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Determination and evaluation of the mineral composition of breadfruit (*Artocarpus altilis*) using multivariate analysis technique

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ABSTRACT

Breadfruit is a fruit originated from South Pacific and afterward it was widespread in rest of Oceania. In Brazil it is known as "*fruta pão*" and has been consumed mainly by humble people. In home use, breadfruit has been consumed cooked, fried or roasted. Industrially, it has been employed to prepare breads and flours.
In the present paper, the mineral composition of breadfruit (*Artocarpus altilis*) was determined and evaluated. Raw breadfruit samples and cooked using domestic methods as boiling and microwave heating were digested in acid media and the elements potassium, calcium, phosphor, magnesium, iron, sodium and manganese were determined employing inductively coupled plasma optical emission spectrometry (ICP OES). Cadmium and lead were also determined using graphite furnace atomic absorption spectrometry (GFAAS), but the concentrations found were lower than the limits of quantification for these elements.
Eleven samples were acquired in several cities from Bahia state, Brazil. All these samples were analyzed in triplicate. The data obtained were evaluated employing the multivariate analysis techniques: Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Both techniques showed that the cooking methods using microwave heating and boiling cause loss of the nutrients. PCA also evidenced that the element concentrations in the samples cooked using microwave heating are higher than in the samples cooked by boiling using stove. The average concentrations of the determined elements (expressed as mg 100 g^{-1} of sample) in raw samples were as follows: 269.4 for potassium, 40.97 for phosphorus, 26.32 for calcium, 24.35 for magnesium, 1.41 for sodium, 0.1891 for iron and 0.0381 for manganese.

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1. Introduction

Breadfruit (*Artocarpus altilis*) in Brazil is known as "*fruta pão*", it is a fruit originating from South Pacific and is now found in Oceania. The breadfruit consumption can be cooked, fried or roasted. Technological processes include the preparation of cookies, breads and flours [1,2]. Additionally, breadfruit has been employed in several applications in medicines: Nwokocha et al. have investigated the use of breadfruit leaves in the preparation of an antihypertensive remedy [3]. Turi et al. recently discussed a number of scientific papers that evidenced the potential of breadfruit to prevent hunger in poor regions and mitigate diabetes [4]. Some authors have identified selected breadfruit cultivars for carotenoids [5,6]. Liu et al. concluded that the breadfruit has great potential to alleviate hunger and increase food security in tropical regions, considering the great productivity of the trees of this fruit. [7]. Ragone and Cavaletto determined the mineral composition of raw breadfruit samples grown in Hawaii, USA. The chemical elements quantified were: phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, copper, zinc and boron [8]. Oladunjoye et al. determined the nutrient composition, energy value and residual antinutritional factors in differently processed breadfruit grown in Nigeria. The concentrations of calcium, phosphor and iron were quantified [9]. Betiku and Taiwo proposed a bioethanol production process using breadfruit as carbon source [10].

In the absence of bioavailability tests, the determination of mineral composition of foods involving different cooking methods is one of the alternatives more recommended for obtaining of data more secure for dietitians involved in menu planning. In the specific case of the potassium, whose control is critical for patients with kidney problems [11,12], the tests using different methods of cooking are the most used. Burrowes and Ramer evaluated two cooking methods for reduction of potassium content in potato [11]. Asiimwe et al. concluded that the cooking method is efficient for it decreases the potassium concentration in bananas [13]. Additionally, several others works have demonstrated that the cooking method reduces the potassium content of plant foods, such as: okra [14], broccoli [15], and sweet chestnut [16].

Principal component analysis (PCA) is a multivariate analysis technique employed to transform the coordinates of the samples into another axis system more convenient for data analysis. This technique

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allows to identify patterns in a data series and expressing them in such a way that similarities and differences can be observed, reducing the dimensionality without losing too much information. Thus, this technique reduces the number of original variables to a few latent variables called principal components (PC). The first PC is chosen in the direction of greatest data variance. The second PC is defined to be orthogonal to the first one and represents the maximum variance not explained by the first component. The others PCs are obtained in the same way with lower order of variance [17].

