# Determination and Evaluation of the Mineral Composition of Obi (*Cola acuminate*)

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**Abstract** The obi (*Cola acuminate*) is a native fruit from Africa, which has been mainly used in the production of soft drinks and also in rituals of African religions. In this paper, the mineral composition of obi collected in seven different cities from Bahia State, Brazil was determined and 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 apple leaves, furnished by National Institute of Standard and Technology. The study involved 46 samples, being 18 of the red specie and 28 for the white specie. The results expressed as milligrams of element per 100 g<sup>-1</sup> of sample demonstrated that the concentration ranges varied of 21.28–548.77 for potassium, 15.73–129.85 for phosphorous, 27.95–286.92 for calcium, 7.67–134.45 for magnesium, 0.05–1.41 for manganese, 0.21–0.94 for iron, 0.11–0.39 for copper, 0.27–1.35 for zinc, and 0.025–0.517 for strontium. The principal component analysis and hierarchical cluster analysis evidenced that the mineral composition of the red specie is different of the white specie. The red obi has mineral content higher than white obi.

Keywords Elements · Mineral composition · Obi · ICP OES · PCA · HCA

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#### Introduction

The determination of the mineral composition of vegetables (fruits, leaves, and roots) that are used as food for humans is a worldwide concern. These studies 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. This way, several studies have been performed with this objective [1-5].

The obi or nut cola (*Cola acuminate*) is a fruit from equatorial forests of West Africa that has been mainly used in the production of several soft drinks [6] and also is consumed in rituals of African religions. For this reason, some papers have been performed in order to evidence the composition of this vegetable. Adeyeye et al. determined the content of amino acids found in this fruit [7]. Atawodi et al. determined the content of polyphenolic compounds present in several species of this vegetable that are used as stimulants in Nigeria [8]. Atolaiye et al. evaluated the potency of some antioxidants contained in these fruits [9]. Adeyeye and Ayejuyo performed the determination of the mineral composition of obi samples collected in Nigeria. The concentrations found (expressed as milligrams of element per kilogram of sample) were 0.60 for zinc, 0.15 for cobalt, 0.50 for manganese, 3.57 for calcium, 14.68 for magnesium, 202.50 for sodium, 87.00 for potassium, 2.50 for iron, 0.33 for copper, 2.66 for lead, and 53.00 for phosphorus [10].

Multivariate analysis techniques—principal component analysis (PCA) and hierarchical cluster analysis (HCA)—have been often employed for evaluation and characterization of analytical results [11–14]. PCA determines the diagonalization of the covariance or correlation matrix transforming the original chemical measurements into linear combinations of these measurements, which are the principal components (PCs) [15]. This corresponds to rotation of the coordinate space axes so that the explained variance of each PC is maximized. This technique allows for data reduction from higher to lower dimensional spaces to simplify their graphical representation. By contrast, HCA determines the similarities between samples by examining the interpoint distances representing all possible sample pairs in higher dimensional space. The sample similarities are represented on two-dimensional diagrams called dendrograms. These techniques complement each other and have been widely used in solving classification problems [16–21].

In Brazil, particularly in some states, obi is consumed raw in religious rituals. Then, this paper had as objective the determination of the mineral composition of this fruit. 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 manufacturer-recommended conditions for power (1.3 kW), plasma gas flow (15.0 L min<sup>-1</sup>), auxiliary gas flow (1.5 L min<sup>-1</sup>), and nebulizer gas flow (0.8 L min<sup>-1</sup>). The elements and analytical wavelengths measured were Ca II (317.933), P I (213.613), K (766.491), Mg II (285.213), Fe II (238.204), Mn II (259.372), Cu I (324.754), and Zn II (202.548).

## Chemicals and Reagents

All chemical reagents used in the experiment were of analytical grade. Ultra-pure water (18.2 M $\Omega$  cm) 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) with a concentration of 1,000 mg L<sup>-1</sup>, except K with concentration of 2,000 mg L<sup>-1</sup>, were used for prepared working standard solutions by diluting appropriate dilution of these solutions with 1% (*v*/*v*) nitric acid. Nitric acids and hydrogen peroxide (all Merck) were used for sample digestion.

