Screening of minerals, proximate composition and physico-chemical characteristics in the discrimination of Oiti (*Licania tomentosa* (Benth.) Fritsch.) using Kohonen self-organizing maps, PCA and HCA

**Emmanuelle Ferreira Requião Silva**  
Doutoranda em Química pela Universidade Federal da Bahia  
Instituição: Instituto de Química - Universidade Federal da Bahia  
Endereço: Rua Barão de Jeremoabo, 147, Ondina, CEP: 40.170-115, Salvador, Bahia, Brasil  
E-mail: emmanuellerequiiao@gmail.com

**Bruna Rosa da Silva Santos**  
Doutoranda em Química pela Universidade Federal da Bahia  
Instituição: Instituto de Química - Universidade Federal da Bahia  
Endereço: Rua Barão de Jeremoabo, 147, Ondina, CEP: 40.170-115, Salvador, Bahia, Brasil  
E-mail: Brunauquimica@hotmail.com

**Geovani Cardoso Brandão**  
Pós-doutorado em Química pela Universidade do Estado da Bahia  
Instituição: Departamento de Ciências Exatas e da Terra - Universidade do Estado da Bahia  
Endereço: Rua Silveira Martins, 2555, Cabula, CEP: 41.150-000, Salvador, Bahia, Brasil  
E-mail: gbrandao14@gmail.com

**Mariângela Vieira Lopes Silva**  
Doutora em Química pela Universidade Federal da Bahia  
Professora Titular em Nutrição e Farmácia pela Universidade do Estado da Bahia  
Instituição: Departamento de Ciências da Vida - Universidade do Estado da Bahia  
Endereço: Rua Silveira Martins, 2555, Cabula, CEP: 41.150-000, Salvador, Bahia, Brasil  
E-mail: mlopes@uneb.br

**Erik Galvão Paranhos da Silva**  
Doutor em Química pela Universidade Federal da Bahia  
Professor Titular em Química Analítica pela Universidade Estadual de Santa Cruz  
Instituição: Departamento de Ciências Exatas e Tecnológicas - Universidade Estadual de Santa Cruz  
Endereço: Campus Soane Nazaré de Andrade, Rodovia Jorge Amado, km 16, Salobrinho, CEP: 40.662-900, Ilhéus, Bahia, Brasil  
E-mail: egpsilva@uesc.br
ABSTRACT
Oiti (*Licania tomentosa* (Benth.) Fritsch.) is a native fruit of Brazil that is not vastly utilized in the human diet due to the scarcity of information about its nutritional characterization. In this paper, the proximate composition, physicochemical characterization and mineral content of oiti pulp were investigated. Multivariate analysis techniques (Kohonen Neural Network, PCA and HCA) were used to evaluate whether there were significant differences among the samples collected in the city of Salvador, Bahia, Brazil, regarding to nutritional composition. The samples were digested in microwave ovens using the HNO₃ and H₂O₂ oxidative mixture for multi-element determination of Ca, Cu, K, Mg, Mn, P and Zn by inductively coupled plasma optical emission spectrometry (ICP OES). Residual carbon content was determined to evaluate the efficiency of the acid digestion procedure. The method accuracy was confirmed by the analysis of the certified reference material of spinach leaves (1570a) and apple leaves (1515) from the National Institute of Standards and Technology (NIST). The results obtained for proximate composition and physical-chemical characterization analysis were in ranges of: 33.2 to 42.2% for moisture; 0.73 to 1.63% for ashes; 1.66 to 4.59% for total lipids; 1.89 to 3.07% for total proteins; 50.6 to 60.6% for carbohydrates; 0.04 to 0.06% for titratable acidity; 6.14 to 6.76 for pH; 0.88 to 5.69 mg 100g⁻¹ for vitamin C; 10 to 14% for soluble solids content (°Brix); 179 to 323 for ratio; 3.93 to 12.4% for reducing sugars; 0.89 to 4.52% for non-reducing sugars; 5.99 to 15.1% for total sugars and 248 to 279 Kcal in 100g for total calories value. The concentration values obtained for the elements in oiti samples in mg 100g⁻¹ were in ranges of: 121 to 259 for Ca; 10.0 to 12.6 for Cu; 394 to 775 for K; 32.9 to 62.7 for Mg; 10.5 to 12.3 for Mn; 48.6 to 70.4 for P and 0.335 to 1.66 for Zn. The results indicate that oiti fruits have a nutritional value and can be used as a potential alternative source of nutrients for human diet and for the development of new food products.
Keywords: Oiti fruit; proximate composition; physical-chemical characteristics; mineral composition; Kohonen self-organizing maps; PCA and HCA.

RESUMO
Oiti (*Licania tomentosa* (Benth.) Fritsch.) é uma das biodiversidades da flora brasileira produzindo fruto com aspecto adocicado empregado na culinária. No entanto, não é considerado como fonte alternativa de alimento devido à escassez de informações sobre a composição nutricional. Neste trabalho, foi investigada a composição centesimal, a caracterização físico-química e a composição mineral da polpa do oiti. Técnicas de análise multivariada (Rede Neural Kohonen, PCA e HCA) foram utilizadas para avaliar se houve diferenças significativas entre as amostras coletadas na cidade de Salvador, Bahia, Brasil, em relação à composição nutricional. As amostras foram digeridas em micro-ondas usando a mistura oxidativa de HNO₃ e H₂O₂ para determinação de elementos múltiplos de Ca, Cu, K, Mg, Mn, P e Zn por Espectrometria de Emissão Óptica por Plasma Acoplada Indutivamente (ICP OES). O teor de carbono residual foi determinado para avaliar à eficiência do processo de digestão ácida. A exatidão do método foi confirmada pela análise do material de referência certificado das folhas de espinafre (1570a) e maçã (1515) do Instituto Nacional de Padrões e Tecnologia (NIST). Os resultados obtidos para composição centesimal e análise de caracterização físico-química foram de: 33,2 a 42,2% para umidade; 0,73 a 1,63% para cinzas; 1,66 a 4,59% para lipídios totais; 1,89 a 3,07% para proteínas totais; 50,6 a 60,6% para carboidratos; 0,04 a 0,06% para acidez titulável; 6,14 a 6,76 para pH; 0,88 a 5,69 mg 100g⁻¹ para vitamina C; 10 a 14% para teor de sólidos solúveis (ºBrix); 179 a 323 para ratio; 73,8 a 87,3% para açúcares redutores e 0,89 a 4,52% para açúcares não redutores; 5,99 a 15,1% para açúcares totais e 248 a 279 Kcal em 100g para o valor total de calorias. Os valores de concentração obtidos para os elementos nas amostras de oiti em mg 100g⁻¹ estavam na faixa de: 121 a 259 para Ca; 10,0 a 12,6 para Cu; 394 a 775 para K; 32,9 a 62,7 para Mg; 10,5 a 12,3 para Mn; 48,6 a 70,4 para P e 0,335 a 1,66 para Zn. Os resultados indicam que os frutos de oiti têm um valor nutricional e podem ser utilizados como uma potencial fonte alternativa de nutrientes para a dieta humana e para o desenvolvimento de novos produtos alimentares.

