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Electronic eye for the prediction of parameters related to grape ripening

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ABSTRACT

An electronic eye (EE) for fast and easy evaluation of grape phenolic ripening has been developed. For this purpose, berries of different grape varieties were collected at different harvest times from veraison to maturity, then an amount of the derived must was deposited on a white sheet of absorbent paper to obtain a sort of paper chromatography. Thus, RGB images of the must spots were collected using a flatbed scanner and converted into one-dimensional signals, named *colourgrams*, which codify the colour properties of the images. The dataset of colourgrams was used to build calibration models to relate the colour of the images with the phenolic composition of the samples – determined by reference analytical methods – and therefore to follow the ripening trend. Satisfactory calibration models were obtained for the prediction of the most important parameters related to phenolic ripening of grapes, such as colour index, tonality, total anthocyanins content, malvidin-3-O-glucoside and petunidin-3-O-glucoside.

1. Introduction

The maturity level of grapes (*Vitis vinifera*) at the harvest is the first factor that influences the quality of the resulting wine [1]. Among the various features, sugar content, pH and acidity levels are the parameters more frequently used to monitor the maturity level of grapes [2]. However, also the phenolic composition of grapes plays an important role on the development of several sensorial attributes of wine, such as colour, body, structure, bitterness and astringency [3].

The determination of the parameters related to phenolic ripening is performed by means of classical analytical procedures, mainly based on UV–Vis spectrophotometry and high-performance liquid chromatography [4–6]. These methods are very accurate but require expensive instrumentations and the involvement of skilled personnel. Alternatively, sensory analysis is frequently used to provide a description of colour, aroma, flavour and texture of the grapes, giving a global characterisation of the maturity level [7]. However, the sensory evaluation requires a lot of training and experience to minimize the inherent subjectivity and the variability of tasters [8].

For these reasons, nowadays increased efforts are devoted to develop easy-to-use, inexpensive and objective methods based on artificial sensors [9], known as electronic nose (EN) [10,11], electronic tongue (ET) [12,13] and electronic eye (EE) [14,15]. These devices are generally used to analyse the sample 'as it is' or after a minimal manipulation. Then, the sensor output is processed by proper chemometric techniques that, following a blind-analysis approach, extract the sought information, such as the amount of specific analytes or the sensory features responsible of smell, taste and colour of the sample.

In a recently published paper, some of us have presented an ET aimed at monitoring grape ripening [16]. In that work it was shown that, by proper fusion of the data measured with two voltammetric sensors, it was possible to quantify the parameters related to the technological maturity of grapes, i.e., pH, total acidity and sugar content, in addition to the anthocyanins content. In the present work, which was conducted on the same grape samples and can be considered as a follow up from our previous investigation, we propose the development of an EE sensing system for the prediction of colour-related parameters, principally suitable to monitor the phenolic maturity.

To this aim, purple grape samples were collected at different harvest times from veraison to maturity, then a drop of the derived must was deposited on a paper sheet to obtain a sort of paper chromatography. The spots of must were imaged by means of a flatbed scanner, and the resulting RGB images were analysed using multivariate methods, in order to use the colour-related information content of the images to predict the phenolic composition of the samples, and therefore to follow the phenolic ripening trend.

To date, few research studies reported the use of image analysis for the evaluation of colour characteristics and colour changes of grape berries during ripening. For instance, in [17,18] RGB images of grape berries have been categorised into different classes according to

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ripening. More in detail, considering the histograms of colour-related parameters such as hue or brightness, proper threshold values have been identified to distinguish grapes into different clusters according to their colour development (e.g., pre-veraison and post-veraison). In other research papers, the digital images of grape seeds have been used to investigate CIELAB parameters and morphological features, such as area, aspect, roundness, length, width and heterogeneity, in order to predict their maturity stage [19,20]. Notwithstanding the satisfactory results obtained in these studies, the proposed methods were limited to the identification of correlations between colour and/or morphological features and the maturity level of grapes, which was determined based on harvest time or on visual inspection by expert assessors.

In the present work, we followed an alternative approach based on the use of RGB images for the quantitative prediction of specific analytical parameters related to the colour properties and the phenolic composition of the grape samples, which could allow to obtain a more detailed and comprehensive overview of the maturity level.

Basically, our method consists in converting each image into a one-dimensional signal, named *colourgram*, which codifies all the colour-related information and represents a sort of fingerprint of the corresponding RGB image [21]. The main advantage of this approach is that the whole dataset of colourgrams can be analysed in the same manner as any other dataset of signals. For instance, exploratory analysis tools like Principal Component Analysis (PCA) can be used to highlight the presence of trends, of clusters or of outlier images, whereas multivariate calibration or classification methods can be used to predict the value of specific parameters or to assign a sample to a specific class, based on its colour-related characteristics.

The colourgram approach has been successfully applied for the automated solution of several colour-related issues concerning food industry, among which the quantification of defective maize kernels, related to the presence of mycotoxins [22], the detection of red skin defect of raw hams [23], the quantification of *Lactobacillus* in fermented milk [24], the prediction of the compositional and sensory characteristics of pesto sauce [25] and the classification of different pesto brands [21].

