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Prediction models for assessing anthocyanins in grape berries by fluorescence sensors: Dependence on cultivar, site and growing season

Patrizia Pinelli[a,*], Annalisa Romani[a], Elisa Fierini[a], Giovanni Agati[b]

[a] DiSIA – Department of Statistics, Computer Sciences and Applications – PHYTOLAB Laboratory, Scientific and Technological Pole, University of Florence, Via Ugo Schiff, 6, 50019 Sesto Fiorentino, Firenze, Italy

[b] Istituto di Fisica Applicata "Nello Carrara" IFAC, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Firenze, Italy

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ABSTRACT

Fluorescence sensors are useful tools for the non-destructive assessment of grape berry anthocyanins. The Multiplex (Mx) sensor here studied provides two anthocyanin indices: $ANTH_R$ = log(1/Chl-fluorescence_R) and $ANTH_{RG}$ = log(Chl-fluorescence_R/Chl-fluorescence_G), based on the chlorophyll (Chl) fluorescence excited with red (R) and green (G) light. These indices were calibrated against wet chemistry. The dependence of anthocyanin prediction models on cultivar, season and site was studied on four cultivars in two Italian regions during three consecutive years. The 2010 global model (all cultivars at both growing sites) gave relative prediction errors on anthocyanin content less than 14.1% ($ANTH_R$) and 19.0% ($ANTH_{RG}$). The $ANTH_{RG}$ was independent of season, maintaining a relative error of about 20% in both 2011 and 2012. In field applications of the calibrated Mx, it showed its ability to detect inter-plot and inter-season differences on both growing sites.

1. Introduction

It is now well accepted that a premium wine trait depends on the quality of the grapes used to produce it, and an important parameter that lends credibility to this premise is the colour of the wine. Anthocyanins (Anths), the red pigments of the berry skins that define the colour of wines, are good indicators of the so-called phenolic maturity of grapes. Phenolic maturity can be assessed by measuring either total phenolics or skin anthocyanin content, which is closely correlated with total phenolics (Kennedy, Matthews, & Waterhouse, 2002). In most red grape varieties, Anths are located in the berry skin and accumulate, starting from véraison, until the grapes ripen fully (Boss, Davies, & Robinson, 1996). In addition to technological maturity, i. e. the acidity and sugar content of berries, phenolic maturity has now become the main concern of viticulturists and oenologists in planning harvest time and in choosing the most appropriate oenological techniques. Therefore, an accurate determination of the Anth content in the berries is fundamental.

This is routinely performed by using destructive 'wet chemistry' procedures, which are costly and time-consuming. Conversely, the non-destructive evaluation of the optical sensors can be extremely advantageous on the large biological variability of grape-berry Anths. In fact, a large number of samplings representative of the whole crop can be measured directly in the vineyard within relatively short time periods. Furthermore, the maturity process can also be followed on the same bunches during the entire season, by repeating the optical measurements at a high frequency. For this purpose, the Multiplex® sensor (FORCE-A, Orsay, France), a no-contact, hand-held optical device, has been developed (Cerovic et al., 2008). It measures Anths in an indirect way that is based on the interference effect exerted by anthocyanin absorbance over chlorophyll fluorescence excitation in the green and red spectral regions (Agati, Meyer, Matteini, & Cerovic, 2007). The sensor provides two Anth indices: the $ANTH_R$ based on a single signal, the far-red chlorophyll fluorescence under red excitation, also called the FERARI (Fluorescence Excitation Ratio Anthocyanin Relative Index) index (Ben Ghozlen, Cerovic, Germain, Toutain, & Latouche, 2010) and the $ANTH_{RG}$ based on two signals, i.e. the ratio between far-red chlorophyll fluorescence under both red and green excitation.

