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Use of near infrared hyperspectral tools for the screening of extractable polyphenols in red grape skins

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ABSTRACT

Hyperspectral images of intact grapes were recorded at harvest time using a near infrared hyperspectral imaging system (900–1700 nm). Spectral data have been correlated with red grape skin extractable polyphenols (total phenolic, anthocyanins and flavanols) by modified partial least squares regression (MPLS) using a number of spectral pretreatments. The obtained results (coefficient of determination (RSQ) and standard error of prediction (SEP), respectively) for the developed models were: 0.82 and 0.92 mg g^{-1} of grape skin for extractable total phenolic content, 0.79 and 0.63 mg g^{-1} of grape skin for extractable anthocyanin content, 0.82 and 0.45 mg g^{-1} of grape skin for extractable flavanol content. The obtained results present a good potential for a fast and reasonably inexpensive screening of the extractable polyphenolic compounds in intact grapes. Moreover, the heterogeneity of extractable polyphenols within the ripeness stage has been also evaluated using the proposed method.

1. Introduction

Red grapes (*Vitis vinifera* L.) contain about four grams of phenolic material per kilo. There is substantial variation in levels of phenolic compounds which depends on a number of factors including the variety of grape, high or low skin:volume ratio, growing region, climate, and growth conditions (Crozier, Clifford, & Ashihara, 2006). Furthermore, similar heterogeneity can be also found within the same physiological stage. Some studies describe a Gaussian bell-shaped distribution of soluble solids and extractable total phenolic content in a sampling point (Kontoudakis et al., 2011; Zouid, Siret, Jourjon, Mehinagic, & Rolle, 2013).

Wine and grape phenolic compounds are grouped into two categories, flavonoids and non-flavonoids. The flavonoids are all polyphenolic compounds, having multiple aromatic rings possessing hydroxyl groups. The majority of flavonoids in red grapes are found in seeds and berry skin and are transferred to the wine from the above mentioned parts during the fermentation process. Among these compounds, flavanols constitute the most abundant class and play a relevant role in the sensory characteristic of red wines whereas anthocyanins are red-coloured phenols that give to red wine its characteristic colour (Waterhouse, 2002). The main non-flavonoids are hydroxycinnamic acids, benzoic acids, hydrolyzable tannins and stilbenes (Harborne, 1994). The origin of the non-flavonoids in wine is more diverse. In grapes and wines not aged in oak, the primary non-flavonoids are derivatives of hydroxycinnamic and hydroxybenzoic acids. They are stored primarily in cell vacuoles of skin and pulp, and are easily extracted on the crushing stage (Jackson, 2000). The influence of phenols (flavonoids or non-flavonoids) in the antioxidant activity of wines has been previously reported (Rice-Evans, Miller, & Paganga, 1997).

Winemakers are continuously looking for high quality wines. One of the major factors affecting wine quality is grape phenolic maturity (Ribéreau-Gayon et al., 2006). This factor shows the amount of phenolic compounds in the skin, pulp and/or seed. It is also really important to know the amount of these phenols that may be extracted from grapes to wine. The extractability of phenolic compounds from skins depends significantly on grape ripeness. Riper grapes have higher cell wall degradation hence they have higher extraction degree (Hernández-Hierro et al., 2014;

Abbreviations: DMACA, p-dimethylaminocinnamaldehyde; EAC, extractable anthocyanin content; EFC, extractable flavanol content; EPC, extractable total phenolic content; *H*, Mahalanobis distance; MPLS, modified partial least squares; MSC, multiplicative scatter correction; NH, neighbourhood Mahalanobis distance; NIRS, near infrared spectroscopy; PC, principal component; PCA, principal component analysis; PLS, partial least squares; RSQ, coefficient of determination; SEC, standard error of calibration; SECV, standard error of cross-validation; SEP, standard error of prediction; SNV, standard normal variate.

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Ribéreau-Gayon et al., 2006). Thus, it is possible to find studies linking phenol extractability to some factors related to grape ripeness. In grape skin, the extracted phenolic compounds (total phenols, total anthocyanins and total flavanols) increase with soluble solids content of grape must (Fournand et al., 2006; Torchio, Cagnasso, Gerbi, & Rolle, 2010; Zouid et al., 2013). In addition, different stages of ripening concern the amount of extractable phenols in the same way that total soluble solids (Canals, Llaudy, Valls, Canals, & Zamora, 2005; Fournand et al., 2006; Hernández-Hierro, Quijada-Morín, Rivas-Gonzalo, Rivas-Gonzalo, & Escribano-Bailón, 2012). Finally, this parameter is also linked to grape maceration conditions (González-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004).

In the abovementioned studies, extractions of different phenols from grape skin were carried out using wine simulated macerations. This technique needs to remove skins manually from the pulp and then immerse them in a model wine hydroalcoholic solution. After a maceration period (around 3–5 days) the supernatant is used for spectrophotometric and chromatographic analysis. Taking that into account, the determination of the amount of the extracted phenols is a destructive and time consuming process.