Palavras-chave: Fruto Oiti; composição centesimal; características físico-químicas; composição mineral; mapas auto-organizados de Kohonen; PCA e HCA.

1 INTRODUCTION

Unconventional food plants – UFP (Plantas Alimentícias Não-Convencionais – PANCs, in Portuguese) are native or exotic plants that have edible parts such as stems, leaves, fruits, flowers, inflorescences, seeds and rhizomes, which can be consumed in natura or processed. However, they are not included in the daily human diet (Kinupp and Lorenzi, 2014; Duhan, Chauhan and Punia, 1992; Leal, Alves and Hanazaki, 2018). Several PANCs have already been used as human food in Brazilian cuisine, but the introducing European cultivars in Brazilian gastronomy caused the devaluation and disuse of these native species (Martinevski et al., 2013). Some studies show that PANCs have high nutritional value, containing high...
contents of minerals, vitamins, fibers and bioactive compounds, which are fundamental to the human health. Among these include: Arumbeva (*Opuntia monacantha*), Begônia (*Begonia cucullata*), Buva (*Conyza bonariensis*), Capuchinha (*Tropaeolum majus*), Mostarda (*Brassica juncea*), Taioba (*Xanthosoma sagittifolium*), among others PANCs (Kelen et al., 2015).

Another important PANC is the *Licania tomentosa* (Benth.) Fritsch., which is a native plant of the Brazilian flora that belongs to the Chrysobalanaceae family. This plant can only be found in the North and Northeast regions of Brazil and is popularly known as “oiti”, “oiti-mirim”, “oiti-da-praia”, “oiti-cagão”, “goiti”, among other terms (Andrade, Zoghbi and Maia, 1998). It is commonly used in the urban and ornamental arborization due to its adaptation to regions of high temperatures (Castilho and Kaplan, 2008). However, it has not been much explored as food in human diet and in agro-industrial activities, causing a high loss of the raw material due to scarcity of researches about its centesimal composition, physical-chemical parameters and mineral content. The pulp of the ripe oiti fruit has yellow color, strong smell and high amount of starch, which allows its use for several industrial applications in food industrial, such as juice, liquor, sweets, breads, biscuits, among others applications. In addition, the pulp can be dehydrated in order to use the fruit as an increment in several food products, which increases the market acceptability (Sousa et al., 2011).

Some phytochemical and pharmacological studies about *Licania tomentosa* (Benth.) show new potential applications of the fruit in pharmaceutical and food industries (Feitosa, Xavier and Randau, 2012). Fernandes et al. (2003) carried out the isolating pentacyclic triterpenes in leaves and fruits of *Licania tomentosa*. The fruit presented cytotoxic activity through two triterpenes (oleanolic and pomolic acids), which inhibited the growth and induced apoptosis of an erythroleukemia cell line (K562), evidencing the potential as antitumor agents for the treatment of leukemia. Castilho, Oliveira and Kaplan (2005) isolated the licanolide (a new lactone of triterpenes), betulinic and palmitoleic acids of the fresh fruits of *Licania tomentosa*. Castilho and Kaplan (2010) reported the identification and isolation of thirteen volatile chemical compounds of the classes of monoterpenes, aliphatic esters, alcohols, ketones and aldehydes which are essential constituents that are common in fruit aromas.

The characterization of physicochemical parameters, as well as the determination of the centesimal and mineral composition of vegetal foods, promotes actions and technological innovations for the elaboration of jellies, ice cream, bread, jam, biscuit, juice, syrup, capsules, among other products (Orsi et al., 2012; Llorent-Martínez et al., 2013). Studies about the chemical composition have demonstrated the nutritional potential of fruits for the elaboration...
and formulation of therapeutic diets. As an example, “atemoya”, for which researchers investigated the physico-chemical parameters, mineral content, phenolic, flavonoids and nutritional properties, concluding that the fruit has antioxidant potential and is a good dietary supplement (Santos et al., 2016). In another work, researchers compared the mineral composition (Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn) and centesimal composition (moisture, ash, protein, lipid and carbohydrate) of white pulp sweet potato roots samples in natura, regarding conventional and organic cultivation. The results demonstrated that the organic cultivation presented higher concentrations of minerals than conventional one (Santos et al., 2019).

Multivariate analysis techniques can be used to discriminate chemical composition data obtained from analysis of vegetable foods, which include artificial neural networks (Kohonen self-organizing maps), principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Santos et al., 2017; Lima et al., 2019). Santos et al. (2018) applied PCA and HCA to evaluate the mineral composition of ten rose petal varieties. The results of this assessment demonstrated that there are differences between samples collected in the summer and the winter. Additionally, the artificial neural nets using Kohonen self-organizing maps allowed the differentiation of the rose petal varieties, according to bioactive compounds.

