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Research Article

Application of multivariate analysis techniques in the evaluation of metal content in industrialized spices sold in sachets

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Abstract

Industrialized powdered spices are widely used by the population in food preparation, although they are associated with several health problems. In this work, six metals (Zn, Fe, Ca, Mg, Na, and K) were determined in samples of dry industrialized spices that were sprayed and sold in sachets. Flame atomic absorption (FAAS) and emission (FAES) spectrometry were used to quantify the metals in the digested samples obtained after the acid decomposition of the samples in a digester block. The following concentration ranges were found for the analyzed metals (mg Kg^{-1}): Zn (<LQ-15.53), Fe (10.82-205.3), Ca (27.45-1842), Mg (114.8-1374), Na (63739-268188) e K (1560-235864). These values were evaluated using principal component analysis (PCA) and Kohonen self-organizing maps (KSOMs) techniques. The multivariate analysis allowed the recognition of grouping trends according to the spice brands, which suggests the possibility of a feedstock, from which the flavors are differentiated.

1. Introduction

The term "spices" is quite broad and refers to natural (herbs, seasonings, salts, etc.) or industrialized food additives that add and enhance specific flavors to foods. It is defined as products obtained from a mixture of spices and other ingredients, fermented or not, used to add flavor or aroma to foods and beverages [1, 2]. In addition to their organoleptic effects, natural spices have physiological benefits, they are rich in antioxidants, and exert beneficial digestive stimulation through enzymes responsible for digestion and/or secretion of bile, which plays an important role in facilitating digestion [3, 4]. On the other hand, industrialized spices are strongly discouraged by nutritionists due to their negative health impacts. They are widely consumed by the population because they offer practicality, easy access, present low price, and diversity of flavors in food preparation [5, 6]. The industrialized powdered spices sold in sachets are made from dehydrated herbs, salt, coloring agents, condiments, and artificial flavor

enhancers such as monosodium glutamate and disodium inosinate. Their use is heavily criticized due to the excessive amounts of preservatives, salt, and other additives used to preserve the product and increase consumer acceptance in terms of flavor. They can increase the risk of developing heart disease, kidney disease, diabetes, and even cancer [7, 8].

The quality of a food depends on several characteristics presented by it, highlighting the diversity and amount of nutrients provided and the absence of harmful contaminating substances [9, 10]. Although the consumption of herbs in nature, such as seasonings in food, brings many health benefits, it is necessary to assess whether nutrient metals and potentially toxic metals are present in safe concentrations for consumption by the population [11, 13]. This concern should also be extended to industrialized seasonings due to their high consumption, even knowing all the disadvantages brought by this type of product, as previously discussed.

Modern analytical techniques in food analysis are capable of generating large amounts of data about the samples of interest. However, extracting relevant information from this dataset may not be an easy task when it is done by relying only on human perception. Thus, multivariate analysis techniques for pattern recognition such as principal component analysis (PCA) [14-16] have been widely applied to reveal latent information in the data that cannot be perceived only by performing a visual inspection. In the food area, PCA has been used, for example, to differentiate gelatine sources based on the molecular weights of polypeptides [17], to evaluate the effect of electron beam radiation on ready-to-eat foods [18], to monitor real-time of the coffee roasting process using near-infrared spectroscopy in diffuse reflectance [19], among others.

Kohonen self-organizing maps (KSOMs) are algorithms based on artificial neural networks with the ability to organize complex data into clusters according to their similarities. They adopt a relatively simple and effective approach by reducing the dimension of the data worked, managing to maintain the real representation about to the investigated properties. This method only requests the input data and hyperparameter tuning, the latter being automatically defined by the computing application package, and this is ideal for its application in problems where the patterns are unknown or indeterminate [20]. KSOMs have been applied for, as examples: evaluation of data generated by the determination of metals in tea samples sold in sachets [21], exploration of macro and micronutrient content in samples of soft drinks of different flavors and manufacturers [22], identification of contamination by pathogens in fresh vegetable packages [23], characterization of pine honey from Greece and Turkey after determination of volatile and semi-volatile compounds by mass spectrometry system [24], evaluation of the special and temporal spread of COVID-19 in Brazil according to the number of cases in regions [25], states and cities, study of the use of bees as markers of space-time pollution by lead [26], evaluation of nutrient profiles in samples of cashew nuts [27], performing screening of mineral nutrient content in different parts and two types of passion fruit samples [28], among others.