In previous works carried out in our laboratory, hyperspectral imaging has been used to develop screening methods in order to measure phenols concentration in grape or grape seeds. Hernández-Hierro, Nogales-Bueno, Rodríguez-Pulido, and Heredia (2013) carried out a screening method to determine the total content of anthocyanins in grapes during ripening for Syrah and Tempranillo varieties. Nogales-Bueno, Hernández-Hierro, Rodríguez-Pulido, and Heredia (2014) used this technology for the screening of pH, total acidity and sugar concentration of must and total phenols concentration in skins of white and red grapes. Finally, hyperspectral imaging has also been used for the screening of total and extractable flavanols in grape seeds in white and red cultivars as reported by Rodríguez-Pulido et al. (2014).

The main aim of this study is to develop a fast and non-destructive hyperspectral method for the screening of the extractable content of anthocyanins, flavanols and total phenolic compounds in grape skins. To our knowledge, near infrared hyperspectral imaging has been applied to grapes to face these goals for the first time.

2. Materials and methods

2.1. Samples

V. vinifera L. cv. Syrah and Tempranillo red grape samples were collected from two vineyards located in the Condado de Huelva Designation of Origin D.O. (Andalusia, Spain). Tempranillo is the most often grown red grape cultivar in Spain for producing quality red wines and Syrah is a resistant cultivar to warm climatic conditions. Syrah represents an important percentage of the grown red grape under the aforementioned climatic conditions (Gordillo et al., 2012).

Tempranillo and Syrah grapes were collected when the vineyards were harvested (August 12 and 27, 2013 respectively). One hundred single berries were collected for each variety. In order to achieve representative samples sets, these were collected from the top, middle and bottom of the cluster and in the sunlight and shade side of this. After that, the samples were refrigerated and they were immediately carried to the laboratory, tempered and subjected to the hyperspectral analysis.

2.2. Hyperspectral image acquisition

Hyperspectral imaging device (Infaimon S.L., Barcelona, Spain) comprised a Xenics® XEVA-USB InGaAs camera (320 × 256 pixels; 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 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. (2013).

After calibration and segmentation processes, the average spectral profile for each 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.

2.3. Determination of reference parameters

Grape skins were separated manually from the whole grapes and they were weighted, then grape skins were immediately frozen and stored an −20 °C until analysis were performed. Reference parameters taken into account were extractable total phenolic content, extractable anthocyanin content and extractable flavanol content in grape skin. Grape skins were immersed in a model wine hydroalcoholic solution (4 g L^{-1} tartaric acid, 12.5% ethanol, adjusted at pH 3.6 with NaOH 0.5 M) for a maceration period of 72 h. This supernatant was used in all the following reference analysis.

Extractable total phenolic content was determined using the Folin–Ciocalteu method (Singleton, 1985). For quantification, results were expressed as mg of gallic acid equivalents per gram of grape skin.

In order to measure the extractable anthocyanin content, the supernatant was diluted 1:2 with 0.1 M HCl, filtered through 0.45 μm pore size filters and directly injected into the chromatographic system. Anthocyanin chromatographic analysis was carried out following a modification of García-Marino, Hernández-Hierro, Rivas-Gonzalo, and Escribano-Bailón (2010) as described elsewhere in Hernández-Hierro et al. (2013). Results were expressed as mg of malvidin-3-*O*-glucoside equivalents per gram of grape skin. All analyses were performed in duplicate. The standard error was generally around 10% so the error and degree of accuracy of the reference method was considered appropriate to use these data as reference values.

Extractable flavanol content was determined following a modification of Vivas, Glories, Lagune, Saucier, and Augustin (1994). Ten microlitres of supernatant was mixed with 190 μL of methanol and 1 mL of p-dimethylaminocinnamaldehyde (DMACA) reagent. The absorbance was recorded at 640 nm after 10 min of reaction. This measure was performed in duplicate on an Agilent 8453 UV–visible spectrophotometer (Palo Alto, USA), equipped with diode array detection (DAD). A calibration curve of (+)-catechin (Sigma–Aldrich, St. Louis, USA) for quantification and all the measures were within the linear range of the calibration curve. All the results were expressed as mg of catechin equivalents per gram of grape skin.

2.4. Data analysis

An unsupervised pattern recognition technique, principal component analysis (PCA), was used in order to provide information about the latent structure of spectral matrix and to find spectral differences among all spectral samples. This method provides not only information related to spectral outliers and the distribution of samples in the newly-created space, but is also an important source of knowledge with which to create cross-validation groups used in the calibration process (Shenk & Westerhaus, 1995; Brereton, 2003). PCA was also used to select representative samples from the spectral data set. Mahalanobis distances (H) for each