The Hierarchical Cluster Analysis (HCA) is another analysis technique which is employed for characterize similarities among samples. This is established by examining of the interpoint distances of all possible sample pairs in high dimensional space. The sample similarities can be observed using diagrams that are named of dendrograms [18]. PCA and HCA are complementary techniques one another that are often utilized in solving classification problems [18]. These chemometric tools have been very employed for data evaluation from food chemistry [18–23]. The geographical discrimination of Italian honeys was performed by determination of fifteen chemical elements in 39 honey samples of different botanical. PCA allowed the discrimination between honeys from Sicilia and Calabria [19]. Cardeal et al. determined 16 polycyclic aromatic hydrocarbons (PAHs) in 29 artisanal cachaca samples collected in Minas Gerais, Brazil. PCA classified the cachaca samples according with their different contamination sources [20]. Marcelo et al. analyzed fifty four yerba mate samples originated from Argentina, Brazil, Paraguay and Uruguay, being that eleven macro elements were determined using inductively coupled plasma optical emission spectrometry and nineteen micro elements by inductively coupled plasma mass spectrometry. PCA was one of the techniques used for classification of the samples according to the origins [21]. Samec et al. evaluated the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using PCA [22]. Boyd et al. classified by geographical origin several white shrimp samples of three regions from Pacific. Twenty chemical elements were determined by ICP OES. The statistical analysis was performed using PCA and also the canonical discriminate analysis (CDA) [23].

In this work, raw breadfruit samples and also cooked in stove and microwave oven were digested in acid media and seven chemical elements were determined by ICP OES. Also cadmium and lead were quantified using GFAAS. The statistical analysis of the results was performed using PCA and HCA.

2. Experimental

2.1. Instrumentation

The determination of chemical elements: potassium, calcium, phosphor, magnesium, iron, sodium and manganese was performed employing an inductively coupled plasma optical emission spectrometer with axial viewing — ICP OES (Vista Pro, Varian, Mulgrave, Australia) equipped with solid state detector, cyclonic spray chamber and concentric nebulizer. The instrumental conditions were established as the manufacturer recommendations, as being: radiofrequency applied power (1.3 kW), plasma (15.0 L min^{-1}), auxiliary (1.5 L min^{-1}) and nebulizer (0.7 L min^{-1}) gas flow-rates. The emission lines chosen were: Ca II (317.933 nm), P I (213.613 nm), K II (766.491 nm), Na I (589.592 nm), Mg II (285.213 nm), Fe II (238.204 nm) and Mn II (259.372 nm).

Cadmium and lead were determined using a ZEEnit 600 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) equipped with a transversely heated pyrolytic graphite coated tubes with the PIN platform, Zeeman-effect background correction and an MPE-60 autosampler (Analytik Jena). The experimental conditions were established as previous works proposed for determination of cadmium [24] and lead [25].

For sample digestion using nitric acid and hydrogen peroxide a digester block (TE 040/25 TECNAL model) with temperature control was used. This digester system is equipped with 40 quartz tubes.

2.2. Reagents and standards

All reagents used in this work were of analytical grade (Merck, Darmstadt, Germany). All the solutions were prepared using water obtained from a Milli-Q system (Millipore, Bedford, USA). Reference analytical solutions for ICP OES analysis were prepared before use by serial dilution of stock reference solutions containing 1000 mg L^{-1} of each analyte in water.

2.3. Samples

The breadfruit samples were purchased in different cities from Bahia State, Brazil, including Salvador, Camaçari, Jequié, Feira de Santana, Candeias and Santo Amaro. In the laboratory, these samples were washed with Extran solution and subsequently with deionized water. After drying at room temperature, these were cut using plastic knives. For cooking, a portion of 1.0 g of sample was placed in a glass beaker with 25.0 mL deionized water, being that the cooking by boiling using stove was performed by 5 min and using microwave oven by a radiation time of 4 min. The cooked breadfruit masses were digested in acid media and the waste solutions were discarded.

The evaluation of method accuracy was performed using a certified reference material from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) of peach leaves (NIST 1547).

2.4. Sample preparation using digester block and cold finger [26]

For digestion, 1.0 g of breadfruit samples (raw or cooked) were placed into digester tubes, and 2 mL of concentrated nitric acid and 1 mL of 30% (*v*/*v*) hydrogen peroxide (Merck) were added. Additionally, the tubes were placed in the digester block. The “cold finger” condensers with water cooling were connected to the tubes. Afterward, the digester block was heated to 150 °C for 90 min. The breadfruit masses were completely digested and the solutions obtained were diluted to 12 mL with ultrapure water. All samples were digested in triplicate.

Table 1
Analytical parameters of the method employed for determination of the chemical elements.