In the present work, multivariate data analysis techniques (PCA and KSOMs) were used to explore data generated by analyzing the content of metals (Zn, Fe, Ca, Mg, Na, and K) by flame atomic absorption (and emission) spectrometry in samples of industrialized spices sold in sachets. These two multivariate tools were also compared in terms of efficiency and ability to recognize patterns of behave about to the analyzed samples.

2. Materials and methods

2.1 Instrumentation

The determinations of the metals in the digests of the industrialized spice samples were carried out using a Perkin Elmer flame atomic absorption spectrometer (Norwalk, CT, USA) with background correction performed by a deuterium lamp. Except for the elements Na and K that were determined in the emission mode, all the other elements absorption measurements were performed using hollow cathode lamps as radiation source. The wavelengths used for the determination of Ca (422.7 nm), Mg (285.2 nm), Fe (248.3 nm), Zn (213.9 nm), Na (589.0), and K (766.5 nm) were those recommended by the equipment manufacturer. The flame was composed of acetylene gas (2.0 L min^{-1}) and atmospheric air (13.5 L min^{-1})

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supplied by a compressor, the sample nebulization flow was 5.0 mL min^{-1} and the burner height was 13.5 L min^{-1}.

The masses of samples and reagents were determined using a Sartorius analytical balance (Model BL D105, Göttingen, Germany). A Tecnal digester block (Modelo TE 0851, Piracicaba, Brazil) supplied with glass tubes, was used to decompose samples of industrialized seasonings.

2.2 Reagents and solutions

Concentrated nitric acid (Exodus Científica, São Paulo, Brazil) and 30% hydrogen peroxide (Química Moderna, São Paulo, Brazil) used in the decomposition of the samples were of analytical grade. To avoid contamination, the glassware was subjected to a 5% nitric acid bath (vv^{-1}) for 24 hours, rinsed with ultrapure water, and dried in a dust-free environment. To obtain ultrapure water, an Elga Purelab Classic system (High Wycombe, UK) was used. Multielement standard solutions of Ca, Mg, Fe, Na, Zn and K were obtained by diluting stock solutions (Titrisol, Merck, Darmstadt, Germany) of each metal in the concentration range from 0.10 to 3.0 mg L^{-1}, making the calibration solutions. A blank solution was also obtained treating the reagents used in decomposition in the digester block in the same way as the sample (but in the absence of the same).

2.3 Sample acquisition and preparation

The samples of industrialized spices in sachets were purchased in supermarkets in the southwest region of Bahia, Brazil. Thirty-two samples belonging to the four most consumed brands were collected, constituting 8 samples per brand. For each brand, samples belonging to 2 batches were analyzed and the seasonings chosen were those recommended for the preparation of meat, chicken, poultry, vegetables, pasta, salad, beans, and rice.

Each sample was mixed so that there where homogenization, and then they were crushed with the aid of a pestle and mortar, later they were sieved so that the smallest granules were selected to increase the contact surface, providing more effective digestion. After this step, the samples were weighed on an analytical balance to determine the mass. Approximately 0.3 g of each sample was digested in a digester block, using 2 mL of concentrated HNO_3 and 1 mL of H_2O_2. The digestion process was carried out at 90ºC for approximately 90 minutes. After being cooled, the solutions were made up to 25 mL.