## Sample Collection and Storage

The samples were collected in the following cities from Bahia, Brazil: Salvador, Santo Amaro, Jequié, Cachoeira, Barreiras, Vitoria da Conquista, and Cruz das Almas. A total of 46 were analyzed, being 18 of red and 28 white.

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. Samples were packed grated into small plastic pots with subsequent mechanical agitation (manual) using plastic pallets in order to homogenize the samples.

## Sample Digestion

About 1.0 g of sample was accurately weighed into glass vessels, and 4 mL of concentrated nitric acid was added. The hot plate temperature was adjusted to  $125^{\circ}$ C, the sample was digested for 30 min, and 3.0 mL of 30% ( $\nu/\nu$ ) hydrogen peroxide was added. Heating was maintained to allow evaporation and yielded a final volume of ~1 mL. Later, the digested samples were transferred to centrifuge tubes of 15 mL and then the volume completed to 12 mL with a 5% ( $\nu/\nu$ ) nitric acid solution.

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 furnished by National Institute of Standard and Technology (NIST; Gaithersburg, MD, USA), Apple Leaves NIST 1515. Digestion of this material was performed with the same decomposition procedure used for the obi samples. The results were in agreement with the certified values as Table 1.

## **Results and Discussion**

Determination of Chemical Elements in Obi Samples

The determination of the elements calcium, copper, iron, potassium, magnesium, manganese, phosphorus, strontium, and zinc were performed in the 46 samples. These results (expressed as milligrams of analyte per 100 g of sample and average of triplicates) are shown in Table 2.

Element	Certified value Ach	
Calcium (%)	$1.526 \pm 0.015$	1.514±0.018
Magnesium (%)	$0.271 {\pm} 0.008$	$0.267 {\pm} 0.009$
Phosphorus (%)	$0.159 {\pm} 0.011$	$0.166 {\pm} 0.004$
Copper ( $\mu g g^{-1}$ )	$5.64 {\pm} 0.24$	$5.3 \pm 1.4$
Zinc ( $\mu g g^{-1}$ )	$12.5 \pm 0.3$	$12.3 \pm 0.4$
Manganese ( $\mu g g^{-1}$ )	54±3	$51.0 {\pm} 0.8$
Iron ( $\mu g g^{-1}$ )	83±5	$85.0 {\pm} 4.7$

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

Data Evaluation Employing Principal Component Analysis

The results of the determination of the elements in the 46 samples analyzed were evaluated using PCA. For this, a data matrix was constructed using the elements as columns and the obi samples as rows (Table 2). This evaluation was performed on auto-scaled data due to the different orders of magnitude of the element concentrations. The loadings of original

Sample	Ca	Cu	Fe	К	Mg	Mn	Р	Sr	Zn
W-CDA1	85.07	0.18	0.22	54.34	14.54	0.09	21.78	0.255	0.60
W-CDA2	85.34	0.22	0.46	65.14	27.63	0.35	31.86	0.184	0.79
W-CDA3	66.69	0.19	0.37	75.75	29.90	0.25	35.51	0.204	0.52
W-VDC1	140.27	0.11	0.40	47.39	24.19	0.15	29.28	0.212	0.60
W-VDC2	63.52	0.28	0.53	72.04	42.28	0.12	28.14	0.072	0.58
W-VDC3	55.85	0.15	0.30	45.86	23.36	0.15	30.94	0.097	0.36
W-VDC4	78.28	0.27	0.43	67.70	37.95	0.21	34.86	0.125	0.46
W-BRR1	35.82	0.37	0.21	51.02	8.45	0.05	20.72	0.155	0.41
W-BRR2	42.43	0.26	0.23	56.31	11.33	0.07	15.73	0.025	0.51
W-BRR3	52.28	0.31	0.21	49.65	8.85	0.05	23.97	0.185	0.54
W-BRR4	65.58	0.32	0.22	45.62	7.67	0.05	19.99	0.150	0.56
W-SSA1	82.68	0.16	0.45	139.77	51.40	0.31	54.11	0.126	0.65
W-SSA2	128.85	0.15	0.56	105.89	39.93	0.64	46.31	0.261	0.50
W-SSA3	66.73	0.18	0.33	90.58	15.41	0.19	28.16	0.142	0.27
W-SSA4	72.38	0.20	0.43	91.93	20.95	0.24	33.42	0.195	0.30
W-SSA5	54.22	0.27	0.57	142.16	49.42	0.16	46.22	0.059	0.67
W-JEQ1	94.23	0.28	0.33	59.81	21.89	0.15	26.60	0.181	0.44
W-JEQ2	74.55	0.27	0.28	54.53	19.80	0.14	29.32	0.092	0.36
W-JEQ3	85.54	0.27	0.28	60.64	21.05	0.14	31.75	0.255	0.37
W-JEQ4	47.31	0.31	0.41	48.25	20.14	0.13	25.42	0.096	0.45
W-CAC1	70.80	0.30	0.58	68.64	31.22	0.43	31.27	0.486	0.52
W-CAC2	108.09	0.28	0.65	22.06	15.40	0.17	19.61	0.402	0.52
W-CAC3	114.31	0.24	0.55	47.64	27.42	0.35	27.91	0.298	0.51
W-CAC4	134.33	0.17	0.40	21.28	16.78	0.19	20.62	0.351	0.54
W-SAM1	35.80	0.26	0.69	69.61	20.17	0.12	33.68	0.067	0.38

