute of Standards and Technology (NIST 1573a, Gaithersburg, MD, USA). Digestion was performed using the same procedure as for the okra samples.

3. Results and discussion

3.1. Determination of elements in raw and cooked okra

The limits of detection and quantification, as well as the results obtained for the certified reference materials of tomato leaves NIST 1573a, are given in Table 2. The results were in agreement with the certified values, as can be seen in Table 2. The relative standard

Table 2
Results obtained for the certified reference materials of tomato leaves NIST 1573a, detection limit, quantification limit, relative standard deviation (RSD) (n = 3).

	Ca	K	Mg	P	Na	Cu	Zn
	(%)				mg kg^{-1}		
Certified value	5.05 ± 0.09	2.70 ± 0.05	1.20	0.216 ± 0.004	136 ± 4	4.7 ± 0.14	30.9 ± 0.7
Found value	4.99 ± 0.07	2.76 ± 0.07	1.08 + 0.02	0.217 ± 0.006	137 ± 2	4.8 ± 0.12	30.3 ± 0.8
Limit of detection (mg kg^{-1})	0.063	0.030	0.008	0.016	0.47	0.001	0.007
Limit of quantification (mg kg^{-1})	0.205	0.100	0.026	0.055	0.16	0.005	0.023
RSD (%) for (n = 3)	0.776	3.27	2.77	1.23	3.6	4.48	5.89

Table 3
Determination of the element concentration samples raw okra.

	Ca	Cu	K	Mg	Na	P	Zn
	mg/100						
1JC	419 ± 8	0.084 ± 0.010	307 ± 13	44.7 ± 1.8	16.2 ± 2.7	54.4 ± 1.4	0.185 ± 0.019
2JC	528 ± 23	0.111 ± 0.003	353 ± 10	52.6 ± 3.3	43.1 ± 4.1	59.2 ± 1.8	0.429 ± 0.032
3JC	313 ± 19	0.089 ± 0.011	290 ± 17	53.0 ± 9.0	8.77 ± 0.6	38.4 ± 4.3	0.241 ± 0.037
1SA	293 ± 28	0.089 ± 0.014	272 ± 10	35.5 ± 2.9	8.60 ± 0.6	53.3 ± 2.4	0.174 ± 0.005
2SA	344 ± 12	0.115 ± 0.007	278 ± 12	64.2 ± 1.6	8.07 ± 0.6	42.6 ± 3.9	0.398 ± 0.047
3SA	284 ± 11	0.107 ± 0.009	224 ± 15	37.1 ± 4.2	20.6 ± 2.0	49.2 ± 3.8	0.330 ± 0.023
1BS	373 ± 8	0.075 ± 0.003	255 ± 4	40.8 ± 0.7	16.2 ± 1.3	40.1 ± 1.5	0.197 ± 0.031
2BS	323 ± 19	0.070 ± 0.004	288 ± 13	38.3 ± 1.3	11.1 ± 1.1	41.7 ± 0.8	0.316 ± 0.031
3BS	308 ± 11	0.070 ± 0.003	268 ± 9	34.7 ± 2.1	12.4 ± 0.6	39.1 ± 3.3	0.120 ± 0.007
1EC	412 ± 13	0.097 ± 0.009	252 ± 12	39.8 ± 2.7	22.1 ± 2.8	46.3 ± 2.5	0.196 ± 0.028
2EC	403 ± 20	0.098 ± 0.005	271 ± 1	41.3 ± 2.3	34.3 ± 2.5	46.5 ± 1.2	0.238 ± 0.027
3EC	273 ± 17	0.214 ± 0.019	288 ± 13	41.9 ± 1.5	15.7 ± 0.6	44.6 ± 2.1	0.226 ± 0.023
1MD	414 ± 6	0.100 ± 0.011	211 ± 11	54.0 ± 2.9	16.8 ± 0.4	39.0 ± 0.8	0.162 ± 0.007
2MD	434 ± 14	0.074 ± 0.011	217 ± 12	52.1 ± 2.7	24.3 ± 2.0	33.9 ± 1.8	0.199 ± 0.013
3MD	374 ± 11	0.129 ± 0.010	238 ± 11	49.3 ± 1.5	18.9 ± 0.6	39.2 ± 2.1	0.168 ± 0.023

JC Conceição do Jacuípe, *SA* Salvador, *BS* Morro do Chapéu, *EC* Euclides da Cunha and *MD* Madre de Deus.
SD: standard deviation.
n: number of replicates (n = 3).

deviation, varied from 0.78 to 5.90%, and according to this data, the method showed good precision.