2.4 Data processing

The data obtained from the determination of metals in samples of industrialized spices were organized in the form of a matrix consisting of 96 lines (samples and their triplicates) and 6 columns (concentration of metals). This data table is presented the in Supplementary material. Before applying the PCA, the values that constituted the matrix were auto-scaled to avoid distortions caused by differences in magnitudes both between absolute values and data variances. The auto-scaling was performed by applying the following expression:

$$x_{ik} = \frac{x_{ik} - \bar{x}_k}{s_k} \qquad \text{with} \quad s_k = \sqrt{\frac{\sum_{i=1}^{n}(x_{ik} - \bar{x}_k)}{n-1}}$$

Where x_{ik} is the value to auto-scaled, x_k is the mean of the values in a given column, s_k is the standard deviation for the column values, and n is the number of objects. Autoscaling was performed using the Microsoft Excel® software. The Statistica® 7.0 program was used to perform the principal components analysis and to generate the score and loading graphs.

Matlab R2016a software (The MathWorks, Co., Natick, MA, USA) together with a toolbox available at www.cis.hut.fi/projects/somtoolbox was used for data analysis, generation of graphs, and related figures with Kohonen's self-organizing maps. The results of the analysis of the spice samples (concentration of metals in mg Kg^{-1}) were trained and processed by implementing the codes developed by the algorithms suggested on the website above.

3. Results and discussion

3.1 Analytical characteristics

The analytical characteristics of the method used to determine the metals were accessed to prove their suitability in the analysis of spice samples. Characteristics such as quantification limits, precisions (expressed as repeatability), sensitivities, and linearity for each metal studied are presented in Table 1. All values of these figures of merit accessed were satisfactory and adequate for the methodology used.

To assess the accuracy of the methodology addiction/ recovery tests and analysis of certified reference

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Table 1. Analytical characteristics of the method using FAAS and FAES in the determination of the metals studied in the spice samples.

Metal	LOQ (mg/Kg)*	% RSD**	Sensitivity (Abs/mg)	Linearity (R^2)
Zn	0,15	2,9	0,373	0,9981
Fe	0,17	1,8	0,0593	0,9993
Ca	9,6	1,3	0,0517	0,9982
Mg	0,66	3,3	0,968	0,9980
Na	0,87	3,5	9752	0,9911
K	2,5	3,1	8952	0,9972

*Expressed for a sample mass of 0.3 g; **Expressed in terms of repeatability for 0.5 mg L-1 solutions of the metal and N=10

material (Apple leaves, NIST 1515) were carried out. Table 2 presents the results of the analysis of this material. The paired t-test (95% confidence level) was applied to the two data sets (certified value and found value) to compare them and verify if they are statistically similar. The calculated t value (0.9168) was found to be located in the acceptance region of the Gaussian curve (critical t = 2.57), proving that the applied methodology has adequate accuracy. Addition/recovery tests were carried out the by addition of analytes standard solutions in the samples. Recoveries in the range of 92-101% were obtained showing the method's accuracy.

Table 2. Results (mean ± standard deviation) of the analysis of certified apple leaf reference material (N=3).

Metal	Certified value (mg Kg^{-1})	Found value (mg Kg^{-1})
Zn	12,45 ± 0,43	11,6 ± 0,6
Fe	82,7 ± 2,6	84,3 ± 0,4
Ca	15250 ± 100	14587 ± 150
Mg	2710 ± 120	3128 ± 181
Na	24,4 ± 2,1	25,6 ± 0,8
K	16080 ± 210	15287 ± 124

3.2 Multivariate analysis

3.2.1 Principal component analysis

Principal component analysis was applied to the data resulting from the analysis and scores (Fig. 1a) and loadings (Fig. 1b) plots were generated allowing the visualization of the behavior of objects (samples) and variables (metals concentrations) after the linear transformation of the data. The first two principal components (PCs) allow the explanation of 58% of the data variance. The score plot reveals that the analyzed samples tend to be grouped according to seasoning brands (identified by codes A, B, C, and D) instead of flavor. This behavior may indicate that manufacturers probably have a base product from which all flavors are developed and differentiated by adding small amounts of a mixture of flavorings and flavor enhancers.