Several applications of the Multiplex (Mx) sensor directly in the vineyards, for the manual determination of Anth evolution in a large

Abbreviations: Mx, multiplex; Chl, chlorophyll; $ANTH_R$, log(1/Chl-fluorescence_R); $ANTH_{RG}$, log(Chl-fluorescence_R/Chl-fluorescence_G); R, red light; G, green light; Anths, anthocyanins; CdG, Casale del Giglio; CB, Castello Banfi; ME, Merlot; PV, Petit Verdot; SH, Syrah; CS, Cabernet Sauvignon; GDD, growing degree-days; RF, red fluorescence; FRF, far red fluorescence; LED, light emitting diode; HPLC, high performance liquid chromatography; RMSEC, root mean square error of calibration; RMSEP, root mean square error of prediction; RE, relative error; SD, standard deviation; $Anth_g$, anthocyanin concentration expressed per berry mass; $Anth_m$, anthocyanin concentration expressed per berry skin mass; CUBA, converter of units of berry anthocyanins; RSM, relative skin mass; DOY, day of the year

* Corresponding author.
E-mail address: patrizia.pinelli@unifi.it (P. Pinelli).

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number of bunches, have been reported (Baluja, Diago, Goovaerts, & Tardaguila, 2012a; Ben Ghozlen et al., 2010; Tuccio et al., 2011). It has been used for assessing the spatial variability of grape colour and for correlating this to several agronomic parameters, such as vine vigour and yield (Baluja, Diago, Goovaerts, & Tardaguila, 2012b).

The use of Mx as an on-the-go sensor mounted on a harvester has also been demonstrated (Bramley et al., 2011). Besides determining Anth, the Mx acts as a tool for characterizing the spatial variability of the vegetative status of the vineyard (Diago et al., 2016).

The Mx sensor can also represent a useful device for the winery laboratories as an alternative to UV–Vis spectroscopy colourimetric analyses of grape berries. In fact, there is a growing interest in adopting non-destructive techniques for the evaluation of grape quality, such as NIR hyperspectral imaging coupled with chemometrics (Chen et al., 2015; Nogales-Bueno, Baca-Bocanegra, Rodríguez-Pulido, Heredia, & Hernández-Hierro, 2015; Zhang et al., 2017).

In general, any sensor aimed at being correctly quantitative must be calibrated against chemical determinations of the target molecules.

Previous studies have reported several calibrations of the Mx for Anth estimation in grape berries (Agati et al., 2013; Baluja et al., 2012a; Ben Ghozlen et al., 2010; Bramley et al., 2011; Ferrandino et al., 2017; Tuccio et al., 2011). The Anth levels investigated ranged from 0.7 to 0.8 mg g^{-1} in Pinot Noir, Pinot Menier, Aleatico and Nebbiolo to about 2 mg g^{-1} in Tempranillo, Merlot and Barbera, and up to 2.5 mg g^{-1} in Shiraz and Cabernet Sauvignon. These prediction models were obtained from single-year determinations and differed as a function of the cultivar. Ferrandino et al. (2017) also reported on the dependence of $ANTH_{RG}$ on the cultivar Anth profile.

The morphological differences among grape varieties in size, weight and berry-skin thickness may have a dissimilar influence on the optical signals acquired by the Mx. Significant differences may also occur for the same variety from one winery to another, as they are influenced both by the terroir and by the different cultural practices. Variability among seasons in the meteorological conditions, particularly temperature and dryness, can induce water stress status in the vines, thus affecting berry size and Anth synthesis (Deluc et al., 2009; Ojeda, Andary, Kraeva, Carbonneau, & Deloire, 2002; Tuccio et al., 2011).

For this reason, an evaluation of both the cultivar and the environmental and growing-place effects on the Mx calibration is required. In this paper, the berry anthocyanin concentrations have been correlated with the Mx indices ($ANTH_R$ and $ANTH_{RG}$) in order to build different calibration models for i) individual cultivars, ii) the same cultivar in two different regions, iii) three cultivars on one site and, lastly, iv) all cultivars on two sites. The seasonal robustness of the models was evaluated by means of validation over three successive years. The Mx calibration models defined were then used to estimate Anth non-destructively from in-vineyard Mx measurements. Examples of applications concerning the comparison of Anth among different plots and years of the same cultivar per site, the comparison of Anth for the same cultivar between different sites and years, and the temporal evolution of Anth have also been reported.

