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Analytical Methods

Characterisation of tea leaves according to their total mineral content by means of probabilistic neural networks

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ABSTRACT

The concentrations of aluminium, barium, calcium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, sodium, strontium, sulphur and zinc in white, green, black, Oolong and Pu-erh teas have been determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Samples were microwave-digested and the performance characteristics of the method were verified by analysing a certified reference material. The measured elemental concentrations in tea leaves were used to differentiate the five tea varieties. Non-parametric analysis was applied to highlight significant differences between types, and pattern recognition methods were used to characterise samples. For this aim, linear discriminant analysis (LDA) and probabilistic neural networks (PNN) were used to construct classification models with an overall classification performance of 81% and 97%, respectively.

1. Introduction

Second only to water, tea is the most consumed beverage throughout the world. Tea comes from the *Camellia sinensis* plant, which makes it highly dependent on the conditions of cultivation and preparation, and these vary between both country of origin and for the type of tea. Since its apocryphal invention in Chinese times – arguably as early as 2700 BC – its potential health effects have been recognized, being recommended for alleviation of minor maladies such as headaches and pains. Many studies have been carried out to pinpoint any potential ramifications of tea on human health such as its anti-oxidative power (Jian, Ping, Lee, & Binns, 2004; Jie et al., 2006; Setiawan et al., 2001; Wu, Yu, Tseng, Hankin, & Pike, 2003), the presence of compounds having a marked anti-Alzheimer's effect (Okello, Savelev, & Perry, 2004), its effect in reducing blood pressure (Yokogoshi et al., 1995) and in preventing obesity (Kim et al., 2009). Due to the accumulation of various minerals in the plant, tea also represents an important form of ingesting bio-essential elements; therefore frequent consumption may contribute significantly to the recommended daily intake (Fernández, Pablos, Martín, & González, 2002).

There are five principal varieties of tea with different qualities due to morphologic and chemical diversities (Chen, Zhao, Fang, & Wang, 2007). White tea comprises of buds and younger leaves. It may also have been shaded from sunlight during its short growth, with the intention of limiting the production of chlorophyll. It is dried soon after harvest to prevent fermentation, which needs to be carefully controlled. Green tea comes from older leaves and is often wilted whilst keeping fermentation to a minimum. Oolong and black, that also come from the older leaves, are fermented teas but in the case of Oolong the fermentation is controlled to anywhere between 10% and 70% and black is fully oxidised. Pu-erh teas, traditionally from the south-western Chinese region of Yunnan, are made with a variant of the tea plant known as broad-leaf tea (*C. sinensis var. assamica*). Whereas black teas are generally processed by a fermentation method that allows for effective action of polyphenol oxidase enzymes, Pu-erh leaves undergo heating to denature these (Liang, Zhang, & Lu, 2005). After drying, the leaves are subjected to humid conditions in which bacterial action changes the chemical composition. This secondary fermentation, analogous to composting, differentiates Pu-erh from black teas in spite of both having similar appearances in terms of leaves and liquors.

Differentiation of tea can be achieved according to several criteria. Indeed, many prior studies have attempted to statistically quantify relationships between a tea and its country of origin (Fernández-Cáceres, Martín, Pablos, & González, 2001; Marcos, Fisher, Rea, & Hill, 1998; Moreda-Piñeiro, Fisher, & Hill, 2003) whilst others look at correlation to the plant's soil (Tokalıoğlu & Kartal, 2004). Differentiation of varieties has been carried out according to amino acid contents (Alcázar et al., 2007), volatile components (Toragi, Kobayashi, & Aishima, 1995), catechins and purine alkaloids (Fernández, Martín, González, & Pablos, 2000).

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The metal content has been also used but neither white nor Pu-erh tea was considered in these previous studies (Fernández et al., 2002; Fernández-Cáceres et al., 2001; Herrador & González, 2001) and only a few number of Oolong samples were analysed by Herrador and González (2001). Discrimination between white and green tea, both made from non-fermented leaves, could be performed thanks to the different age of the leaves used in the elaboration of these products. On the other hand, the use of broad-leaf tea for Pu-erh elaboration should result in the possible differentiation between this type and other fermented teas, such as black and Oolong. This work seeks to confirm if such conclusions can be drawn with regards to metal concentrations in the five types of tea. The concentration in aluminium, barium, calcium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, sodium, strontium, sulphur and zinc was determined by inductively coupled atomic emission spectrometry (ICP-AES). Different pattern recognition methods such as linear discriminant analysis (LDA) and probabilistic neural networks (PNN) were used in order to characterise the five tea varieties by using the mineral content as input variables.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade 65% nitric acid (Merck, Darmstadt, Germany) and 30% hydrogen peroxide (PanReac Química SA, Barcelona, Spain) were used for the microwave digestion of the samples. All solutions were prepared with ultrapure water (MilliQ, Millipore, Bedford, MA, USA). A multi-element standard (Merck, Darmstadt, Germany) was used to obtain the ICP-AES calibration curves.