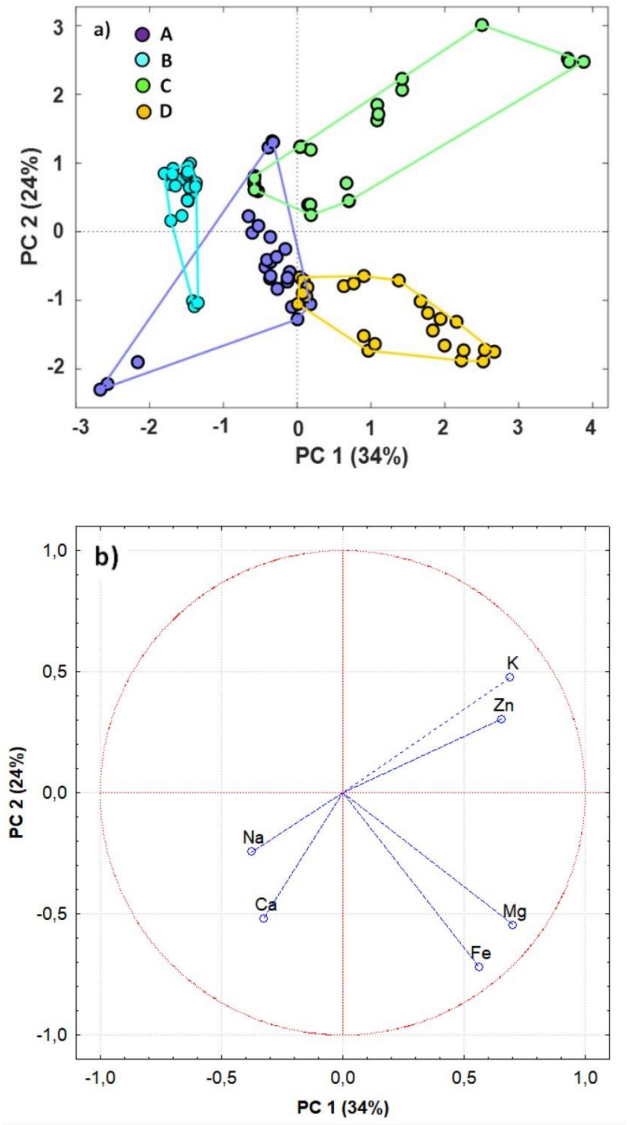

Figure 1. (a) Score plot for data obtained after analysis of samples of industrialized seasonings sold in sachets; (b) Loadings plot for data obtained after analysis of samples of industrialized seasonings sold in sachets.

The loadings plot reveals which variables were responsible for separating each group. The concentrations of Fe and Mg were the variables responsible for the formation of the group corresponding to brand D. The concentrations of K and Zn were responsible for the grouping of the samples of brand C. The group formed by the brand B

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and three samples of the brand B were grouped due to the concentrations of Ca and Na, in addition to the low concentration of Zn and K. Most of the samples of brand A are represented by points that are located near the center of the Cartesian axis, revealing that the concentrations of the variables studied contribute little to differentiate it from the others.

3.2.2 Kohonen self-organizing maps (SOMs)

SOM is an artificial neural model based on mammals' brain characteristic of having specific parts that are activated by specific stimuli, being interpreted as a map organized in areas, each area is activated by a specific input that does not activate others areas [24]. Kohonen's SOM is an artificial neuronal lattice, generally two dimensional, with artificial neurons organized in areas that are automatically trained to be activated by specific characteristics of input data. Each input data is applied to all neurons and the neuron with higher output is more representative with respect to that input characteristics. The lattice is self-organized in the sense that neurons activated by similar inputs are closer and compound a lattice area (or class). Besides that, other areas in the vicinity have low output for the same class of input, evidencing specialized areas.

The routine adopted by Gomes et al. [21] was applied to the data obtained by the analysis of the samples by FAAS in the implementation of the analysis by KSOM both in the training stage and the stage of formation of the sample clusters. Fig. 2b shows the contextual map (8 x 6 dimensions) obtained and its neural components. Except for two samples (A211 and A111), it is possible to evidence the formation of four groups related to the different brands of the samples, regardless of the flavors, as was also observed by the application of PCA. This map presents the group of neurons that defines the clustering of samples for various input data due to its ability to reduce dimensionality and eliminate irrelevant and/or redundant information. Unlike A111, which approaches a group to which it does not belong, sample A211 forms its own group with characteristics completely different from the others.