In this study, after an exploratory analysis of the colourgrams matrix by PCA, multivariate calibration models were developed using a feature selection/calibration algorithm, namely interval-Partial Least Squares (iPLS) [26]. The purpose was to define the correlation between the images of must spots and a series of twelve parameters related to the phenolic composition of the samples. The use of iPLS was particularly profitable for identifying the colourgram features related to the changes in colour and phenolic composition of must samples during the grape berries ripening.

Moreover, by using a proper algorithm [23] the selected features were displayed in the original image domain, allowing to evaluate the relevant colour features automatically selected by iPLS.

2. Materials and methods

2.1. Samples

In this study, three Italian purple grape varieties were considered: *Ancellotta* (A), *Lambrusco Marani* (L) and *Malbo Gentile* (M). The samples were collected in Reggio Emilia (Italy) during vintage 2015.

For each variety, grape sampling was conducted on three grapevines (field replicates) in order to account for the vineyard variability. More in detail, about 100 individual grape berries per grapevine were randomly gathered for each one of 5 subsequent harvest times (T0, T1, T2, T3, T4) at about 10-day intervals, starting from veraison and ending at harvest of the mature grapes. Therefore, 45 grape samples were collected on the whole, resulting from (3 grape varieties × 3 field replicates × 5 harvest times) and were immediately carried to the laboratory under refrigerated conditions. Each grape sample was crushed into a falcon tube under nitrogen atmosphere to prevent the oxidation of phenolic compounds. The crushed berries were left to macerate for 60 min at 4 °C in the dark, then centrifugation at 4000 rpm for 15 min was performed (refrigerated centrifuge 4237R, ALC, Cologno Monzese, MI). The supernatant –called "must" from here onwards– was divided into two different aliquots to perform replicate determinations.

Each aliquot was stored at − 20 °C and unfrozen just before spectrophotometric and chromatographic analyses, that were performed in parallel with image acquisition. All the analyses were replicated twice for each must sample: in the first measurement session the acquisition order was randomized, and the first aliquot of each sample was analysed. Then, the order was shuffled again and the second aliquot of each sample was analysed. The overall number of analyses was therefore equal to 90 (3 grape varieties × 5 harvest times × 3 field replicates × 2 analytical replicates).

2.2. Determination of parameters related to phenolic ripeness

The following twelve parameters were measured by means of spectrophotometric and chromatographic assays:

- total flavonoids content (TF) was determined by UV spectroscopy as reported in the literature [27]: after a proper dilution, the absorbance of the sample was measured at 280 nm and TF was expressed as mg of (+) catechin/L. All the UV–Vis measurements were performed by means of a Perkin Elmer Lambda 650 spectrophotometer using a 10 mm quartz cuvette as sample holder. Before spectrophotometric analysis the samples were diluted 50 times in hydrochloric acid-ethanol solution (ethanol:H2O:HCl 70:30:1 v/v/v);
- total anthocyanins content (TAnt) was determined by UV–Vis spectroscopy according to the method described in [28]: the absorbance value was measured at 540 nm and TAnt was expressed as mg of oenin chloride/L;
- colour index (CI) was calculated as the sum of the absorbance values measured at 420 nm (corresponding to a yellow-orange sample colour), at 520 nm (corresponding to a red-purple sample colour) and at 620 nm (corresponding to a blue sample colour) [29]. In oenology, CI is used to evaluate the colour of red wines: if a wine needs a colour correction it is blended with a different wine up to the desired CI value;
- optical density values (OD420%, OD520%, OD620%) defined as the percentage contribution of each absorbance, at 420, 520 and 620 nm, to the colour index [30];
- tonality (Ton) was calculated as the ratio between the absorbance values measured at 420 nm and at 520 nm. In oenology, Ton is a parameter frequently used to assess the oxidation of wine during aging [30]. In this case, Ton is used as a parameter suitable to describe the colour variation which occurs during grape ripening;
- the five major anthocyanins in the form of 3-O-monoglucoside, i.e. malvidin (Mv-3-glc), petunidin (Pt-3-glc), peonidin (*P*n-3-glc), delphinidin (Df-3-glc) and cyanidin (Cn-3-glc), were separated and quantified by reverse phase-high performance liquid chromatography with a diode array detector (RP-HPLC-DAD) following the chromatographic method described in [31] and adjusted to our equipment, as reported by Vasile Simone and co-authors [32]. The concentration of the anthocyanins was determined by measuring the absorbance at 520 nm by Total-Chrom Workstation version 6.2.1 chromatography system software (PerkinElmer, Inc.), and was expressed as malvidin-3-O-glucoside equivalents.

2.3. Image acquisition

From each aliquot of must sample obtained as described in Section 2.1, 50 µl drops of must were deposited on A4-sized sheets of white absorbent paper. For the deposition of the must drops, a precise scheme was used: 8 drops of each must sample were put on each sheet of absorbent paper following a 4 × 4 chessboard scheme, alternated to 8