Analytical Methods

Front-face fluorescence excitation-emission matrices in combination with three-way chemometrics for the discrimination and prediction of phenolic response to vineyard agronomic practices

Manuel Cabrera-Bañegil[a,*], Esperanza Valdés-Sánchez[b], Daniel Moreno[b], Diego Airado-Rodríguez[c], Isabel Durán-Merás[b,d]

[a] Department of Analytical Chemistry, Faculty of Sciences, University of Extremadura, 06006 Badajoz, Spain
[b] Technological Institute of Food and Agriculture (CICYTEX-INTAEX), Junta of Extremadura, 06007 Badajoz, Spain
[c] Department of Sciences and Mathematics Education, Education School, University of Extremadura, 06006 Badajoz, Spain
[d] Research Institute on Water, Climate Change and Sustainability (IACYS), University of Extremadura, 06006 Badajoz, Spain

ARTICLE INFO

ABSTRACT

Phenolic extracts from *cv* Tempranillo grapes subjected to water stress and irrigation treatment, both of them with high and low crop load, were analyzed by front-face fluorescence. Excitation-emission matrices (EEMs) were analyzed by means of unsupervised parallel factor analysis (PARAFAC), PARAFAC supervised by linear discriminant analysis, and discriminant unfolded partial least-squares. All algorithms allowed to differentiate between water stress and irrigation grape samples when the fluorescence maxima region of catechin and epicatechin, and resveratrol was considered. A central composite design was employed for the calibration of catechin, epicatechin and resveratrol. Resveratrol was quantified by U-PLS in both, irrigated and water stressed samples, and levels between 3.46 ± 0.22 and $4.67 \pm 0.08\,\mu g\,mL^{-1}$ and 2.43 ± 0.60 and $3.03 \pm 0.10\,\mu g\,mL^{-1}$, respectively, were found. PARAFAC only allowed the determination of the sum of catechin plus epicatechin ($R^2 = 0.9397$). The determination of total catechin plus epicatechin by means of PARAFAC was successfully validated by liquid chromatography.

1. Introduction

The possibility of monitoring the agricultural techniques is essential for achieving the desirable quality and to give response to the consumers' demands. The water regime in grapevine crops is crucial for obtaining grapes with determined physical-chemical and sensory features (Kyraleou et al., 2016). Different researches show that the water status of the vineyard affects to the chemical composition of grapes influencing the color intensity, sugar accumulation and concentration of polyphenols and anthocyanins (Romero et al., 2013) and its control is necessary for reducing the excessive vine vigor, improving the correct compromise between yield and quality (Gil et al., 2013). In other words, a water deficit during berry growth reduces yield and, depending on variety, it is usually considered beneficial for wine quality (Valdés et al., 2009). The effect of irrigation on consumer acceptability of red and white wines has been studied (Mirás-Avalos et al., 2017). In semiarid climates, the main goal when cultivating grapes for wine production should be to obtain the best grape composition with the

highest possible yield while maximizing the available water resources. This can be achieved with an appropriate balance between vegetative growth and yield. One purpose of a modern viticulture is establishment practices for being able to limit vineyard yield and improve grape composition. In this sense, the cluster thinning and leaf removal are some interesting practices to balance the source-sink relationship by increasing carbohydrate sources in relation to sinks (Uriarte et al., 2015). In both cases, an increase and better distribution phenolic composition of grape berries has been demonstrated (Gatti, Bernizzoni, Civardi, & Poni, 2012; Moreno et al., 2015).

The consumption of the phenolic compounds has a positive effect on human-health due to their antioxidant activity, preventing illnesses (Saiko, Szakmary, Jaeger, & Szekere, 2008). In addition to this, among the typical kinds of phenolic compounds present in the most of grape varieties, it is important to highlight the presence of stilbenes, non-flavonoids compounds, which present beneficial and interesting biological properties. The synthesis of stilbenes is carried out when grapevine defense response is stimulated with exogenous molecules often

---

originated from microbes or plants (Delaunois et al., 2014). The most well-known bioactive stilbene is resveratrol, which possesses to biological and pharmacological properties (Witte, Kerti, Margulies & Flöel, 2014). On the other hand, another interesting phenolic compounds present in grapes are the flavanols, specifically monomeric flavan-3-ols such as catechin and epicatechin. These contribute to wine sensory perception, such as astringency, bitterness and mouth-feel.