Fig. 2a presents the distance matrix (U matrix) that shows the formation of groups. In this graph, the proximity of the samples is based on the color scale, where blue indicates close proximity between the samples. In it, one can notice the formation of the four groups related to the brands of industrialized seasonings although the C group presents heterogeneity.

Fig. 2c shows the influence of each determined metal on the separation of groups according to the seasoning brand. Note that the group formed by samples of brand D tend to have the highest concentrations of Fe and Mg. The groups formed by the samples of brand C had the highest concentrations of K and the group formed by the majority of samples A and B had high concentrations of Na. Two samples (A211 and B141) formed a separate group due to their high concentrations of Ca and Na.

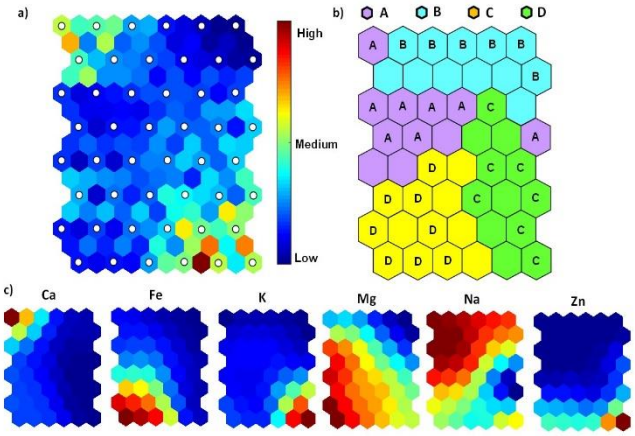

Figure 2. a) Distance matrix (U matrix) for the concentration data generated by the determination of the studied metals in samples of industrialized seasonings after treatment by KSOM; b) Components of the neural map showing the neurons responsible for the formation of the groups for the concentration data generated by the determination of the studied metals in the samples of industrialized spices after the treatment by KSOM; c) Component maps for the concentration data generated by the determination of the metals studied in samples of industrialized seasonings after KSOM treatment.

Although PCA and KSOM revealed very similar latent information in relation to the analyzed data set, the latter proved to be a more powerful processing tool, for quick visualization of the formed clusters and for identifying the variables that influenced the similarity between the samples. Despite these advantages, KSOMs are still little applied in data pattern recognition in Analytical Chemistry in relation to PCA, the latter being much better known and applied.

