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Geographical characterization of Spanish PDO paprika by multivariate analysis of multielemental content

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ABSTRACT

A multielemental analytical method has been proposed to determine the contents of Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr and Zn in paprika samples from the two Protected Designations of Origin recognized in Spain, such as Murcia and La Vera (Extremadura). The samples are mineralized by acid wet digestion using a mixture of perchloric and nitric acids and analyzed by means of inductively coupled plasma atomic emission spectroscopy. The method performance has been checked studying the absence of matrix effect, trueness, precision, linearity, limit of detection and limit of quantification. The proposed method has been applied to analyze samples of sweet, hot and hot/sweet paprika from the considered production areas. Differences between paprika samples from Murcia and Extremadura were found and pattern recognition methods, such as linear discriminant analysis, linear support vector machines, soft independent modeling of class analogy and multilayer perceptrons artificial neural networks, has been used to obtain classification models. Sweet and hot/sweet paprika types were differentiated by means of linear models and hot paprika was differentiated by using artificial neural networks. A model based on artificial neural networks is proposed to differentiate the geographical origin of paprika, with independence of the type, leading to an overall classification performance of 99%.

1. Introduction

Paprika is a red powder made from grinding the dried pepper pods of some varieties of *Capsicum annuum* L. [1]. This natural food product is commonly used as spice and natural colorant in cookery and to provide redness to meat products and commercial sauces [2]. There are different types of paprika according to its pungency, named sweet, sweet/hot and hot. This variety of tastes makes this product very common in different traditional cuisines. The adequate consumption of pepper fruits is also related to potential health effects due to the presence of antioxidant compounds [3]. Although paprika is original from America, it is also produced in Europe, particularly in Hungary, Turkey and Spain. In Spain, paprika is called “pimentón” and it is cultivated and produced in two main areas namely Murcia and La Vera [4]. Paprika from both regions is recognized under Protected Designation of Origin (PDO) by the European Commission of Agriculture and Rural Development [5], being these products of great importance for the local economies. The production region of the paprika protected by La Vera PDO is located in the centre-west of Spain. This PDO includes some towns of the province of Cáceres in the region of Extremadura. La Vera PDO uses peppers belonging to the variety Bola and Ocales from the species *C. annuum* L. and *C. longum* L., respectively [6]. The other pepper growing region which is currently protected under the Murcia PDO comprises the homonymous region, located to the south east of Spain. In this case, the peppers used belong to the variety Bola from *C. annuum* L. [7].

Nowadays, consumers relate quality of foodstuff to certain characteristics influenced by the production areas, the harvesting practices and the raw materials. For this reason, it is of great importance the development of suitable methodologies allowing the characterization of different products. Within this context, several studies have been focused on the compositional profiling of paprika samples. Mateo et al. [4] identified volatile compounds influencing the overall flavor of paprika from La Vera, whilst volatile composition of paprika from Murcia has been studied by Guadayol et al. [8]. Kocsis et al. [9] researched on the volatile component composition of Hungarian red paprika as an important parameter of quality and identity. Due to the use of red paprika as a source of pigments to enhance or change food color, the study of constituents related to this characteristics, such as carotenoids, is valuable. In fact, color is used as a parameter to monitoring the quality changes during the elaboration or storage process. Techniques based on the spectrophotometric measurement of color

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and HPLC separation with UV–vis detection have been developed aiming this purpose [10,11]. On the other hand, antioxidant activity attributed to paprika powder is mainly related to the presence of polyphenols and several authors consider these compounds as useful variables to characterize paprika [3,11]. Metals have been also used to characterize paprika. The content of Cu, Fe, Mn, K and Na, determined by absorption and emission flame atomic spectrometry, has been used to characterize paprika samples cultivated in Venezuela [12]. Arc atomic emission spectrometry has been also used to determine B, P, Fe, Mg, Si, Mn, Al, Ca and Cu with the aim to differentiate parts of the paprika plant [13]. Multielemental profile, determined by inductively coupled plasma-mass spectrometry (ICP-MS) has been used to establish the authenticity of Hungarian paprika Szegedi Füszerpaprika (PDO) by means of pattern recognition techniques [14]. Trace elements are important parameters for establishing the PDO of foods by using appropriate chemometric data. In this realm, ICP-MS and inductively coupled plasma optical emission spectrometry (ICP-OES) are powerful analytical tools due to their low detection limits and feasibility to perform multicomponent determinations in a relatively short time. Gonzalvez et al. [15] and Jakubowski et al. [16] have reviewed the usefulness of these techniques to authenticate the origin of food matrices, including vegetable-type products.