Analyte	LOD (mg 100 g^{-1})	LOQ (mg 100 g^{-1})	Certified values[a]	Obtained value[a]
Potassium	0.018	0.059	2.43 ± 0.03 (%)	2.32 ± 0.09
Calcium	0.260	0.860	1.56 ± 0.02 (%)	1.51 ± 0.09
Phosphor	0.059	0.197	0.137 ± 0.007 (%)	0.135 ± 0.005
Magnesium	0.061	0.203	0.432 ± 0.008 (%)	0.425 ± 0.015
Iron	0.006	0.020	218 ± 14 (μg g^{-1})	201 ± 15
Sodium	0.080	0.260	24 ± 2 (μg g^{-1})	25.4 ± 1.4
Manganese	0.002	0.005	98 ± 3 (μg g^{-1})	96 ± 6

[a] Results of CRM of peach leaves NIST 1547 analyzed for method evaluation expressed as interval confidence at 95% level.

Table 2
Determination of chemical elements in raw and cooked breadfruit samples (mg analyte per 100 g^{-1}).

Element	Raw samples		Samples cooked in stove		Samples cooked in microwave oven	
	Average[a]	Concentration range	Average[a]	Concentration range	Average[a]	Concentration range
Potassium	269.4 ± 10.1	218.6–319.7	140.8 ± 9.1	111.4–197.1	182.2 ± 16.1	131.3–290.1
Calcium	26.32 ± 1.61	18.19–35.25	15.49 ± 1.77	10.00–28.18	19.78 ± 1.84	13.62–31.00
Phosphor	40.97 ± 3.59	27.85–64.10	21.82 ± 2.27	14.91–33.97	28.51 ± 2.94	15.99–40.92
Magnesium	24.35 ± 1.93	15.49–34.30	15.65 ± 1.99	7.23–28.84	19.74 ± 2.10	9.99–31.00
Iron	0.1891 ± 0.0114	0.0944–0.2191	0.0965 ± 0.0070	0.0426–0.1111	0.1273 ± 0.0083	0.0677–0.1575
Sodium	1.406 ± 0.269	0.1625–2.3952	0.7292 ± 0.1509	0.1011–1.6513	1.0217 ± 0.2174	0.1363–1.9547
Manganese	0.0381 ± 0.0077	0.0050–0.0710	0.0131 ± 0.0022	0.0050–0.0250	0.0262 ± 0.0050	0.0050–0.0523

[a] Expressed as confidence interval at 95% level.

3. Results and discussions

3.1. Analytical parameters of the method used for the determination of the elements

The analytes: potassium, calcium, phosphor, magnesium, iron, sodium and manganese were determined in breadfruit samples employing inductively coupled plasma optical emission spectrometry (ICP OES) after acid digestion. The limits of detection (LOD) and quantification (LOQ) for the seven analytes were calculated as being the analyte concentration that corresponded to three and ten times, respectively, the standard deviation of ten independent measurements of the blank, divided by the slopes of the calibration curves. Table 1 shows the limits of detection and quantification for each chemical element.

The accuracy of the ICP OES method was confirmed by analysis of the CRM of peach leaves, being that this material was decomposed utilizing the same digestion procedure used for the breadfruit samples. The results obtained can be seen also in Table 1.

3.2. Determination of the chemical elements in breadfruit samples

Firstly, three breadfruit samples were digested and the elements: calcium, magnesium, potassium, phosphor, sodium, iron, manganese, zinc, copper, cadmium, lead and barium were preliminarily determined using ICP OES. The results showed that the concentrations obtained for the elements: zinc, copper, lead, cadmium and barium are smaller than the limits of quantification of the analytical method employed.

Afterward, eleven samples were acquired and the elements quantified in the raw and cooked samples were: calcium, magnesium, potassium, phosphor, sodium, iron and manganese. All samples were digested and analyzed in triplicate.

The results obtained expressed as average and concentration range for the elements are shown in Table 2. In this can be seen that the chemical element of greater concentration is potassium and the element with lower content is manganese. Also, it was observed that the cooking processes induce the extraction of the elements into the aqueous phase. The release of the elements using cooking by boiling in stove is greater than using microwave oven. This phenomenon was observed for all elements. For magnesium, the release rate by the cooking process using stove was quite variable. This may be associated with the fruit ripeness.

The results as can be seen also in Table 2 which showed that this fruit (in raw form) has a high potassium content, which is critical for patients with kidney problems [11–12]. However, the cooking processes using stove and microwave oven allow a significant reduction of the potassium content in breadfruit, as have been observed in other plant foods [13–16].