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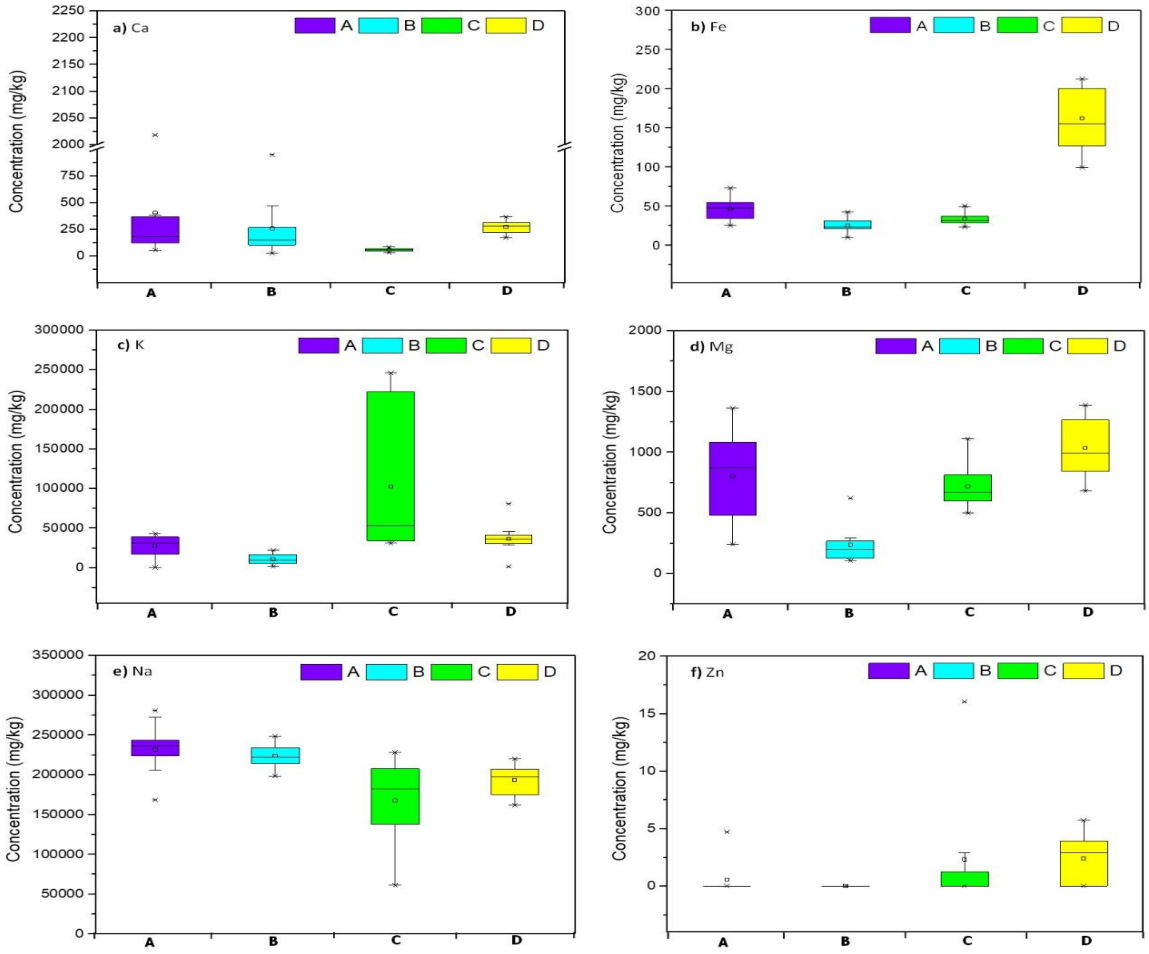

Figure 3. Mean, median and standard deviation of the concentrations for the elements determined in the spice samples according to the brands analyzed

3.3 Metal concentrations in spice samples

The concentration averages and dispersion parameters of the six metals determined in the samples of seasonings in sachets are presented in Fig. 3. The following general observations can be made: (1) the concentrations found for Zn were below the limit of quantification in the most samples mainly for brands A and B; (2) samples of brand D presented the highest concentrations of elements Zn, Mg and Fe and (3) the highest concentration ranges occur in relation to Na and K for brand C.

4. Conclusions

The determination of the six metals studied in the samples of industrialized dry spices in sachets and the use of multivariate analysis techniques allowed to investigate and relate their contents to the manufacturers who produced them. This behavior may indicate that each manufacturer uses a base material and differentiates the purpose of the seasoning by adding small amounts of the ingredients that are responsible for the flavor. Both principal component analysis and Kohonen's self-organizing maps allow reaching the same result, that is, the formation of four groups related to brands. However, KSOMs allow greater precision and security when grouping the samples, in addition to allowing a quick visualization of the formed groups and their relationship with the variables responsible for the formation of the clusters.

Authors' contributions

Performed the experiments, wrote and revised the manuscript, IS.G. and UM.F.M.C.; Responsible for the statistical treatment, writing and review of the manuscript, V.C.C.C, F.A.C.A. and T.P.C.; Conceived the research project, guided the students in carrying it out and coordinated the writing of the article, M.A.B.

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and C.G.N.

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Availability of data and materials

All data will be made available on request according to the journal policy

Conflicts of interest

The authors have no conflicts of interest or competing interests to declare that are relevant to the content of this article.

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