In this way, the objective of this study was to determine the proximate composition (moisture, ashes, proteins, lipids and carbohydrates), physical-chemical characteristics (titratable acidity, pH, vitamin C, soluble solids content, ratio, reducing sugar, non-reducing sugar, total sugar and total caloric value) and mineral contents (Ca, Cu, K, Mg, Mn, P and Zn) in samples of oiti pulp collected in the city of Salvador, Bahia, Brazil. Multivariate analysis techniques (Kohonen self-organizing maps, PCA and HCA) were applied to separate and classify the samples, according to proximate composition, physical-chemical characteristics and mineral content, in order to investigate the nutritional properties of the fruit.

2 MATERIALS AND METHODS

2.1 MATERIALS, REAGENTS AND SOLUTIONS

To prepare all solutions, the ultrapure water with resistivity 18.2 MΩ cm obtained from the Milli-Q water purification system (Millipore, MA, USA) was used. All reagents and solvents used in all experiments were of high grade of purity obtained from Merck (Darmstadt, Germany) and the certified reference materials of apple leaves (CRM 1515) and spinach leaves.
(CRM 1570a) were acquired from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

The following reagents were used to determine the proximate composition and physical-chemical characteristics: ascorbic acid, ammonium sulfate, boric acid, bromocresol green, buffer solution (pH = 4.0, 7.0 and 9.0), calcium carbonate, copper sulfate, sodium and potassium tartrate, D-glucose monohydrate, hydrochloric acid, methyl orange, methyl red, methylene blue, petroleum ether, phenolphthalein, potassium biphthalate, potassium iodate, potassium iodide, potassium sulfate, sodium hydroxide, sulfuric acid and starch.

To determine the mineral composition in the samples and validation of the analytical method, nitric acid, hydrogen peroxide, CRMs of apple leaves (1515) and spinach leaves (1570a) were used. The standard solutions used to prepare the calibration curve were made by appropriate dilutions of the stock solutions of the elements Ca, Cu, K, Mg, Mn, P and Zn (1000 to 4000 mg L\(^{-1}\)). To determine the residual carbon content, a stock solution of 20000 mg L\(^{-1}\) of carbon was prepared with citric acid. The glassware was maintained in a 10% (v v\(^{-1}\)) citric acid solution for decontamination for 24 h.

2.2 INSTRUMENTATION

An analytical balance (model ALC/210.4, Sartorius, Goettingen, Germany) was used to weigh the samples and CRM. For the proximate composition, the following equipments were used: greenhouse (model Q317M, QUIMIS, São Paulo, Brazil) for the determination of moisture, muffle (model Q318S, QUIMIS, São Paulo, Brazil) for the quantification of the ash content, extraction battery of the sebelin type (model Q308-26B, QUIMIS, São Paulo, Brazil) for the determination of the lipid content, and nitrogen distiller (model MA-036, Marconi, São Paulo, Brazil) for the determination of the total protein. To determine the physical-chemical characteristics, an abbe refractometer was used (model Q767-2, QUIMIS, São Paulo, Brazil) to quantify the soluble solids, and a pH-meter Digimed DM20 (São Paulo, Brazil) was used for pH measurements of the samples of oiti pulp at 25 °C.

For the acid decomposition of oiti pulp samples, CRMs (apple leaves and spinach leaves) and blank, a microwave oven (model Milestone Start E, Sorisole, Italy) was used. Multi-element determination was performed by using an inductively coupled plasma optical emission spectrometer (OPTIMA 7300 DV, PerkinElmer, USA), with an axially and radial viewed, charge-coupled device detector, cyclonic chamber and a GemCone\textsuperscript{TM} nebulizer - Low Flow. The instrumental parameters of the equipment were: measured power of 1300 W; signal
integration time of 1 s; plasma gas flow of 15 L min\(^{-1}\); auxiliary gas flow of 1.5 L min\(^{-1}\); 0.70 L min\(^{-1}\) nebulization gas flow; and 0.70 mL min\(^{-1}\) sample pump flow. The atomic (I) and ionic (II) analytical wavelengths (nm) chosen were: C I (193.030), Ca II (422.673), Cu I (327.393), K I (766.490), Mg I (279.077), Mn II (259.372), P I (214.914) and Zn I (213.857).

2.3 COLLECTION AND PREPROCESSING OF THE SAMPLES

The oiti fruits were collected in eleven different locations in the city of Salvador, in the state of Bahia, Brazil. Samples were collected in the following neighborhoods: Dois de Julho (DJ), Bonfim (BON), Comércio (COM), Corredor da Vitória (CVI), Engelinho Velho de Brotas (EVB), Graça (GRA), Nazaré (NAZ), Ondina (OND), Pernambués (PER), Pituba (PIT) and Santo Inácio (STI), during the months of January and February in 2017. In the laboratory, the fruits selected were pre-treated by washing with running and ultrapure water, followed by the process of separating the pulp from the seed using a plastic grater. Subsequently, they were packed in plastic bags and stored in a freezer for two days and lyophilized for four days. The grinding was performed with a blender, and the products stored in plastic containers and kept in a desiccator until the moment of analysis.

2.4 PROXIMATE COMPOSITION AND PHYSICO-CHEMICAL CHARACTERISTICS IN OITI PULP

The proximate composition (moisture, ash, lipid, protein and carbohydrate) and physico-chemical characteristics (titratable acidity, pH, vitamin C, soluble solids content, ratio, reducing sugar, non-reducing sugar, total sugar and total caloric value) of oiti pulp were determined according to the analytical methodology described by the Adolfo Lutz Institute (IAL, 2008), and the caloric value was determined according to Atwater conversion factors. All determinations were performed in triplicate.