Concentrations of calcium, magnesium, sodium, phosphorus, potassium, copper and zinc were determined in raw and cooked okra. The concentrations of these macro- and micronutrients, expressed as mg of analyte per 100 g of sample, are shown in Tables 3 and 4.

3.2. Data evaluation employing principal component analysis

The mineral concentrations in okra samples were evaluated using two exploratory analysis techniques: PCA and HCA. A 90 × 7 data matrix was constructed, with the elements in columns and okra samples in rows. The data were auto-scaled because of the great variation in element concentrations. After pre-processing, the computer programme *Stat Soft Statistic version 6* was used to calculate scores and loadings.

The loadings of the original variables in the first three principal components, and the variance explained by each component, are given in Table 5. The first two principal components had substantial loadings for all seven variables. They accounted for 79.7% of total variance. The dominant variables for the first principal component (PC1) were K, P, Mg, Zn, Na and Cu, representing 65.1% of total variance. These six elements contributed most of the variability among the samples and were positively correlated with PC1. Examining loadings, K, P, Mg and Zn were the dominant variables, with smaller contributions from Na and Cu. The second principal component (PC2) accounted for 14.6% of total variance and included Ca as the dominant variable.

Fig. 1 shows a projection of the first two PCs. In PC1, two clusters of points can be observed. The cluster on the right is cooked samples with positive scores; the clusters on the left are raw samples with negative scores. Comparing loadings, in PC1 all elements had negative weights. As such, we can infer that the raw samples had the highest concentrations of all seven elements, indicating a loss of nutrients during cooking, possibly by leaching into the cooking water. This inference is corroborated by Table 6, which presents the averages and ranges of the concentration of each mineral in raw and cooked okra.

As shown in Table 5, PC1 had large negative loadings to K, P, Mg, Zn, Na, and Cu and a lower negative loading to Ca. Elements with high negative loadings showed the greatest declines in concentration after cooking, while elements with low negative loadings had the lowest declines. Average percentage loss after cooking was 63, 61, 60, 59, 47 and 42% for K, Na, Zn, Mg, Cu and P, respectively, and 11% for Ca. Thus, it was possible to differentiate the elements according to the variations observed after cooking. One possible explanation for the different reductions observed in these minerals after cooking is that different interaction occurs with the macromolecules in okra. Such macromolecules may be present in fibre, protein or crude fat, which represents 31.4%, 27% and 21.72% of okra's structure, respectively [6].

Table 4
Determination of the element concentration in samples cooked okra.

	Ca	Cu	K	Mg	Na	P	Zn
	mg/100 g						
C1JC	385 ± 17	0.057 ± 0.004	120 ± 2.0	22.2 ± 0.054	5.13 ± 0.5	30.7 ± 2.1	0.054 ± 0.002
C2JC	451 ± 12	0.063 ± 0.007	118 ± 2.0	20.0 ± 2.3	17.1 ± 1.5	32.7 ± 2.1	0.142 ± 0.011
C3JC	286 ± 4	0.036 ± 0.003	93.4 ± 3.3	24.8 ± 0.6	6.32 ± 0.8	33.8 ± 0.8	0.098 ± 0.015
C1SA	274 ± 14	0.072 ± 0.005	134 ± 10	21.6 ± 3.8	2.46 ± 0.4	32.3 ± 1.8	0.166 ± 0.007
C2SA	238 ± 17	0.026 ± 0.003	98.0 ± 6.8	12.7 ± 0.4	1.96 ± 0.4	23.4 ± 2.2	0.105 ± 0.011
C3SA	264 ± 11	0.065 ± 0.008	71.6 ± 5.5	10.2 ± 0.4	4.52 ± 0.4	24.1 ± 2.3	0.093 ± 0.011
C1BS	367 ± 9	0.053 ± 0.003	104 ± 6	22.5 ± 1.8	4.63 ± 0.7	28.2 ± 2.0	0.129 ± 0.012
C2BS	279 ± 15	0.021 ± 0.003	84.1 ± 9.2	8.69 ± 0.7	2.01 ± 0.1	20.0 ± 1.2	0.076 ± 0.007
C3BS	298 ± 5	0.030 ± 0.002	94.9 ± 2.9	11.2 ± 0.4	4.83 ± 0.6	23.2 ± 1.2	0.024 ± 0.002
C1EC	400 ± 14	0.064 ± 0.005	94.5 ± 6.9	22.0 ± 2.0	9.24 ± 0.7	24.9 ± 1.9	0.102 ± 0.006
C2EC	396 ± 5	0.051 ± 0.003	105 ± 9	17.4 ± 0.8	13.6 ± 0.8	27.7 ± 0.9	0.045 ± 0.004
C3EC	244 ± 9	0.102 ± 0.008	96.9 ± 9.4	17.4 ± 1.4	4.85 ± 0.4	23.5 ± 1.7	0.112 ± 0.004
C1MD	400 ± 15	0.069 ± 0.004	79.6 ± 6.9	31.1 ± 1.7	8.70 ± 0.7	20.3 ± 1.6	0.080 ± 0.007
C2MD	373 ± 12	0.046 ± 0.004	71.9 ± 5.8	20.2 ± 1.9	9.18 ± 0.8	18.9 ± 1.8	0.034 ± 0.004
C3MD	226 ± 6	0.031 ± 0.001	98.6 ± 3.7	13.0 ± 1.1	10.6 ± 0.8	23.6 ± 1.0	0.073 ± 0.006

