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Comparative study on the use of anthocyanin profile, color image analysis and near-infrared hyperspectral imaging as tools to discriminate between four autochthonous red grape cultivars from La Rioja (Spain)

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ABSTRACT

Three independent methodologies were investigated to achieve the differentiation of red grapes from different grape varieties (Garnacha, Graciano, Mazuelo and Tempranillo) collected from five vineyards located in the D.O.Ca. Rioja. Anthocyanin chromatographic analysis, color image analysis and near infrared hyperspectral imaging were carried out for the grapes. Then, a Stepwise Linear Discriminant Analysis (SLDA) was developed for each data set in order to discriminate grapes according to their grape variety. As a result, using anthocyanin profile, color image analysis and near infrared hyperspectral imaging respectively, 88%, 54% and 100% of the samples were correctly classified in the internal validation process and 86%, 52% and 86% were correctly classified in the leave-one-out cross-validation process.

1. Introduction

The major factors impacting on wine quality are related to winemaking process and cultivar features. Grape composition – which depends on climate, soil where grapevines are grown and grape varieties – mainly influences wine composition and therefore wine sensory parameters such as aroma, color and flavor. Consumers value the quality characteristics of wine produced from high-quality grapes enough to pay higher prices, so for each harvest, grape price are closely linked to grape quality [1].

In a cellar it is really important to know the characteristics of grapes that are taken by the vine growers. Grape variety, maturity or sugar content are typically analyzed in order to determine grape quality, set grape price and classify grapes for the various wines produced. The grape maturity and sugar content are usually determined using analytical methods recommended by the O.I.V. [2], which are simple and reliable methods. However, in cellars, grape variety is typically determined by means of visual methods based on the staff experience and knowledge. Furthermore, in situ vine analyses, such as ampelometry are also used. However, it is not possible to have a panel to classify the berries according to their variety, essentially because this is expensive, there are many samples to be classified, and there is insufficient time to carry out the abovementioned task at harvest time. It would be appropriate to have rapid and inexpensive analytical methods to classify grapes according to their variety.

There are several studies that try to classify grape and wine samples according to their grape variety. A number of them use polyphenol content or polyphenol profiles to classify or authenticate samples [3–7]. In addition, some studies only use anthocyanin profile to carry out these classifications [8–10]. These studies generally achieve very accurate results although they are destructive and time consuming analyses that also require the use of chemical reagents. Other authors have classified wine and grape samples using spectroscopic tools in the ultraviolet–visible and near-infrared regions in order to discriminate samples according to their grape variety and origin [11–13]. Urbano et al. [11] use ultraviolet–visible spectroscopy in order to discriminate wine samples according to their origin, grape variety and ageing process. The classification rates obtained by these authors were 90%, 75% and 75%, respectively. Ferrer-Gallego et al. [12] classify grape samples using NIR spectroscopy according to their origin

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obtaining a prediction rate of entire grapes correctly classified of about 93%. Using MIR/NIR traditional spectroscopy, up to seven different grape variety wines are discriminated and over 95% of the samples were correctly classified [14]. Spectroscopy achieves good results in a fast and reasonably inexpensive way. Furthermore, the use of appropriate chemometric tools is needed to classify grape and wine samples according to their grape variety [14].

The area where the present study was conducted involving the wine-producing region of D.O.Ca. Rioja, in Northern Spain, which has vineyards extending over 63,593 ha. *Vitis vinifera* L. cv Tempranillo is the most widely grown red grape variety in Spain and it is cultivated in 75% of the D.O.Ca. Rioja. This variety is capable of producing wines with a good balance of alcohol content, color and acidity, and a fruity mouthfeel that turns velvety as it ages. Graciano, which is an autochthonous cultivar to the aforesaid region, is less known, but it is used as an integral component of many Rioja wines because it is considered to contribute significantly to the quality of the wine. It offers wines with a marked acidity and polyphenolic content, ideal for ageing, with a unique aroma. Furthermore, cultivars such as Garnacha and Mazuelo are also grown in this area. Garnacha is a high-yielding grape that produces vigorous wines of great texture, body and color. Finally, although short on flavors, Mazuelo produces wines with abundant tannins, high acidity and stable color [15,16].

Taking into account the importance of these grape varieties in the D.O.Ca. Rioja and the differences between wines produced from these varieties, it would be very useful to have a fast and inexpensive method to discriminate between these four autochthonous red grape cultivars from La Rioja.