Cadmium and lead were also determined in eight different breadfruit samples using GFAAS as detection technique. The raw samples in triplicate were decomposed in acid media using the same sample preparation procedure [26]. Cadmium was determined using aluminum as chemical modifier [24]. For determination of lead the chemical modification was established employing a reagent mixture containing tartaric acid–citric acid–sucrose [25]. However, the cadmium and lead concentrations found in all eight samples analyzed were always lower than the limits of quantification obtained for these elements, which were 45 and 60 ng g^{-1}, respectively, for sample masses of 1.0 g. Obviously, the samples cooked using stove and microwave oven were not analyzed for these elements.

3.3. Evaluation of the results employing multivariate analysis techniques

3.3.1. Evaluation using principal component analysis (PCA)

The analyses of the eleven samples (all in triplicate), in the raw forms and cooked using stove and microwave oven, involving the determination of the elements (potassium, calcium, manganese, magnesium, phosphor, iron and sodium) resulted in a (7 × 99) data matrix using the elements as columns and the 99 samples as rows. Firstly, the data were auto-scaled because the element concentrations are of different orders of magnitude. So, the data were evaluated employing the PCA technique. Seven dimensions are necessary for the total explanation of the data variability. Table 3 shows the loadings of the original variables on the first three principal components and the variances explained by each principal component. The dominant variables for the first principal component (PC1) are the potassium, phosphor and iron concentrations that represent 54.73% of the total variance.

These three elements contribute to the major variability shown in the breadfruit samples, and they are positively correlated. The second principal component (PC2) accounts for 15.34% of the total variance, having the sodium concentration as dominant variable. The third principal component PC3 describing 11.89% of the total variance was associated with variations in the calcium and magnesium concentrations. The score plot of the first two components is shown in Fig. 1.

Considering that the dominant variables (potassium, phosphor and iron concentrations) have negative loadings on the PC1, Fig. 1 shows clearly that the raw samples have higher concentrations of these elements, demonstrating that both cooking processes by microwave oven or stove cause loss of these nutrients. Fig. 1 evidences also that the element concentrations in the samples cooked using microwave oven are higher than the element concentrations in the samples cooked using stove.

PCA demonstrated that the element concentrations in a breadfruit sample depend of the cooking process. This way, the element contents

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

Element	PC1	PC2	PC3
Ca	−0.6189	0.4222	**0.6303**
Fe	**−0.8619**	0.2761	0.0428
K	**−0.9300**	−0.1953	0.0588
Na	−0.3693	**−0.8174**	0.1359
P	**−0.8701**	0.0080	−0.1925
Mg	−0.6867	0.2215	**−0.6072**
Mn	−0.6891	−0.2538	0.0764
Total variance (%)	54.73	15.34	11.89
Cumulative variance (%)	54.73	70.07	81.96

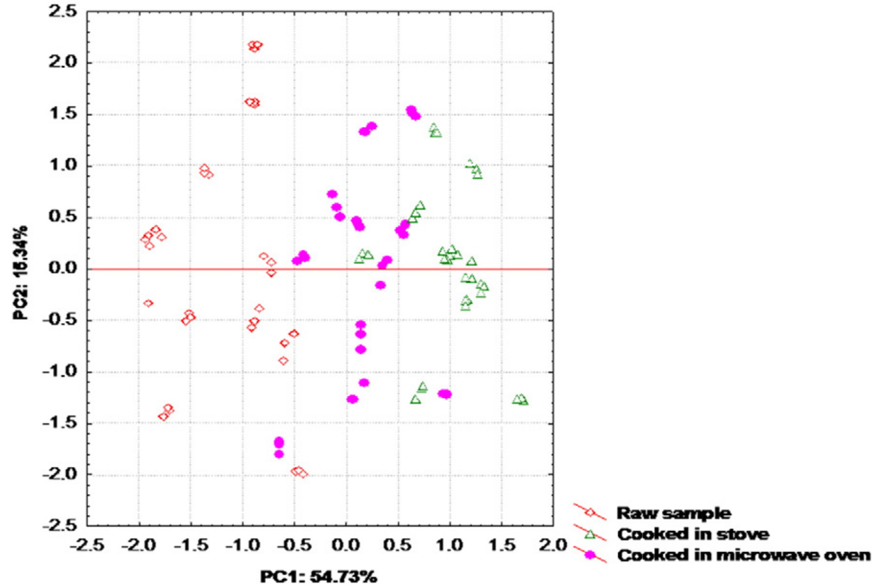

Fig. 1. Plot of the first principal component (PC1) versus the second principal component (PC2) for the breadfruit samples.

in the samples analyzed should be calculated separately for each cooking process as shown in Table 2.