The moisture was determined by the direct drying method in greenhouse until constant weight at 105 °C. The ash content was quantified after calcination of the samples in a muffle heated at 550 °C. The lipid content in the dry sample was determined by intermittent hot extraction using petroleum ether as solvent in a Soxhlet apparatus. The protein quantification was performed by the Kjeldahl method using the 6.25 factor for the conversion of nitrogen and the recovery test using ammonium sulfate standard. The percentage of total carbohydrates was calculated by the difference between 100 and the sum of moisture, ash, lipid and protein.
The titratable acidity was determined by titration using 0.0998 mol L\(^{-1}\) NaOH standard solution, and 1.0% m v\(^{-1}\) phenolphthalein in ethanol. The pH of the samples was measured by direct reading in a digital potentiometer previously calibrated with buffer solutions of pH 4.0, 7.0 and 9.0. The determination of vitamin C was carried out by the method of iodometric titration with potassium iodate, and the validation of the analytical method was performed using ascorbic acid standard. The soluble solids content (ºBrix) was measured in the refractometer. The ratio was calculated by dividing the soluble solids content (ºBrix) by acidity. The reducing and non-reducing sugars were determined by the Eynon-Lane method based on copper oxidation titration. The total sugars were calculated by the sum of the reducing and non-reducing sugars. The total caloric value was calculated by summing the multiplication of the Atwater conversion factors by 4 kcal g\(^{-1}\) for the carbohydrate and protein content, and by 9 kcal g\(^{-1}\) for lipids.

2.5 MICROWAVE-ASSISTED DIGESTION AND CERTIFIED REFERENCE MATERIAL

Approximately 150 mg of each lyophilized oiti pulp sample were placed into the microwave oven digestion vessels. Then, 2.5 mL of concentrated nitric acid were added, left under pre-digestion for 1 hour at room temperature. Thereafter, 1.0 mL of 30% (v v\(^{-1}\)) hydrogen peroxide and 4.5 mL of ultrapure water were added. Subsequently, the samples were digested in the heating program with maximum temperature of 200 °C and power of 500 W for 30 minutes, and ventilation of 30 minutes. After cooling, the digests were transferred and diluted with ultrapure water to 12 mL in centrifuge tubes. The results were converted from a dry basis to a wet basis. The certified reference material of oiti was not found for purchase. The accuracy of the method was evaluated by analysis of the certified reference materials of apple leaves (CRM 1515) and spinach leaves (CRM 1570a) of the National Institute of Standards and Technology (NIST).

2.6 CHEMOMETRIC ANALYSIS

The results obtained by the determination of proximate composition and physico-chemical characteristics of the fruit were evaluated by Artificial Neural Networks with Neural Network MATLAB (R2013a – The MathWorks, Co., Natick, MA, USA) and Kohonen self-organizing maps using a somtoolbox available in http://www.cis.hut.fi/projects/somtoolbox/download/. The mineral composition data were
evaluated by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) through the Statistica software 10.0 (StatSoft Inc., USA).

3 RESULTS AND DISCUSSION

3.1 APPLICATION OF ARTIFICIAL NEURAL NETWORKS IN THE EVALUATION OF PROXIMATE COMPOSITION AND PHYSICAL-CHEMICAL CHARACTERISTICS

The experimental data were analyzed by Kohonen artificial neural networks (KNN) using a competitive algorithm, known as a self-organizing map (SOM) (Annas, Kanai and Koyama, 2007). The choice of KNN only for centesimal composition and physical-chemical characteristics as input, was due to the number of partial inputs forming less complex models in the network structures. The increase in the quantity of inputs favors a greater number of parameters that will be adjusted, incurring in the formation of high computational complexity models and the inclusion of poor inputs, causing the reduction of network performance (Haykin, 2009).

The dimension map [6x5] allows to visualize the correlations between the various input attributes coded by scores of the values relating colors to the values of each variable of the weight vector in Figure 1A. By analyzing the U-matrix Figure 1B, it is possible to observe the distance of structures of the input data with the formation of two distinct groups. The neural map of studied variables is shown in Figure 1C, evidencing the concentration range of mean values for the proximate composition and physical-chemical characteristics analyzed. From the results of the statistical quality measures for the separation of the clusters, a quantization error of 1.66 was observed. Low values are required since they indicate that the winning neuron is closer to the input vectors, which increases the generalization capacity of the network. The topographic error is calculated by checking which is the best and the second best fit neuron in all entries. The value found was 0.00, showing a good topology of the input data (Novaes et al. 2017).
The comparison between the Figures 1A with 1C can indicate the action of external factors on the oiti plants, such as water content, pH, salinity, microbiologic activities, among others factors, which promote the chemical reactions in the presence of specific enzymes providing metabolic pathways for the absorption of nutrients by the plants, which can influence in the discrimination of the oiti samples. Metabolic pathways aim the obtaining nutrients for the cell activity, promoting the producing primary metabolites that are responsible for the biosynthesis of essential macromolecules (carbohydrates, lipids, proteins, among others compounds) for the performing the vital functions of plants (Gobbo-Neto and Lopes, 2007; Pereira and Cardoso, 2012). This way, there was a formation of a group containing the neighborhoods of STI, OND, PIT, EVB and COM with the characteristics of total caloric value (Cv), carbohydrate (Car), vitamin C (Vic), total protein (Pro), reducing sugar (Rs), total sugar (Ts) and non-reducing sugar (Nrs), and low concentrations of soluble solids content (BRI), moisture (MOI) and pH. The second group made of NAZ and DJ presents low concentrations of reducing sugar (Rs) and total sugar (Ts) and relatively high values of titratable acidity (Aci). Using Kohonen artificial neural networks, it was possible to classify samples of oiti collected in different places in the Salvador City, which can be recommended as a nutritional supplement and processing new food products.

3.2 FIGURES OF MERIT FOR ANALYTICAL METHOD

The results obtained for the addition/recovery tests of total proteins and vitamin C were 111.74% and 100%, respectively, which are in accordance with the recovery range of 80-120% recommended by USEPA (1989). All figures of merit obtained for the analytical method are
Shown in Table 1. These parameters, such as limit of detection (LOD) and limit of quantification (LOQ), were obtained according to recommendations of the International Union of Pure and Applied Chemistry (IUPAC, 2002).