**Table 2** Determination of the mineral composition of obi samples (milligrams of analyte per  $100 \text{ g}^{-1}$ )

Sample	Ca	Cu	Fe	К	Mg	Mn	Р	Sr	Zn
W-SAM2	60.09	0.23	0.44	98.57	33.13	0.25	41.04	0.090	0.69
W-SAM3	52.71	0.24	0.63	79.28	26.13	0.18	42.64	0.078	0.54
W-SAM4	69.65	0.22	0.43	71.63	17.06	0.16	29.67	0.173	0.43
R-SSA1	90.95	0.19	0.62	378.42	77.89	0.21	56.13	0.036	0.68
R-SSA2	171.54	0.11	0.66	408.42	96.20	0.47	83.46	0.248	0.94
R-SSA3	186.51	0.31	0.58	386.77	71.87	0.26	71.41	0.268	0.78
R-SSA4	144.88	0.27	0.69	408.63	90.55	0.66	71.69	0.082	0.93
R-SSA5	113.55	0.24	0.57	412.10	69.08	0.17	82.31	0.031	1.06
R-JEQ1	220.61	0.39	0.88	547.25	134.45	1.14	123.00	0.486	1.17
R-JEQ2	263.40	0.38	0.94	548.77	127.75	1.40	129.85	0.517	1.35
R-JEQ3	139.33	0.39	0.73	476.26	107.53	1.41	97.26	0.396	1.16
R-JEQ4	238.68	0.34	0.89	530.59	126.06	1.19	117.14	0.478	1.20
R-CAC1	27.55	0.19	0.51	421.54	58.12	0.21	54.45	0.206	0.77
R-CAC2	252.47	0.30	0.60	383.04	84.08	1.38	85.70	0.209	0.96
R-CAC3	97.70	0.31	0.59	422.75	85.77	0.29	76.23	0.168	0.98
R-CAC4	85.35	0.22	0.48	379.44	71.74	0.58	59.80	0.056	0.70
R-SAM1	64.04	0.26	0.62	484.23	87.98	0.52	74.23	0.040	0.91
R-SAM2	35.06	0.22	0.59	494.68	67.98	0.33	61.08	0.041	1.01
R-SAM3	53.57	0.24	0.55	482.95	81.05	0.47	64.23	0.022	0.90
R-SAM4	196.40	0.27	0.62	379.37	103.27	1.10	71.27	0.086	1.11
R-CAS1	286.92	0.18	0.65	391.66	100.45	0.19	76.56	0.291	0.94

Table 2 (continued)

SSA Salvador—Feira de São Joaquim, CAS Salvador—Casa Branca, SAM Santo Amaro, JEQ Jequié, CAC Cachoeira, BRR Barreiras, VDC Vitoria da Conquista, CDA Cruz das Almas, W white obi, R red obi

variables on the first three principal components and the variances explained by each component are given in Table 3.