As state above, hyperspectral imaging and digital imaging may provide fast, non destructive and green chemistry determinations compared with traditional methods. Furthermore, the number of samples that might be evaluated using these methods is higher than using the traditional ones and also the determination could be automated. Moreover the use of hyperspectral imaging over conventional spectroscopy in samples having small size, such as individual berries, provides the individual spectrum of each berry using the whole grape without any contact. This may be an advantage in order to use this device at line or in line at winery belts. Nevertheless, the availability of these new devices in conventional laboratories at that time due to their high cost could be a drawback. Digital imaging is a surrogate to human inspection in order to provide an objective analysis. Moreover, the digital image device is cheaper than the hyperspectral device and should be tested in order to find the cheapest way to perform the analysis.

The main aim of this work is to compare the use of anthocyanin profile, color image analysis and near infrared hyperspectral imaging as tools to discriminate between Tempranillo, Graciano, Garnacha and Mazuelo grapes in order to select the more useful and accurate tool. In a second strand, a second level data fusion has also been tried in order to improve the results. To our knowledge, this is the first time that near infrared hyperspectral imaging has been applied to red grapes to discriminate between cultivars. Furthermore, this is also the first time that a comparative study on the use of the three aforesaid analytical tools has been performed.

2. Material and methods

2.1. Samples

Vitis vinifera L. cv. Tempranillo (two different vineyards), Graciano, Garnacha and Mazuelo were collected from five vineyards located in the D.O.Ca. Rioja. Red grapes were collected at two different developmental stages during berry maturity in the 2012 vintage: harvest time and over-ripening. Harvest time and over-ripening dates were selected in agreement with the cellar staff. Individual grapes were randomly separated from the top, middle and bottom of the cluster. Five berries were taken into account for each cultivar and developmental stage with the exception of Tempranillo. In the case of Tempranillo, 20 berries were used since two different vineyards were taken into account (i.e. 5 berries $\times$ 2 vineyards $\times$ 2 developmental stages). A total of 50 samples were used. Berries were immediately frozen and stored at -20 °C until analyses were performed.

2.2. Image analysis

An individual image for each grape was taken. For acquiring images, the DigiEye® system based upon a calibrated digital camera was used [17]. This system includes an illumination box specially designed by VeriVide Ltd. (Leicester, UK) to illuminate the samples consistently and a digital camera connected to a computer. The digital camera used for image acquisition was a 10.2-MP Nikon® D80 with Nikkor® 35 mm f/2D objective. In order to calibrate the digital camera, a color chart (DigiTizer, VeriVide Ltd., Leicester, UK) is used to characterize its response by relating its RGB signals to CIE specifications. The cabinet is equipped with two fluorescent tubes that emulate the CIE standard illuminant D65 and offer stable lighting conditions. They were switched on at least 10 min before being used, according to manufacturer indications, to stabilize them.

Image processing was carried out using MATLAB (The Mathworks, 2009). For obtaining CIELAB coordinates from RGB color space, the software DigiFood® [18] was used. The obtained CIELAB coordinates were used in the subsequent analyses.

2.3. Near-infrared hyperspectral imaging

Hyperspectral imaging device (Infaimon S.L., Barcelona, Spain) comprised a Xenics® XEVA-USB InGaAs camera (320 $\times$ 256 pixel; Xenics Infrared Solutions, Inc., Leuven, Belgium), a spectrograph (Specim ImSpector N17E Enhanced; Spectral Imaging Ltd., Oulu, Finland) covering the spectral range between 900 and 1700 nm (spectral resolution of 3.25 nm). The images were recorded using the abovementioned mirror scanner pushbroom device, a 50 Hz frame rate, an exposure time of 9 ms and the instrument acquisition software SpectralDAQ v. 3.62 (Spectral Imaging Ltd., Oulu, Finland). The samples were thawed and tempered at room temperature and individual hyperspectral image of each grape was recorded. Equipment and procedure used to image recording are described in detail elsewhere in Hernández-Hierro et al. [19].

After calibration and segmentation processes, the average spectral profile for each individual grape was saved. Noisy wavebands at both extremes of the spectra range were removed and only spectral data in the resulting effective wavelength 950–1650 nm regions were used in data analysis due to reduced efficiency outside this range in the used device. Principal component analysis (PCA) was applied to reduce the dimension of the aforementioned spectral matrix. The software used for PCA analysis was Win ISI® (v1.50) (Infrasoft International, LLC, Port. Matilda, PA, USA). Overall, the spectral variability explained was 99.9% using 8 principal components. The obtained scores were used in the subsequent analyses.

2.4. Anthocyanins extraction and chromatographic analysis

After image acquisition process, grape skins were separated manually from the whole grapes. Individual grape skins were macerated twice at 4 °C in 10 mL of methanol containing 0.1% of