JC Conceição do Jacuípe, *SA* Salvador, *BS* Morro do Chapéu, *EC* Euclides da Cunha and *MD* Madre de Deus.
SD: standard deviation.
n: number of replicates (n = 3).

Table 5
Loadings of the variables for the three first PCs.

	PC1	PC2	PC3
Ca	−0.4985	0.8017	0.2605
Cu	−0.7227	−0.3706	0.4964
K	−0.9214	−0.1906	−0.2317
Mg	−0.8996	−0.0406	0.0648
Na	−0.7648	0.4110	−0.2093
P	−0.9072	−0.1168	−0.2508
Zn	−0.8482	−0.1511	0.0638
Total variance (%)	65.1	14.6	6.9
Cumulative variance (%)	65.1	79.7	86.6

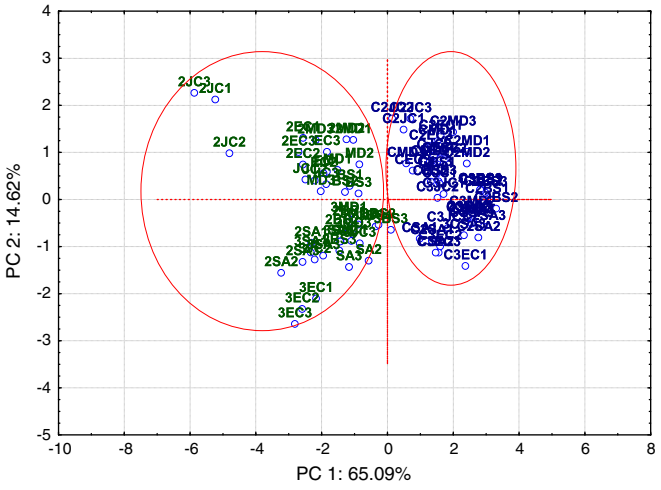

Fig. 1. Plot of the first principal component (PC1) versus the second principal component (PC2).

The raw samples 2JC1, 2JC2 and 2JC3 separated from the main cluster in PC1 scores (Fig. 1). The high concentrations of K, P, Na and Zn meant the loadings for these elements were highly negative for this PC. This was confirmed, as these elements are shown in average concentrations (2JC) in Table 3. The great reduction of potassium during cooking may be advantageous for people requiring restriction of dietary potassium. For example, patients with acute or chronic renal failure (ARF or CRF) undergoing hemodialysis require controlled potassium intake [23]. Cuppari et al. [24], in a study of food pretreatment and preparation techniques such as peeling, chopping, moisturising and boiling, showed that it is possible to reduce potassium levels up to 60% by discarding the cooking water. Similar results were found for okra in this work.

In PC2 (Fig. 1), two clusters were separated, neatly discriminating these samples from the rest. The raw samples 2JC3, 2JC1, 3EC1, 3EC2 and 3EC3 forming this group had large positive and negative scores for PC2, respectively. These samples 2JC3 and 2JC1 appeared to be richer in Ca, and samples 3EC1, 3EC2, and 3EC3 poorer in Ca, than the others. This was confirmed, as these samples had average Ca concentrations of 5.28 and 2.73 $mg \cdot g^{-1}$ for 2JC and 3EC, respectively, as shown in Table 3.

In Fig. 2, we plot the scores for the samples to compare the results for the two types of cultivation. No tendency towards separation of organic and conventional samples was observed in PC1 or PC2.

Fig. 3 shows the dendrogram for the HCA results. The results were separated into two groups, at linkage distances between 2000 and 5000, which confirmed the result obtained in PCA: two clusters of points can be observed, separating cooked and raw samples. This shows that the two groups in the two-dimensional projection (Fig. 1) are even more separate in real space once the dendrograms are based on real distances between samples; principal component analyses are only projections.