Table 1. Analytical parameters of the mineral determination method by ICP OES.

| Ion      | LOD (mg 100g\(^{-1}\)) | LOQ (mg 100g\(^{-1}\)) | Certified value              | Achieved value   |
|----------|--------------------------|--------------------------|------------------------------|------------------|
| Ca (II)  | 0.003                    | 0.010                    | 1.527 ± 0.041 (26\(^{a,b}\))| 1.620 ± 0.242    |
| Cu (II)  | 0.00008                  | 0.00026                  | 12.2 ± 0.6 (mg kg\(^{-1}\)) | 11.3 ± 0.3       |
| K (I)    | 0.003                    | 0.010                    | 2.903 ± 0.052 (26\(^{a}\))  | 2.460 ± 0.432    |
| Mg (II)  | 0.001                    | 0.004                    | 0.271 ± 0.008 (26\(^{b}\))  | 0.259 ± 0.007    |
| Mn (II)  | 0.000003                 | 0.000008                 | 75.9 ± 1.9 (mg kg\(^{-1}\)) | 72.2 ± 1.8       |
| P (I)    | 0.00009                  | 0.00029                  | 0.518 ± 0.011 (26\(^{b}\))  | 0.524 ± 0.012    |
| Zn (II)  | 0.000006                 | 0.000020                 | 82 ± 3 (mg kg\(^{-1}\))     | 81 ± 3           |

\(^{a}\) CRM Spinach Leaves (1570a).
\(^{b}\) CRM Apple Leaves (1515).

The concentration values achieved by analysis of CRM employing the proposed method were expressed at 95% confidence level (n = 3).

Limit of detection (LOD); Limit of quantification (LOQ).

The precision of the analytical method was calculated as relative standard deviation (RSD) for the seven elements, considering a sample mass of 100 mg (n = 10). The RSD values were as follows (%): Ca (6.4), Cu (1.8), K (1.2), Mg (7.9), Mn (5.1), P (7.8) and Zn (4.9). Once there are not CRMs available for this matrix kind, CRMs of vegetable matrix were analyzed to evaluate the method accuracy. The concentration values found by the analysis of the CRMs employing the proposed method were in agreement with the certified values, at 95% confidence level. The residual carbon contents (RCC) varied in range from 12 to 20% (m m\(^{-1}\)) in oiti pulp samples collected in different neighborhoods of Salvador, after the acid digestion procedure. These RCC values are similar to data reported in literature. This fact demonstrates that the acid digestion procedure was efficient for the decomposition of the samples, in this study (Araújo et al., 2002; Bressy et al., 2013). All these results indicate that the analytical method is suited for the analysis of the oiti pulp samples employing ICP OES to simultaneously determine the elements (Table 2).
Table 2. Determination of the mineral composition of oiti pulp by ICP OES.

| Sample | Ca    | Cu    | K     | Mg     | Mn     | P      | Zn     |
|--------|-------|-------|-------|--------|--------|--------|--------|
| DJ     | 121±8 | 10.0±0.5 | 617±8 | 47.0±2.4 | 10.5±0.5 | 59.8±2.9 | 0.583±0.032 |
| BON    | 142±8 | 11.2±0.1 | 596±35 | 32.9±1.5 | 10.5±0.1 | 69.7±3.3 | 0.541±0.023 |
| COM    | 130±6 | 12.1±0.3 | 744±14 | 32.9±3.4 | 11.2±0.2 | 67.9±2.6 | 1.66±0.05  |
| CVI    | 238±14| 12.0±0.5 | 775±79 | 60.7±3.6 | 11.7±0.5 | 65.6±4.2 | 0.770±0.083 |
| EVB    | 168±4 | 10.4±0.1 | 552±28 | 35.9±2.0 | 11.0±0.1 | 48.6±0.8 | 0.335±0.026 |
| GRA    | 248±6 | 12.0±0.2 | 555±15 | 62.7±5.1 | 11.6±0.2 | 62.2±4.3 | 0.719±0.032 |
| NAZ    | 158±10| 10.1±0.1 | 466±13 | 34.6±0.9 | 10.5±0.1 | 61.4±0.3 | 0.321±0.007 |
| OND    | 143±2 | 12.6±0.2 | 654±5  | 51.3±1.0 | 12.3±0.2 | 68.9±0.8 | 1.66±0.03  |
| PER    | 259±8 | 10.9±0.3 | 394±15 | 47.2±4.4 | 11.2±0.4 | 54.3±2.2 | 0.604±0.051 |
| PIT    | 165±13| 10.6±0.4 | 711±22 | 35.3±1.4 | 11.1±0.4 | 49.8±2.8 | 0.339±0.039 |
| STI    | 162±5 | 12.4±0.6 | 673±39 | 33.3±2.2 | 11.6±0.6 | 70.4±2.1 | 0.481±0.031 |
| Average| 176   | 11.3   | 612   | 43.1   | 11.2   | 61.7   | 0.728  |

Mean ± confidence interval, n = 3.

Location of sample collections in Salvador-Bahia: Dois de Julho (DJ), Bonfim (BON), Comércio (COM), Corredor da Vitória (CVI), Engenho Velho de Brotas (EVB), Graça (GRA), Nazaré (NAZ), Ondina (OND), Pernambués (PER), Pituba (PIT) and Santo Inácio (STI).

3.3 EVALUATION OF THE MINERALS COMPOSITION EMPLOYING MULTIVARIATE ANALYSIS TECHNIQUES

The composition mineral data were evaluated by principal components analysis (PCA) and hierarchical cluster analysis (HCA). The results obtained from autoscaling allowed to generate a 33x7 data matrix. The samples collected in locations of Dois de Julho (DJ), Ondina (OND), Comércio (COM), Santo Inácio (STI), Bonfim (BON), Graça (GRA), Corredor da Vitória (CVI), Engenho Velho de Brotas (EVB), Pituba (PIT), Nazaré (NAZ) and Pernambués (PER) were arranged in lines, whereas the analytes (Ca, Cu, K, Mg, Mn, P and Zn) were arranged in columns. The PC1, PC2 and PC3 were responsible for explaining 83.99% of data total variance, with auto-values higher than 0.6, and each PC contributed with 47.00% (3.3), 27.41% (1.9) and 9.58% (0.7), respectively. The linear equations for the three first principal components were: PC1 = -0.10Ca - 0.59K - 0.46Mg - 0.69P - 0.95Cu - 0.86Mn - 0.78Zn, in which all the elements presented negative loadings, mainly the analytes Cu, Mn, P and Zn, with high positive correlations; PC2 = 0.93Ca - 0.42K + 0.74Mg - 0.38P + 0.01Cu + 0.31Mn - 0.25Zn, with expressive positive correlations also for the analytes Ca and Mg; and PC3 =
0.04Ca - 0.62K - 0.07Mg + 0.48P + 0.10Cu - 0.14Mn + 0.12Zn, in which the most significant variable was the K.