Spanish paprika from La Vera and Murcia PDO has not been yet characterized according to their elemental composition. The metal content in paprika could be influenced by different factors such as the level of these elements in soil, fertilizing practices and the processing conditions and some differences are expected between these two PDOs. The presence of some major elements such as Ca, K, Mg, P, Al, Fe and Na in vegetable-type products is highly related to those factors. These elements are valuable chemical descriptors to perform the geographic differentiation of tea, coffee and other food products [15–18]. Consequently, it could be expected that these elements would be useful for paprika geographical differentiation. Other minor constituents, such as B, Cu, Mn, Ni, Pb, Sr or Zn, are also influenced by geographical factors and could be relevant to obtain classification models [17,18].

The main objectives of this work are the development and validation of a multielemental analytical method to determine the mineral content of paprika samples and the use of that chemical information to obtain adequate classification models to authenticate PDO Spanish paprika samples. Accordingly, an ICP-OES method has been proposed and the contents of Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr and Zn have been determined in sweet, sweet/hot and hot paprika from the two Spanish PDOs. In order to differentiate the geographical origin of the considered paprika samples, pattern recognition techniques such as principal component analysis (PCA), linear discriminant analysis (LDA), support vector machines (SVM), soft independent modeling of class analogy (SIMCA) and multilayer perceptron artificial neural networks (MLP-ANN) have been applied.

2. Materials and methods

2.1. Chemicals and reagents

Nitric (65%), sulfuric (96%), perchloric (60%) acids and hydrogen peroxide (30%) were used in the mineralization of samples. All of them were of analytical grade and obtained from Merck (Darmstadt, Germany). Standard solutions of 1000 mg l^{-1} (Merck) were used to prepare working solutions. Ultrapure water (Milli-Q, Millipore, Bedford, MA) was used throughout.

2.2. Samples

Samples of paprika belonging to two different origins: La Vera (Extremadura) ($n=72$) and Murcia ($n=72$) were obtained from local stores. Within these classes there are three different types of paprika: sweet, hot/sweet and hot, being 24 the number of samples of each pair of type-origin.

2.3. Apparatus and methods

ICP-OES analysis was performed using an ULTIMA 2 atomic emission spectrometer (Horiba Jobin Yvon, Kyoto, Japan). The ICP operating conditions are shown in the electronic Supplementary material (Table S1).

Three mixtures of acids were tested to carry out wet ashing mineralization of paprika samples: M1 (2 ml of H_2SO_4 and 15 ml of HNO_3), M2 (5 ml of H_2O_2 and 20 ml of HNO_3) and M3 (2 ml of $HClO_4$ and 20 ml of HNO_3). The acids were gradually added to 1 g of paprika (weighted with precision of 0.1 mg) placed in a beaker and the mixture was heated till complete mineralization. All obtained solutions were cooled to room temperature, filtered (0.45 μm) and transferred to 50 ml volumetric flasks. Adequate blanks were prepared for each method.

2.4. Chemometrics

A data matrix consisting of 14 columns (the determined elements) and 144 rows (the paprika samples) was created for the chemometrics calculations. Two-way nested analysis of variance (ANOVA) was used to compare recoveries of the mineralization methods. Non-parametric comparison test such as Kruskal–Wallis and Mann–Whitney tests were applied in order to draw attention to significant difference in elemental content between the paprika types and origins, respectively. PCA was used to visualize data trends and to get a first evaluation of the discriminant power of the variables. LDA, SVM, SIMCA and MLP-ANN were applied to obtain classification models. The STATISTICA 8.0 software package (StatSoft, 2007) was used for the statistical analysis.

3. Results and discussion

3.1. Methods comparison

Three mineralization methods were proposed with the aim of determining metals in paprika samples by means of ICP-OES. In order to compare among the selected methods of mineralization, and taking into account that there are no CRMs available for the trueness study, recovery assays with spiked samples was carried out [19]. A control sample was prepared by mixing paprika samples from each considered origin and type. This control sample was used to prepare the spiked samples at the expected concentrations for each element [20]. The control sample was mineralized according to the method i, fortified in j levels and analyzed in k replicates. The corresponding elemental recovery of each ijk value, R_{ijk}, was obtaided. A two-way nested ANOVA was performed for each element. The fortification level was nested in the oxidant mixture used for digestion. Variance due to the factor oxidant mixture was compared with pure error variance and Tukey-HSD *post-hoc* analysis [21] was used to detect the methods responsible of bias. The obtained results are shown in Table 1. There are significant differences for many of the determined elements for at least a pair of methods. The three methods are equivalent in the case of B, Ca, K, Mn and Ni. There are significant differences between all the possible comparisons for Sr. For the remaining elements one of the methods differs for the other two, except for Na and P, which only shows differences in the recoveries obtained by M1 and M2. In order to select the best mineralization method, the uncertainty associated to each method, $u(\overline{R}_i)$, was evaluated and their recoveries statistically compared to 100. The uncertainty