2. Material and methods

2.1. Plant material and growing sites

The experiment was conducted over three consecutive years (2010–2012) at the Casale del Giglio (Aprilia, LT, Italy; 41°30′44.0″N, 12°44′44.2″E) and Castello Banfi (Montalcino, SI, Italy; 42°58′49.22″N, 11°23′55.59″E) wine estates. Casale del Giglio (CdG) is located in the Agro-Pontino Valley, 10 km from the Tyrrhenian Sea coast on a flat area (0–20 m of elevation) that is characterized by a maritime climate. Three cultivars from among the 160 ha of vineyards cultivated at CdG – namely, Merlot (ME), Petit Verdot (PV) and Syrah (SH) – were considered. The three varieties were bred using the Guyot or spur-pruned cordon system and the vines spaced 2.2 m × 0.8 m (inter- and intra-row). Two to three plots, which differed as regards clones and soil texture, were selected for each variety: 3A and 20 for Merlot, 30, 7 and 1B for Petit Verdot, and 2B, 6 and 21B for Syrah.

At the Castello Banfi (CB) estate, located in a territory that occupies 2830 ha, 850 ha of which are for specialized vineyards, three red varieties and two to three plots per cultivar were considered. In particular: plots 23.04, 37.05 and 7.04 for cv. Merlot (ME), plots 12.07 and 10.13 for cv. Syrah (SH), and plots 24.09 and 7.03 for cv. Cabernet Sauvignon (CS). The three varieties were bred using the low spur-pruned cordon system and the vines were spaced 3.0 m × 0.8 m (inter- and intra- row). CB is located on an inner hillside (200–600 m a.s.l.) in southern Tuscany. The entire area is characterized by a temperate climate, with high exposure to the sun and to breezes and considerable variations between daytime and night-time temperatures.

The climatic conditions, global radiation, air temperature and rainfall recorded in the two regions during the 2010–2012 seasons are reported as online Supplementary materials (Fig. S1 and Table S1). The heat accumulation index for *Vitis vinifera* L., that is, the growing degree-days (GDD) index, was calculated according to Winkler (1974).

2.2. Optical sensor and indices

The Multiplex® (Mx) fluorimetric sensor (FORCE-A, Orsay, France) is described in detail elsewhere (Ben Ghozlen et al., 2010). It measures fluorescence emitted by chlorophyll in the 670–690 nm red (RF) and 720–780 nm far-red (FRF) spectral regions, under excitation with different light-emitting diode (LED) sources in the UV (375 nm) and visible (blue at 450 nm, green at 515 nm and red at 630 nm). Since the LED sources are pulsed and synchronized with the detection, the sensor is insensitive to ambient light and can be used directly in the vineyard. The wide detection area of the sensor (8-cm diameter) makes it possible for a signal to be acquired from a large area in each bunch. The acquisition time for a single bunch sample is less than 1 s. The collected data, which are visible on a real-time display, are stored on a secure digital card for data elaboration.

The fluorescence indices used in this work are defined as:

$$ANTH_{RG} = \log(FRF_R/FRF_G) \quad (1)$$

$$ANTH_R = \log(1/FRF_R) \quad (2)$$

where FRF_R and FRF_G are the far-red chlorophyll fluorescence signals excited by red and green light, respectively (Ben Ghozlen et al., 2010). Signals were corrected for residual electronic offsets and normalized to a fluorescence standard (blue plastic foil, FORCE-A, Orsay, France).

The selected bunches were extracted and analysed in the laboratory by means of spectrophotometric analysis, in order to evaluate the anthocyanin content. These data were then used to calibrate and validate the Mx optical sensor, as described here as follows.

2.3. Sampling for calibration and in field measurements

Samples of grapes were collected at different stages of ripening, from green to véraison until full ripeness, in order to cover as broad as possible a range of Anths content.

At CdG, the sampling dates were 20 and 26 August, 2, 9, 16 and 23 September for the 2010 campaign, 24 and 31 August, 8 and 14 September for the 2011 campaign, and 23 and 30 August, 12 September for the 2012 campaign. At CB, the sampling dates were 17 and 31 August, 10, 20 and 27 September, 5 and 12 October for the 2010 campaign; 29 August, 6, 12 and 21 September for the 2011 campaign; and 29 August and 11 September for the 2012 campaign.

The samples collected (2–3 bunches) were transported under cool conditions to the laboratory for further analysis.

On the same dates of sampling, in-field Mx measurements on bunches attached to the vines were performed as follows. At the beginning of the 2010 campaign, 2–4 rows per plot, depending on the plot size,