From the comparison between the scores and loadings plots of PC1xPC2 (Figures 2A and 2B), it was observed that the oiti pulp samples formed two groups: DJ, BON, EVB, PIT and NAZ, which had high correlations for Ca and Mg; and other one group formed by CVI, COM, GRA, PER, STI and OND, with high correlations for Cu, K, Mn, P and Zn. According to the map from the Brazilian Agricultural Research Corporation (EMBRAPA), all eleven neighborhoods of Salvador City, where the samples of oiti fruit were collected, have the same soil type that is classified as red-yellow latosol. This way, the predominant mineral concentration found in fruit pulp for the two different groups formed can be probably attributed to the differences of soil composition due to presence and absence of concrete. The concrete contains cement, which has limestone as one of the main raw materials for its production. The limestone is composed of calcium carbonate and magnesium oxide that increase the soil pH. This process causes the decreasing the availability of some elements in soil, such as Cu, Mn e Zn, through the formation of hydroxides slightly soluble (inorganic complexes) (Camargo, Valadares and Dechen, 1982). Furthermore, the presence of concrete decreases the availability of water in soil, which has influence on the K and P absorption. This fact can change the ion/root interaction process and, consequently, the absorption and distribution processes of ions for the several parts of plant (Medeiros, Soares and Guimarães, 2005).

By analyzing the dendrogram obtained by Ward’s method and Euclidean distance (Figures 2C and 2D) with linkage of 20, two homogeneous groups were observed with low linkage distance, indicating great similarity among the samples that formed the group. A group was subdivided into two other groups: the first formed by the samples PER, CVI and GRA, being discriminated by the high Cu and Mn contents; and the second formed by the samples STI, COM and OND, being discriminated by the analytes K, P and Zn. This behavior observed by HCA is confirmed by sample projections in first two PC’s. Therefore, the multivariate analysis allowed the discrimination of oiti pulp samples collected in the city of Salvador, Bahia, Brazil.
3.4 COMPARING THE CONCENTRATIONS OF PROXIMATE COMPOSITION, PHYSICAL-CHEMICAL CHARACTERISTICS AND MINERAL CONTENTS OF THE OITI WITH OTHER FRUITS

According to the results obtained of mean concentration for proximate composition, physical-chemical characteristics and mineral contents in oiti pulp in this study, the moisture content of 37.4% and vitamin C of 3.56 mg 100g⁻¹ were found. In the case of the fruit, the moisture is relatively low (37.4%), which can be proven by its thick and dense pulp. When compared to the results from the Brazilian table of food composition (TACO) for ciriguela (Spondias purpurea L.), mango (Mangifera indica L.) and cajá-manga (Spondias dulcis Parkinson.), the oiti presented lower values (Table 4). The oiti total ashes value (1.22%), total
protein (2.37%), total lipids (3.03%), carbohydrates (56.0%) and caloric value (261 Kcal) were all higher than the three fruits previously cited. It can be observed that there is a significant presence of total protein and total lipids in the oiti pulp, which is an interesting result, since these nutrients can be found in low concentration in most the fruits. In addition, the ethereal extract showed intense yellow coloration, indicating the extraction of liposoluble pigments such as carotenoids.

According to the Table 3, when the mean concentration values of the physicochemical characteristics of the oiti pulp were evaluated in this study, the following values were found: 0.06% (titratable acidity), 6.45 (pH), 12.1 °Brix (soluble solids content), 234 (ratio), 8.18% (reducing sugar), 2.39% (non-reducing sugar) and 10.6% (total sugar). The Brazilian legislation (Brasil, 2009) does not establish the physical-chemical parameters for the oiti pulp in the General Technical Regulation for Fixing the Identity and Quality Standards for Fruit Pulp (original title in Portuguese). This way, the physical-chemical parameters of cajá (Spodias lutea L.) and mango (Mangifera indica L.) presented in this legislation of 0.9 and 0.30 g% (total acidity expressed as citric acid), 2.2 and 3.5 (pH) and 9 and 12 °Brix (soluble solids content), respectively, were compared with those results obtained for oiti pulp in present study. It is noted that the oiti pulp present lower values for total acidity and similar pH for the soluble solids. In addition, the oiti pulp ratio was higher when compared with the yellow mombin (Spondias mombin L.) pulp, which has a ratio of 10.2, according to Tiburski et al. (2011). Finally, the value of the reducing, non-reducing and total sugars for the oiti pulp is close to the one obtained for mango, which present contents of 3.32% of glucose, 11.5% of glucose, and 13.7% of the total sugar by the USDA. The found sugars values indicate that the oiti pulp has a great potential for its use in fermentation processes.
Table 3. Determination of the proximate composition and physical-chemical characteristics of oiti pulp.

| Location                  | DJ       | BON      | COM      | CVI      | EVB      | GRA      | NAZ      | OND      | PER      | PIT      | STI      | Average |
|---------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Mean ± confidence interval| 39.8±1.2 | 40.1±1.0 | 35.5±1.1 | 35.9±1.6 | 37.3±0.9 | 36.8±1.2 | 40.4±1.2 | 33.4±1.0 | 42.2±0.1 | 37.6±0.4 | 33.2±0.1 | 37.4±0.4 |

