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Classification of red wine based on its protected designation of origin (PDO) using Laser-induced Breakdown Spectroscopy (LIBS)

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ABSTRACT

This work reports on a simple and fast classification procedure for the quality control of red wines with protected designation of origin (PDO) by means of Laser Induced Breakdown Spectroscopy (LIBS) technique combined with Neural Networks (NN) in order to increase the quality assurance and authenticity issues. A total of thirty-eight red wine samples from different PDO were analyzed to detect fake wines and to avoid unfair competition in the market. LIBS is well known for not requiring sample preparation, however, in order to increase its analytical performance a new sample preparation treatment by previous liquid-to-solid transformation of the wine using a dry collagen gel has been developed. The use of collagen pellets allowed achieving successful classification results, avoiding the limitations and difficulties of working with aqueous samples. The performance of the NN model was assessed by three validation procedures taking into account their sensitivity (internal validation), generalization ability and robustness (independent external validation). The results of the use of a spectroscopic technique coupled with a chemometric analysis (LIBS-NN) are discussed in terms of its potential use in the food industry, providing a methodology able to perform the quality control of alcoholic beverages.

1. Introduction

The certification of the protected designation of origin (PDO) is one of the most important parameters to be controlled in order to protect the production and origin of agroalimentary products. Since the introduction of European regulations control [1] on this matter, many wine companies have adopted different strategies on the confirmation of wine authenticity improving the PDO controls. The type of grape, geographical origin, harvest, and vintage are parameters that determine the quality of wine products. Nevertheless, these indicators are not easily recognizable for the consumers and the PDO is considered as a single and unambiguous sign of quality that companies use to promote their brand in the market influencing the customer final decision [2]. The wine industry has seen an important growth in the last decade associated with an increase in the wine consumption. This is a strategic sector due to its importance in economic, environmental and social terms, as well as its importance representing the country's image abroad. Since 2008, the wine industry has put an important effort in the control of counterfeiting of wines with the objective of protecting the trade-mark quality wines and to prevent their illegal adulteration [3,4]. The wine adulteration consists of the addition of any substance to the natural wine, which changes its composition and may occur in many different forms. The greater part of the adulteration consists of addition of water and sugar, mixing with lower quality wines and label replacing [3]. The two main constituents of wine are water (81%) and ethanol (between 11% and 15%). Two types of flavonoids, the anthocyanins and flavanols, are key compounds for color and astringency, being responsible for the organoleptic properties and quality of wine. Other organic compounds in small amounts, such as acids, alcohols, phenols, nitrogenous compounds and inorganic substances represent the remaining 7%, making wine a complex sample and difficult to analyze [5,6].

The sensorial analyses together with chemical assays, and mineral content analysis may not be adequate for determining the PDO of wine [3]. Chromatographic techniques [7–9] require to conduct separate analysis of each component in the wine being slow and expensive process. The identification of grape variety by means of isotopic analysis [4,10], nuclear magnetic resonance (NMR) [11,12] or ADN/aRNA [13] techniques are generally used, due to their capacity of generating a fingerprint of wine providing the identification. Although these techniques produce accurate results, a large amount of sample and the use of expensive consumables are required, increasing the cost and duration of the analysis.

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This work evaluates the Laser Induced Breakdown Spectroscopy (LIBS) technique for the discrimination and the determination of geographical origin of red wines. LIBS technique is based on the interaction of a laser beam with a material target generating a plasma, the emission of the plasma contains spectroscopic information of excited atoms and ions present in the sample and reflecting its elemental composition [14]. Although there is a loss of molecular information in plasma, LIBS has provided excellent results in the identification of samples with complex matrixes [15,16]. LIBS provides spectral fingerprints characteristic of each sample based on the composition of wine The presence of mineral elements in wine is generally related to soil composition, grape variety, climate conditions, yeasts and winemaking [8]. The combination of LIBS with supervised classification methods such as NN has already shown successful results in many areas of knowledge for sample classification [17–20].

One of the major advantages of LIBS is that it does not require sample preparation, providing an economic and fast analysis, however in some cases avoiding a sample preparation goes in detrimental of the technique limiting its analytical performance. The change of the physical state of the sample transforming the liquid into solid has already been described as sample preparation [21]. Although the liquid-to-solid process produces an increase in the time analysis and an alteration of the original chemical composition, significant improvements such as the increase of the ablation rate, higher plasma temperature and electron density as well as a better laser-to-solid interaction has been observed in literature [22–24]. Moreover, avoiding the inherent drawbacks of working with liquids such as splashing and surface ripples produces lower limit of detection, better repeatability and sensitivity [25,26].