2.2. Samples

Samples of tea belonging to five different varieties: white ($n = 28$), green ($n = 24$), black ($n = 36$), Oolong ($n = 20$) and Pu-erh ($n = 27$) were obtained from local stores. A certified reference material (CRM) of tea, NCS ZC73014 (China National Analysis Centre for Iron and Steel, Beijing, China), was used to carry out the method validation.

2.3. Apparatus and methods

An Ethos 900 MILESTONE (Microwave Laboratory System, Sorisole, Italy) was used for the digestion of the samples. With this aim, 6 ml of nitric acid and 1 ml of hydrogen peroxide were added to 0.2500 g of tea and the microwave program shown in the electronic supplementary material (Table S1) was applied. The digested samples were left to cool to room temperature and were then transferred to 25 ml volumetric flasks. Blank samples were prepared with each run. ICP-AES analysis was performed using an ULTIMA 2 atomic emission spectrometer (Horiba Jobin Yvon, Kyoto, Japan). The ICP operating conditions are shown in electronic supplementary material (Table S2).

2.4. Chemometrics

A data matrix consisting of 14 columns (the determined elements) and 135 rows (the tea samples) was created for the chemometric calculations. Kruskal–Wallis test was applied in order to draw attention to significant differences in elemental content between the types of tea. Linear discriminant analysis (LDA) and probabilistic neural networks (PNN) were applied to obtain classification rules. The STATISTICA 8.0 software package (Statsoft, 2007) was used for the statistical analysis and the PNN model was constructed with STATISTICA Neural Networks 4.0 (Statsoft, 1999).

3. Results and discussion

3.1. Method performance

The performance characteristics of the method, such as trueness, repeatability, intermediate precision, limit of detection (LOD) and quantification (LOQ) and linearity in the calibration range, were tested. The CRM NCS ZC73014 was used to study trueness, repeatability and intermediate precision.

The trueness of the method was evaluated by quintuplicate determination of the studied elemental concentrations in the CRM. Recoveries (R) were calculated dividing the mean value of the determined element by the certified value. Applying the law of propagation of errors the standard uncertainty of the recovery is calculated and the expanded uncertainty is obtained by multiplying by a coverage factor ($k = 2$). The obtained results are shown in Table 1. The recoveries can be deemed to be not significantly different from 100% as the inequality $|R - 100| \leqslant U(R)$ holds true for all of the studied elements. It can thus be stated that insignificant bias was detected and that the trueness of the method is assured.

The repeatability was evaluated by analysis of the CRM over a short period of time, and subject to the same operating conditions. Five digestions of the reference material were performed and the relative standard deviation (RSD_{rep}) was calculated. Furthermore, by analysing the CRM under intermediate precision conditions the relative standard deviation (RSD_{ip}) was also calculated. Results are shown in Table 1. Repeatability varied from 2.01% to 10.9% and intermediate precision present values in the range 1.05–12.09%. These results are in accordance with those obtained from the Horwitz (1982) function depending on the analyte level. Only in the case of sodium a poor intermediate precision was obtained.

Linearity in the calibration range were calculated as $100(1 - s_b/b)$ (Cuadros, García, & Bosque, 1996), being b the slope of the calibration curve and s_b its standard deviation. As can be seen in Table 1, linearity of magnesium and manganese were 95.3% and 97.2%, respectively, and the remaining elements presented values higher than 98%.

The LOD and LOQ were calculated as the concentration corresponding to a signal of 3 and 10 times the standard deviation of the blank sample, respectively. The obtained results are shown in Table 1. Aluminium, barium, calcium, iron, magnesium, manganese, strontium and zinc presents LOD values in the range 0.001–0.002 mg L^{-1} and LOQ from 0.003 to 0.007 mg L^{-1}. The LOD for the remaining elements are lower than 0.012 mg L^{-1}, except for potassium, which has a LOD of 0.045 mg L^{-1}. The LOQ varies from 0.02 in the case of nickel to 0.15 in the case of potassium.

3.2. Sample analysis

Teas belonging to white, green, black, Oolong and Pu-erh varieties were analysed and median concentrations and ranges are shown in Table 2. A complete data set is included in the electronic supplementary material (Table S3). As can be seen, potassium is the most abundant of the 14 elements, where barium, copper, nickel, strontium and zinc exhibit the lowest quantities. Manganese, nickel, and sulphur contents do not show large differences throughout the five types, contrasted by aluminium, barium, copper, magnesium, sodium and zinc. Aluminium levels in green and Pu-erh teas are comparable, with median values of approximately 1400 mg kg^{-1}, and higher than those present in the other three types. A similar conclusion was pointed by Fung, Carr, Poon, and Wong (2009) in the analysis of different types of Chinese teas. Oo-