3.3.2. Evaluation using hierarchical cluster analysis (HCA)

The results obtained during the determination of the seven elements in the 99 samples were also evaluated by HCA. These data were also auto-scaled using the single linkage method, and the Euclidean distances were used to calculate sample interpoint distances and similarities. The obtained dendrogram is shown in Fig. 2. In this, it can be seen that the raw samples are completely separated of the samples cooked in stove. These results are compatible with those found by PCA technique.

4. Conclusions

The digestion procedure of breadfruit samples using the reflux system with "cold finger" was very opportune considering the economy of reagents and consequently the decrease of the limits of detection and quantification of the analytical methods.

The PCA technique demonstrated that the elements of greater variability in the samples are potassium, phosphor, iron and sodium.

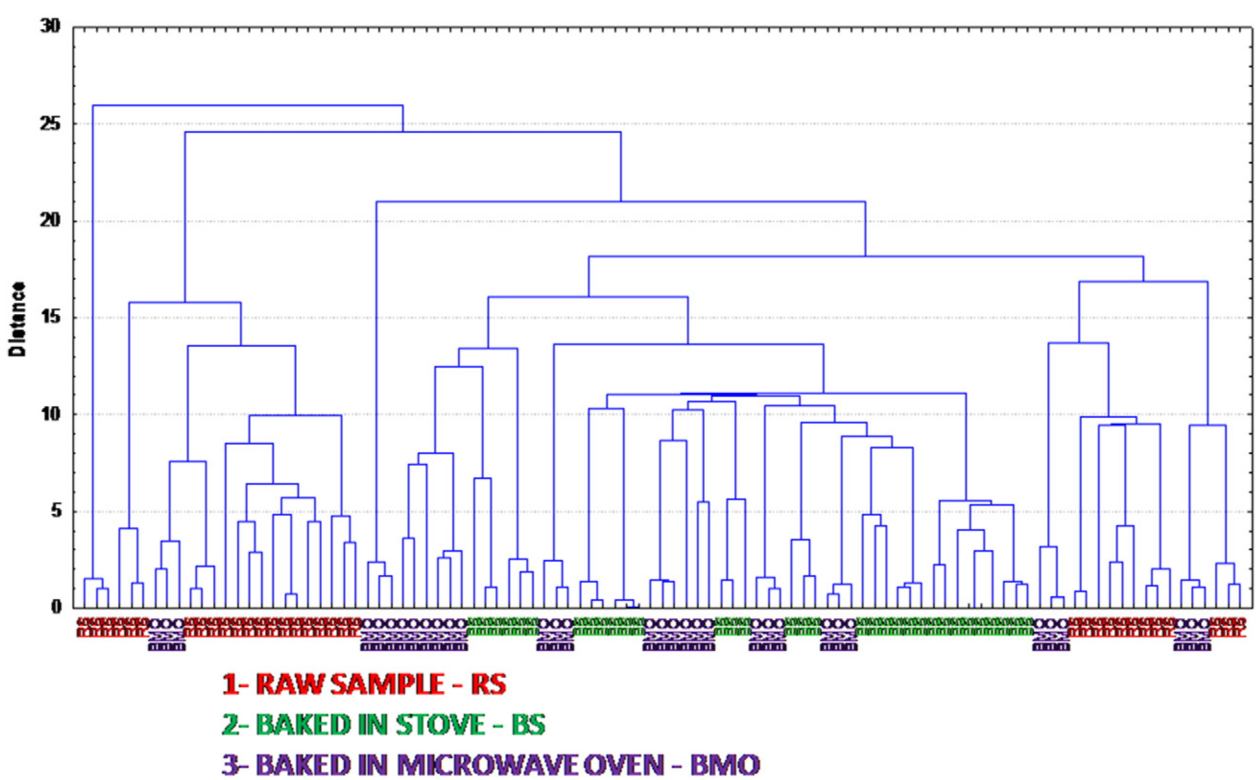

Fig. 2. Dendrogram for breadfruit samples showing single linkage with Euclidean distances.

Additionally, this technique also evidenced that the cooking processes using microwave oven or stove cause loss of nutrients. However, the loss of nutrients is higher when the cooking process is performed using stove.

This study showed that the breadfruit consumption by patients with kidney problems should be done after cooking using stove. This process reduces significantly the potassium concentration in this fruit.

The determination of the mineral composition of breadfruit consumed in Brazil was important considering the multiple scientific and technological applications that this fruit has had in recent years.

The high contents of phosphorus, calcium and magnesium in breadfruit suggest this as a good alternative for nutritional supplementation.

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