Different liquid-to-solid matrix conversion protocols have been described in the literature involving precipitation, filtering and pellets formation procedures [25,27,28]. Herein, the transformation of the liquid wine sample into gels by adding a natural collagen and its subsequent dried in an air assisted oven has been used as a new sample preparation protocol.

The aim of this work was to identify the adulteration of wines collected from Spanish local markets and evaluate the capacity of LIBS coupled with NN to detect the PDO of wines with negligible compositional and spectral differences and to improve the recognition capacity of extremely similar samples that have fewer physical and spectral differences between them.

2. Materials and methods

2.1. Wine samples

Thirty-eight Spanish red wines from eleven protected designation of origin, three foreign red wines and four table wines were purchased in retail stores. These wine samples were selected to cover the main Spanish wine regions (Fig. 1) including La Mancha, Ribera de Duero, Rioja, Valdepeñas, Vinos de Madrid, Cariñeña, Ribeiro, Ribera del Guadiana, Navarra and Somontano and Toro. Moreover a German, French and Italian wine was also included in the study. Most of the wines included in the study were elaborated with Tempranillo grapes, although Cabernet Sauvignon, Garnacha, Tinta de Toro and Shyrah were also considered. All samples belong to the 2011 vintage and were not affected by ageing period (young wine). Table 1 shows sample information including sample ID, commercial brand and type of grapes.

2.2. Sample preparation

A methodology based on the formation of a gel of wine using a commercial collagen was applied. 50 mL of wine sample were introduced into a beaker and 1 g of collagen gel was added and dissolved in the wine sample. 2.4 mL of this solution were allowed to stand 15 min until the formation of a gel on a square petri dish of 4×4 cm. Then, samples were introduced in a forced ventilation oven at 35 ± 2 °C during 12 h to evaporate the water, obtaining a dry solid. The final sample was completely flat with a thickness of approximately 0.35 mm. Fig. 2 shows an example of dry gel and the craters formed by single laser shots. In this process not only LIBS analysis was simplified but also pre-concentration of the sample (pre-concentration factor of 1:5) is performed allowing an improvement in the limits of detection. The gels for all samples were prepared at the same time to maintain the same conditions and avoiding the degradation and oxidation of the wine components.

2.3. LIBS set-up

The LIBS technique and the methodology used in the present work together with the most significant experimental conditions have been previously described [29]. Thus, only the experimental conditions relevant to this study are presented here. LIBS measurements were obtained using a Q-switched Nd: YAG laser (Quantel, Brio model) operating at 1064 nm, with a pulse duration of 4 ns full width at half maximum (FWHM), 4 mm beam diameter and 0.6 mrad divergence. Samples were placed over an X–Y–Z manual micro-metric positionator with a 0.5 μm stage of travel at every coordinate to ensure that each laser pulse impinged on a fresh position. The laser beam was focused onto the sample surface with a 100 mm focal-distance lens, producing a spot of 100 μm in diameter. The best signal-to-background ratio was achieved at 42 mJ of pulse energy with a repetition rate of 1 Hz. The laser crater profile was measured by means of a confocal microscope after laser pulse irradiation on a fresh position. A narrow crater was created with a diameter of 450 μm and 140 μm in depth. Emission from the plasma was collected with a 4-mm aperture, and 7 mm focus fused silica collimator placed at 4 cm from the sample, and then focused into an optical fiber (1000 μm core diameter, 0.22 numerical aperture), coupled to a spectrometer. The spectrometer system was an EPP2000, StellarNet (Tampa, FL, U.S.A.) with a gated CCD detector. A grating of 300 l/mm was selected; a spectral resolution of 0.5 nm was achieved with a 7 μm entrance slit. The wavelength range used was from 200 to 1000 nm. Therefore, 2048 data points were recorded for each sample. The detector integration time was set to 1 ms, to prevent the detection of bremsstrahlung, the detector was triggered by a 2 μs delay time between the laser pulse and the acquired plasma radiation using a digital delay generator (Stanford model DG535). The spectrometer was computer-controlled using an interface developed in Matlab.

2.4. LIBS analysis

Wine samples were measured directly in air at room condition. Each LIBS spectrum was acquired from a single shot measurement. A total of 100 spectra were recorded for each wine sample by moving the sample stage about 0.25 mm to expose a fresh portion of the sample surface and avoiding areas irradiated by previous shots. Only in the case of the M1, D1, R1, V1 and VM1 samples, four data sets of 100 spectra were obtained: the first data set (training library) was used to calculate the model of the NN; whereas the last three data sets (replicate libraries) were used for validation purpose. In order to avoid data variations due to changes in the laser pulse energy, each spectrum was normalized by the intensity of one specific spectral line, *i.e.*, K (I) 766.49 nm [30]. The spectral information of each sample was obtained in less than 2 min considering the integration time of the spectrometer and the frequency of laser pulses that was fixed to 1 Hz.