The first three principal components were chosen for modeling the data because they describe almost 89% of the total variance. Phosphorus, magnesium, zinc, potassium,

Variable	CP1	CP2	CP3
Ca	-0.7829	0.2630	-0.3819
Cu	-0.4122	0.4514	0.7797
Fe	-0.8610	-0.0297	-0.0629
К	-0.8820	-0.3827	0.0876
Mg	-0.9589	-0.2308	-0.0124
Mn	-0.8715	0.1620	0.0418
Р	-0.9693	-0.1398	0.0091
Sr	-0.4749	0.7812	-0.2775
Zn	-0.9218	-0.1999	0.0574
Total variance (%)	66.62	12.99	9.42
Cumulative variance (%)	66.62	79.61	89.03

Table 3 Loadings of the variables for the first three principle components

manganese, iron, and calcium are the dominant variables for the first principal component (PC1) that represent 67% of the total variance. These seven elements contribute to the major variability presented in the samples and are positively correlated. The second principal component (PC2) accounts for 13% of the total variance and strontium is the dominant variable. The third principal component (PC3) presents 9% of the total variance, being copper the dominant variable. The score plot of the first two components is shown in Fig. 1.

In this, it can be seen a separation between the species of white and red obis. Samples with high concentrations for these seven elements have low scores on PC1, since the loadings are negative. Then, red obi samples R-JEQ2, R-JEQ1, and R-JEQ4 collected in Jequié City have the greatest concentrations for these elements. Obviously, samples with higher scores on PC1 have the lesser concentrations of these elements. This way, white obi samples present the lesser concentrations for these nine elements. Samples with high scores on PC2 are W-CAC1 and W-CAC2, which have high concentrations of strontium. The samples with lower scores on PC2 (R-SAM2 and R-SAM3) have lower concentrations, suggesting that red obi samples have lower concentrations of this element.

The results found for the 18 red obi samples showed in Table 2 were also separately evaluated using the PCA. This analysis demonstrated that all the nine elements influence in the variability of the samples, considering that the PC1 represents 71% of the total variance, and all the nine elements are dominant variables. The PC2 accounts for 11% of the total variance and calcium and potassium are the dominant variables, being negatively



Fig. 1 Plot of the first principal component versus the second principal component for the 46 obi samples

Variable	CP1	CP2	CP3
Ca	-0.6953	0.6870	0.0406
Cu	-0.7588	-0.2109	-0.5066
Fe	-0.9425	-0.0199	0.2179
К	-0.7102	-0.6374	0.2353
Mg	-0.9298	0.1077	0.1349
Mn	-0.8078	0.0518	-0.4572
Р	-0.9699	0.0046	0.0899
Sr	-0.8684	0.1636	0.1676
Zn	-0.8796	-0.1534	-0.0058
Total variance (%)	71.53	10.97	6.94
Cumulative variance (%)	71.53	82.50	89.44

Table 4 Loadings of the variables for the first three principle components for the red obi samples

correlated. The PC3 represents 7% of the total variance and copper is the dominant variable. The loadings of original variables on the first three principal components are given in Table 4. Figure 2 shows the score plot of the first two components.

In this, it can be observed that the red obi samples R-JEQ2, R-JEQ1, R-JEQ4, and R-JEQ3 collected in Jequié City, which have lower scores on PC1, have highest concentrations



Fig. 2 Plot of the first principal component versus the second principal component for the 18 red obi samples

for all the nine elements. On the other hand, the samples R-CAC1, R-CAC4, and R-SSA1 have lower concentrations for these elements. Samples R-CAS1 and R-CAC2 with high scores on PC2 have higher concentrations of calcium, and the samples R-SAM2, R-SAM3, and R-SAM1 that have lower scores on PC2 have higher concentrations of potassium and lower concentrations of calcium. It can be explained considering that these elements are negatively correlated.

The PCA was also employing for evaluation of the results achieved during the determination of the mineral contents in the 28 white obi samples. Figure 3 shows the score plot of the first two components, and Table 5 shows the loadings of original variables on the first three principal components.