3.3. Determination of the mineral content of raw and cooked okra

The PCA and HCA results revealed a systematic difference in the mineral composition of raw and cooked okra collected in Bahia. The average concentrations of the elements were calculated for 90 samples. The average concentrations and ranges (per gramme of fresh weight) are shown in Table 6.

The concentrations of macro and micronutrients in raw okra demonstrate that it is a nutritious food. The levels of calcium, copper, magnesium, phosphorous, potassium, sodium and zinc are consistent with prior reports. We observed a clear loss of minerals after baking. These reductions indicate that the consumption of raw or sautéed food is the best option to maintain nutritional value.

4. Conclusions

The determination of minerals in okra by ICP OES gave a satisfactory quantification of Ca, Cu, K, Mg, Na, P and Zn. PCA and HCA demonstrated that K, P, Mg, Cu, Na and Zn contribute most of the variability between raw and cooked samples, with a minor contribution from Ca. The raw samples had the highest concentrations of seven elements, indicating a loss of nutrients during cooking; the nutrients possibly leached into the cooking water.

Table 6
Averages and ranges of concentrations of minerals in raw and cooked okra samples (milligrammes of analyte per 100 g of sample).

Elements	Okra samples	
	Raw mg/100 g	Cooked mg/100 g
Ca_{MD}	366	325
(min–max)	(273–528)	(226–451)
Cu_{MD}	0.102	0.052
(min–max)	(0.070–0.214)	(0.021–0.102)
K_{MD}	267	97.7
(min–max)	(211–353)	(71.6–134)
Mg_{MD}	45.3	18.3
(min–max)	(34.6–64.2)	(8.69–31.1)
Na_{MD}	18.3	7.00
(min–max)	(8.1–43.1)	(1.96–17.1)
P_{MD}	44.5	25.8
(min–max)	(59.1–33.9)	(18.9–33.8)
Zn_{MD}	0.233	0.094
(min–max)	(0.08–0.429)	(0.024–0.166)

(MD) — Average concentration.
(Min–max) — Ranges of concentration.

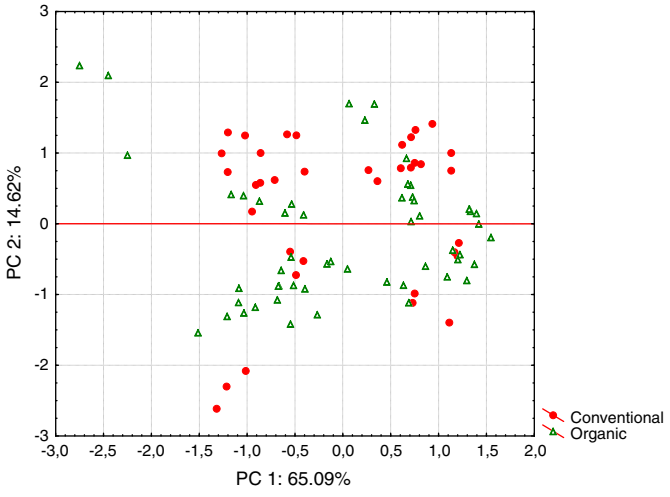

Fig. 2. Scores plot of PC1 × PC2 for conventional and organic samples.

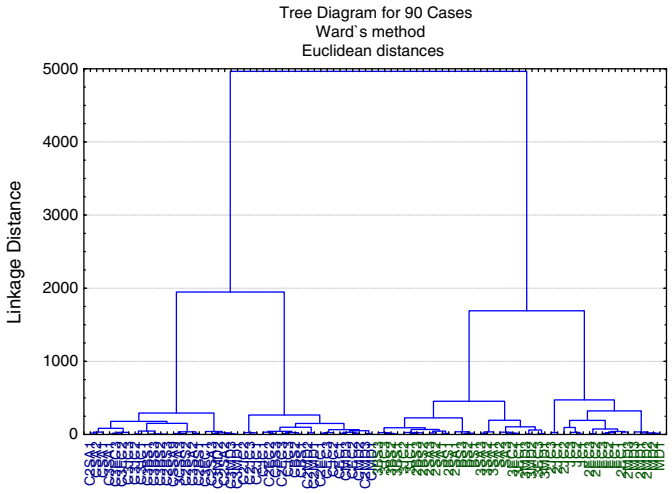

Fig. 3. Dendrogram for okra samples showing Ward's method with Euclidean distances.

In comparing organic and conventional samples, we did not observe a tendency towards separation in PC1 or PC2.

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