In relation to the minerals, when the ranges of average concentration of oiti pulp were evaluated, in this work, the following values were found, in mg 100g⁻¹: Ca (121 to 259), K (394 to 775), Mg (32.9 to 62.7), Cu (10.0 to 12.6), Mn (10.5 to 12.3), P (48.6 to 70.4) and Zn (0.335 to 1.66). Comparing these results with the ciriguela, mango and cajá-manga fruits, in Table 4, it is observed that all the results found for macro and micro elements were higher for oiti. Therefore, the results obtained indicate that oiti is a fruit that presents a significant nutritional value and can be exploited for consumption as well as for the development of new products through the pulp flour, or concentrated pulp of the fruit, and can be added as a potential nutritional supplement in the human diet.
Table 4 Comparison of mineral and proximate compositions of oiti pulp among some fruits.

| Analyte                  | Oiti* | Ciriguela, rawa | Mango, Tommy Atkins, rawa | Cajá-Manga, rawa |
|--------------------------|-------|-----------------|---------------------------|------------------|
| Proximate composition and physical-chemical characteristics |       |                 |                           |                  |
| Moisture (%)             | 37.4  | 78.7            | 85.8                      | 86.9             |
| Ash (%)                  | 1.22  | 0.7             | 0.3                       | 0.4              |
| Protein (%)              | 2.37  | 1.4             | 0.9                       | 1.3              |
| Lipid (%)                | 3.03  | 0.4             | 0.2                       | Tr               |
| Carbohydrate (%)         | 55.8  | 18.9            | 12.8                      | 11.4             |
| Vitamin C (mg 100g⁻¹)    | 3.56  | 27              | 7.9                       | 26.7             |
| Total caloric value (kcal in 100g) | 261   | 76              | 51                        | 46               |

| Mineral composition      |       |                 |                           |                  |
| Ca (mg 100g⁻¹)           | 176   | 27              | 8                         | 13               |
| Cu (mg 100g⁻¹)           | 11    | 0.12            | 0.06                      | 0.02             |
| K (mg 100g⁻¹)            | 612   | 248             | 138                       | 119              |
| Mg (mg 100g⁻¹)           | 43    | 18              | 7                         | 11               |
| Mn (mg 100g⁻¹)           | 11    | 0.06            | 0.34                      | 0.05             |
| P (mg 100g⁻¹)            | 62    | 49              | 14                        | 24               |
| Zn (mg 100g⁻¹)           | 0.7   | 0.5             | 0.1                       | 0.2              |

* This work
a (TACO, 2011)
Tr: Trace
The results for proximate composition and physical-chemical characteristics and mineral composition of oiti pulp were expressed as wet basis.

4 CONCLUSIONS

The centesimal composition, physical-chemical parameters and minerals in the oiti pulp, indicate its nutritional relevance and possibilities of use of this PANCs, previously considered inedible, in the Brazilian diet. The Kohonen self-organizing maps discriminated the samples according to the centesimal composition and the physical-chemical parameters. The PCA and HCA showed that differences in mineral composition occur due to the soil type and external factors. The result of this study aims to contribute to the valorization of this new alternative in the food menu, making the improvement of the nutritional status of the Brazilian population possible.

ACKNOWLEDGEMENTS

The authors are grateful to the Brazilian agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), by the grants and fellowships.
REFERENCES

Andrade, E.H.A.; Zoghbi, M.G.B.; Maia, J.G.S. Volatile constituents of the fruits of *Licania tomentosa* Benth., Acta Amaz. 28 (1998) 55-55.

Annas, S.; Kanai, T.; Koyama, S. Principal component analysis and self-organizing map for visualizing and classifying fire risks in forest regions, Agr. Inf. Res. 16 (2007) 44-51.

Araújo, G.C.L.; Gonzalez, M.H.; Ferreira, A.G.; Nogueira, A.R.A.; Nóbrega, J.A. Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials, Spectrochim. Acta B 57 (2002) 2121-2132.

BRASIL, Ministério da Agricultura, Pecuária e Abastecimento. Decreto no° 6.871, de 4 junho de 2009, referente as normas reguladoras dos padrões de identidade e qualidade de polpa de fruta. Diário Oficial da República Federativa do Brasil. Brasília, DF, 1 de setembro de 2016. Seção 1, p. 2-5. http://www.agricultura.gov.br/acesso-a-informacao/participacao-social/consultas-publicas/documentos/01_09-secao-1-portaria-58.pdf.

Bressy, F.C.; Brito, G.B.; Barbosa, I.S.; Teixeira, L.S.G.; Korn, M.G.A. Determination of trace element concentrations in tomato samples at different stages of maturation by ICP OES and ICP-MS following microwave-assisted digestion, Microchem. J. 109 (2013) 145-149.

Camargo, O.A.; Valadares, J.M.A.S.; Dechen, A.R. Efeitos do pH e da incubação na extração do manganês, zinco, cobre e ferro do solo, Rev. Bras. Cienc. Solo, 6 (1982) 83-88.

Castilho, R.O.; Kaplan, M.A.C. Chemical constituents of *Licania tomentosa* Benth. (Chrysobalanaceae), Quím. Nova 31 (2008) 66-69.

Castilho, R.O.; Kaplan, M.A.C. Volatile Components of Oiti Fruit (*Licania tomentosa* Benth.), Rec. Nat. Prod. 4 (2010) 238-241.

Castilho, R.O.; Oliveira, R.R.; Kaplan, M.A.C. Licanolide, a new triterpene lactone from *Licania tomentosa*, *Fitoterapia* 76 (2005) 562-566.
Duhan, A.; Chauhan, B.M.; Punia, D. Nutritional value of some non-conventional plant foods of India, Plant Foods Hum Nutr. 42 (1992) 193-200.

EMBRAPA, Brazilian Agricultural Research Corporation.
http://www.uep.cnps.embrapa.br/solos/index.php?link=ba. Accessed 4 June 2019.

Feitosa, E.A.; Xavier, H.S.; Randau, K.P. Chrysobalanaceae: traditional uses, phytochemistry and pharmacology, Braz. J. Pharm. 22 (2012) 1181-1186.

Fernandes, J.; Castilho, R.O.; Costa, M.R.; Wagner-Souza, K.; Kaplan, M.A.C.; Gattass, C.R. Pentacyclic triterpenes from Chrysobalanaceae species: cytotoxicity on multidrug resistant and sensitive leukemia cell lines, Cancer Lett. 190 (2003) 165-169.