This investigation demonstrated that the main elements that influence the variability for these samples are magnesium, phosphorus, manganese, potassium, and iron, being that the PC1 represents 41% of the total variance. Considering that all these elements have positive scores, in Fig. 3, it can be seen that the samples W-SSA1 and W-SSA2 have higher concentrations for these elements. The samples W-BRR1 and W-BRR4 have lower concentrations for these elements. The PC2 represents 24% of the total variance and the dominant variables are strontium and calcium, both with positive scores. Then, the samples W-CAC4 and W-CAC2, which have higher scores on PC2, have higher concentrations of these two elements. The sample W-SSA5 has lower score on the PC2, and it has the lowest concentration of strontium between the white obi samples.



Fig. 3 Plot of the first principal component versus the second principal component for the 26 white obi samples

Variable	CP1	CP2	CP3
Ca	0.3108	0.8505	-0.2212
Cu	-0.5011	-0.3411	0.7244
Fe	0.6383	0.1008	0.5316
К	0.7549	-0.5262	-0.1107
Mg	0.9012	-0.1953	0.0821
Mn	0.7690	0.3894	0.0975
Р	0.8962	-0.3141	-0.0810
Sr	0.0406	0.8627	0.3081
Zn	0.4199	0.0492	0.2075
Total variance (%)	41.22	24.02	11.44
Cumulative variance (%)	41.22	65.24	76.68

Table 5 Loadings of the variables for the first three principle components for the white obi samples

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. 4 shows the dendrogram that was obtained.



Fig. 4 Dendrogram for the 46 obi samples showing single linkages with Euclidean distances

Firstly, it can be seen the formation of two great groups, where the separation between all the red obi samples and all white obi samples can be observed. Other results found by PCA were also achieved using HCA. For instance, using PCA, it was observed that the samples R-JEQ2, R-JEQ1, and R-JEQ4 have the greatest concentrations for the elements phosphorus, magnesium, zinc, potassium, manganese, iron, and calcium. In Fig. 4, it can be seen that these samples also formed a small group at a distance of linking above of 35. Another similarity can be found in the samples W-CAC4 and W-CAC2, which have high concentrations of strontium and calcium. By HCA (Fig. 4), it can be seen a group formed by these two samples, and in Fig. 3 (PC1×PC2 for white obi samples), these samples are also separated forming a small group.

Determination of the Mineral Composition of Red and White Obi

Considering that PCA and also HCA clearly demonstrated that the mineral composition for red obi is different for white obi, the average content for the elements was calculated separately for each specie obi. The results for the average content and concentration range for the elements are shown in Table 6. It can be seen that the concentration of all elements determined are always higher for red obi.

## Conclusions

The method developed for the determination of minerals in obi by inductively coupled plasma optical emission spectrometry was satisfactory for the quantification of K, Mg, Ca, Mn, Fe, Cu, Zn, P, and Sr. The PCA and HCA chemometric techniques demonstrated that there is a significant difference in the mineral content between red obi and white obi. PCA showed also that the elements that contribute to the major variability between the obi varieties are phosphorous, magnesium, zinc, potassium, manganese, iron, and calcium.

Element	White obi		Red obi		
	Average	Concentration range	Average	Concentration range	
Calcium	75.84	35.80-140.27	148.25	27.95-286.92	
Copper	0.24	0.11-0.37	0.27	0.11-0.39	
Iron	0.41	0.21-0.69	0.65	0.48-0.94	
Potassium	67.97	21.28-142.16	440.94	378.42-548.77	
Magnesium	24.41	7.67-51.40	91.21	58.12-134.45	
Manganese	0.20	0.05-0.64	0.67	0.17-1.41	
Phosphorous	30.73	15.73-54.11	80.88	54.45-129.85	
Strontium	0.179	0.025-0.486	0.203	0.022-0.517	
Zinc	0.50	0.27-0.79	0.98	0.68-1.35	

 Table 6
 Concentrations of chemical elements in white and red obi samples (milligrams of element per 100 g of sample)

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