In this sense, the determination of total phenols and their distribution is essential for the quality control of red grapes. In addition, the amount and distribution of polyphenols in the grape determines the harvest time. So, faster and easy to perform methodologies are, therefore, necessary for making decisions in the vineyard. Traditionally, the determination of phenols has supposed long times and reagent consuming reactions. The individual phenolic information of grapes can be obtained by means of a liquid chromatography (LC) (Da Silva Padilha et al., 2017) which is not available for most vine growers and small winemarkers. LC, among other techniques requires qualified personnel, high elution times and high organic solvents consumption. In this context, the development of new, fast, cost effective methodologies with simple sample preparation would be very welcome in winemaking sector (viticulture and enology sectors). Near infra-red spectroscopy (NIRS) coupled with chemometric algorithms has been successfully employed for predicting major sugar and organic acids of homogenized extract of grape berries at various developmental stages (Musingarabwi, Nieuwouldt, Young, Eyéghè-Bickong, & Vivier, 2016). NIRS has also been used for evaluation of phytosanitary status of intact grapes (Beghi, Giovenzana, Brancadoro, & Guidetti, 2017). On the other hand, NIR hyperspectral imaging with chemometrics has been used for the determination of anthocyanins and total phenolic in intact grapes berries (Diago, Fernández-Novales, Fernandes, Melo-Pinto, & Tardaguila, 2016; Martínez-Sandoval et al., 2016; Nogales-Bueno et al., 2015) and UV–visible spectroscopy with chemometrics has allowed to determine the phenolic profiles of grapes (Aleixandre-Tudo, Nieuwoudt, Olivieri, Aleixandre, & du Toit, 2018). However, fluorescence spectroscopy has hardly ever been used for studying the effects of irrigation and water stress conditions in phenol content of red grapes. Several studies show that fluorescence excitation emission (EEMs) can be a useful tool for quality control of red wine (Airado-Rodríguez, Durán-Merás, Galeano Díaz, & Petter Wold, 2011; Airado-Rodríguez, Galeano-Díaz, Durán-Merás, & Petter Wold, 2009; Cabrera-Bañegil, Hurtado-Sánchez, Galeano-Díaz, & Durán-Merás, 2017) and it can be used also for the classification of white wines according to grape variety (Azcarate et al., 2015) and grape ripening stage (Le Moigne et al., 2008). The information provided by fluorescence spectroscopy has been commonly analyzed by second order multivariate algorithms such as parallel factor analysis (PARAFAC) and unfolding partial least squares (U-PLS) (Durán-Merás, Domínguez Manzano, Airado Rodríguez & Muñoz de la Peña, 2018). Furthermore, the application of PARAFAC can be carried out in unsupervised way or supervised by linear discrimination analysis (LDA) to maximize the separation of groups (Muñoz de la Peña et al., 2016).

Considering the potential fluorescence information obtained from excitation emission matrices data, the main goal of this study was the development of a fast and simple front-face fluorescence methods in combination with PARAFAC, alone and combined with LDA, and discriminant unfolding partial least square (DU-PLS).

2. Materials and methods

2.1. Reagents, solvents and phenolic standards

Catechin, epicatechin and resveratrol were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Diethyl ether (Panreac, Barcelona, Spain) was used for phenols extraction and for preparing the calibration samples. Oxalic acid was purchased from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q water system (Millipore S.A.S., Molsheim, France).

2.2. Samples

2.2.1. Vineyard site, sampling and preliminary selection

The experiment was carried out in an experimental *Vitis vinifera*, L. cv. Tempranillo vineyard located in Badajoz, Extremadura (western Spain) (lat. 38°51′N; long. 6°40′W; elevation 198 m above sea level. Tempranillo cultivar, grafted on rootstock 110R, were planted in 2001 and trained to a vertical trellis on a bilateral cordon system east/west oriented. Vines were spaced 1.20 m within the row and 2.50 m between rows (3333 vines/ha). Vines were trained in a vertical shoot-positioning system oriented north-west to southeast. The vineyard soil was a silt-loam with 37.3% sand, 25.5% clay, 36.1% silt, and 1.1% organic matter. Volumetric water content was 0.30 m^3/m^3 at field capacity and 0.16 m^3/m^3 at the permanent wilting point.

The irrigation regimes were as follows: Non irrigated (only rain-fed, NI) and full irrigated vines (FI), corresponding to 100% of crop evapotranspiration (ETc). ETc is a parameter related with water requirements and it was calculated by means of a weighing lysimeter installed in the experimental vineyard under study (Picón-Toro, González-Dugo, Uriarte, Mancha & Testi, 2012).

The experimental orchard was divided in 12 randomised experimental plots, six non irrigated, and other six full irrigated. For each irrigation regime, two shoots load levels were established: Control with 12 shoots/vine (high crop level), and shoot-thinned treatment, in which the load was adjusted at 6 shoots/vine (low crop level).

Grapes of each experimental plot were randomly sampled by picking berries from the top, central and bottom parts of the cluster. Four samples were sampled from each experimental plot, and after sampling, grapes were immediately transported to the laboratory. With the aim to improve the physiological homogeneity of the different samples, berries were calibrated according to their density (Carbonell-Bejerano et al., 2016). The berries used for the different analysis were those which floated in 150–170 g/L sodium chloride solution, corresponding to berries with a total soluble solid content about 23–24 °Brix.

2.3. Extraction and HPLC analysis of phenolic compounds

Phenolic extraction was carried out according to a modification of the methodology previously described (Kontoudakis, Esteruelas, Fort, Canals & Zamora, 2010). 250 g of berries were crushed and homogenised in a blender (Worwek Model TM-31, Germany, speed 3, 1 min). Three replicates of 50 g of the homogenized sample were then used to extract total phenolic compounds after maceration with 50 mL of oxalic acid 0.3 M (pH 1.00). All samples were macerated during 16 h at 22–24 °C. The extracts, previously filtered (0.25 µm diameter Chromafil filters, Düren, Germany), were analyzed by HPLC (1200 LC; Agilent Technologies, Palo Alto, CA). The HPLC system is equipped with a photodiode array detector. The analytical column employed was a Kromasil® column (100-5-C18 250 × 4.6 mm, Akzonobel, Bohus, Sweden) and during the analysis, the temperature was maintained at 40 °C. The elution was carried out as previously described (Natividade, Corrêa, Souza, Pereira, & Lima, 2013). The injection volume was 10 µL. Detection was performed at 280 for catechin and epicatechin and 320 nm for resveratrol.

2.4. Front-face fluorescence

Two dimensional fluorescence spectra, as excitation-emission matrices (EEMs), were recorded using a Fluorescence Spectrophotometer Varian Model Cary Eclipse (Agilent Technologies, Madrid, Spain). Measurements were made with a variable front-face accessory, and in a 10-mm quartz cell at room temperature. The slits of excitation and emission monochromators were set at 5 and 5 nm, respectively, and the scan rate was 300 nm min^{-1}. For each sample, EEM was collected as a