Gobbo-Neto, L.; Lopes, N.P. Medicinal plants: factors of influence on the content of secondary metabolites, Quim. Nova 30 (2007) 374-381.

Haykin, S. Neural Networks and Learning Machines. 3rd ed, Pearson Education, New Jersey, 2009.

IAL, Adolfo Lutz Institute (2008). Physicochemical methods for food analysis, http://www.crq4.org.br/sms/files/file/analisedealimentosial_2008.pdf. Accessed 12 March 2020.

IUPAC, International Union of Pure and Applied Chemistry, Harmonized guidelines for single laboratory validation of methods of analysis, Pure Appl. Chem. 74 (2002) 835-855.

Kelen, M.E.B.; Nouhuys, I.S.V.; Kehl, L.C.; Brack, P.; Silva, D.B. Plantas alimentícias não convencionais (PANCs): hortaliças espontâneas e nativas, 1ª ed., UFRGS, Porto Alegre, 2015.

Kinupp, V.F.; Lorenzi, H. Plantas Alimentícias Não Convencionais (PANC) no Brasil: guia de identificação, aspectos nutricionais e receitas ilustradas. São Paulo: Instituto Plantarum de Estudos da Flora, 2014.
Leal, M.L.; Alves, R.P.; Hanazaki, N. Knowledge, use, and disuse of unconventional food plants, J. Ethnobiol. Ethnomed. 14 (2018) 1-9.

Lima, A.M.S.; Santos, L.O.; David, J.M.; Ferreira, S.L.C. Mineral content in mustard leaves according to the cooking method, Food Chem. 273 (2019) 172-177.

Llorent-Martínez, E.J.; Fernández-de Córdova, M.L.; Ortega-Barrales, P.; Ruiz-Medina, A. Characterization and comparison of the chemical composition of exotic superfoods, Microchem. J. 110 (2013) 444-451.

Martinevski, C.S.; Oliveira, V.R.; Rios, A.O.; Flores, S.H.; Venzke, J.G. Utilização de bertalha (Anredera cordifolia (TEM.) Steenis) e ora-pro-nobis (Pereskia aculeata Mill.) na elaboração de pães, Braz. J. Food and Nutrition 24 (2013) 1-6.

Medeiros, R.D.; Soares, A.A.; Guimarães, R.M. Soil compaction and water management. I: effects upon uptake of N, P, K, root and shoot dry matter of rice plants, Ciênc. Agrotec. 29 (2005) 940-947.

Novaes, C.G.; Romão, I.L.S.; Santos, B.G.; Ribeiro, J.P.; Bezerra, M.A.; Silva, E.G.P. Screening of Passiflora L. mineral content using principal component analysis and Kohonen self-organizing maps, Food Chem. 233 (2017) 507-513.

Orsi, D.C.; Carvalho, V.S.; Nishi, A.C.F.; Damiani, C.; Asquieri, E.R. Use of sugar apple, atemoya and soursop for technological development of jams - chemical and sensorial composition, Ciênc. agrotec. 36 (2012) 560-566.

Pereira, R.J.; Cardoso, M.G. Vegetable secondary metabolites and antioxidants benefits, J. Biotechnol. Biodivers. 3 (2012) 146-152.

Santos, A.M.P.; Lima, J.S.; Santos, I.F.; Silva, E.F.R.; Santana, F.A.; Araujo, D.G.G.R.; Santos, L.O. Mineral and centesimal composition evaluation of conventional and organic
cultivars sweet potato (*Ipomoea batatas* (L.) Lam) using chemometric tools, Food Chem. 273 (2019) 166-171.

Santos, W.N.L.; Sauthier, M.C.S.; Cavalcante, D.D.; Benevides, C.M.J.; Dias, F.S.; Santos, D.C.M.B. Mineral composition, nutritional properties, total phenolics and flavonoids compounds of the atemoya fruit (*Annona squamosa L. x Annona cherimola Mill.*) and evaluation using multivariate analysis techniques, An. Acad. Bras. Cienc. 88 (2016) 1243-1252.

Santos, W.N.L.; Sauthier, M.C.S.; Santos, A.M.P.; Santana, D.A.; Azevedo, R.S.A.; Caldas, J.C. Simultaneous determination of 13 phenolic bioactive compounds in guava (*Psidium guajava L.*) by HPLC-PAD with evaluation using PCA and Neural Network Analysis (NNA), Microchem. J. 133 (2017) 583-592.

Santos, A.M.P.; Silva, E.F.R.; Santos, W.N.L.; Silva, E.G.P.; Santos, L.O.; Santos, B.R.S.; Sauthier, M.C.S.; Santos, W.P.C. Evaluation of minerals, toxic elements and bioactive compounds in rose petals (*Rosa spp.*) using chemometric tools and artificial neural networks, Microchem. J. 138 (2018) 98-108.

Sousa, F.C.; Sousa, E.P.; Silva, L.M.M.; Martins, J.J.A.; Gomes, J.P.; Rocha, A.P.T. Mathematical modeling for description of the pulp drying kinetics of pulp of the Licania tomentosa, Rev. Educ. Agríc. Super. 26 (2011) 108-112.

TACO, Brazilian table of food composition (2011), http://www.nepa.unicamp.br/taco/contar/taco_4_edicao_ampliada_e_revisada.pdf?arquivo=taco_4_versao_ampliada_e_revisada.pdf, Accessed 6 April 2020.

Tiburski, J.H.; Rosenthal, A.; Deliza, R.; Godoy, R.L.O.; Pacheco, S. Nutritional properties of yellow mombin (*Spondias mombin L.*) pulp, Food Res. Int. 44 (2011) 2326-2331.

USDA, United States Department of Agriculture, Agricultural Research Service, USDA Food composition database, https://ndb.nal.usda.gov/ndb/nutrients/index. Accessed 4 June 2019.
USEPA, US Environmental Protection Agency (1989), Guidance for methods development and methods validation for the RCRA program SW-846 methods, https://www.epa.gov/sites/production/files/2015-10/documents/methdev.pdf. Accessed 03 June 2019.