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Application of Proximal Sensing in Viticulture: Comparison of Different Berry State Conditions

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Previous works have analyzed the relation between the total anthocyanins content in fresh grape berries and some fluorimetric indices.
This study aimed to investigate the different fluorescence sensor responses when used to analyze anthocyanins content in grape berries under different conditions: in open field or laboratory, on fresh or frozen berries.
The study was conducted in 2016 in a commercial vineyard, cv. Cannonau, located in Nurra Valley (Sardinia, Italy) during the ripening period. A portable fluorescence-based optical sensor (Multiplex®3) was used to measure the "Excitation Fluorescence Anthocyanin ratio Relative Index" (FERARI) on 12 vines in the field (Field Berries = BF), and on 36 Multiplex plates in laboratory on fresh (Fresh Berries = BSH) and frozen (Berries Frozen = BZ) samples. Analysis were performed using two instrument configurations, CFG 2 and CFG 4.
A statistical correlation between the fluorescence index and the anthocyanins content in berry skin was observed in all treatments. The highest values were obtained for the frozen samples (CFG 2 ρ = 0.89 R2 = 0.80; CFG 4 ρ = 0.89 R2 = 0.80), while the lowest were acquired for BF (CFG 2 ρ = 0.64 R2 = 0:42; CFG 4 ρ = 0.79 R2 = 0.62).
In conclusion, we observed that the CFG4 configuration on frozen fruits gave the best agreement between FERARI index and total anthocyanins content in Cannonau berries.

1. Introduction

During the last years, several authors have studied precision viticulture, both in Australia and in Europe (Aho JE., 2002; Bramley et al., 2003, 2004). The high heterogeneity of vineyards is caused by the pedo-morphological characteristics, cropping practices and seasonal weather (Bramley, 2003). This environmental variability causes different vine physiological responses that may influence grape quality (Smart, 1985).
Non-invasive techniques, such as hyperspectral imaging (Fernandes et al. 2011) and near infrared spectroscopy (Ferrer-Gallego et al. 2011), were recently used to evaluate the anthocyanin content of grapes in the laboratory. The anthocyanin content of the berries is an important element in viticulture, especially for choosing the correct harvest time. Generally, the berries anthocyanin content was measured using destructive 'wet chemistry' procedures (Glories 1984, Iland et al. 2004), which are time-consuming and labour-intensive.
To manage the vineyard variability (Calderon-Orellana et al., 2014) as better as possible, precision viticulture provides several support tools (Baluja et al., 2012; Bramley et al., 2011; Ferrandino et al., 2010).
During the last years, several instruments were developed in order to monitored the fruit quality (Matese and Di Gennaro, 2015). The fluorimetric probe called Multiplex3® (Force-A, Paris, France) is an optical portable sensor successfully used for the non-destructive and fast monitoring of grape maturity (Agati et al., 2013) that finds large application in precision viticulture (Bramley et al., 2011).
In order to measure the anthocyanin content of the grape skin indirectly (Agati et al., 2007), a rapid and non-invasive techniques have studied the fall in the chlorophyll content of the fresh and preserved grapes (DeEll et al., 1999; Kolb et al., 2006) and developed a specific index (FERARI - Fluorescence Excitation Ratio Anthocyanin Relative Index) to reveal that correlation.

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Several authors found a good correlation with the anthocyanin content in the peel of red grape and FERARI index (Tuccio et al., 2011; Baluja et al., 2012, Gambella et al., 2016). However, few authors have analyzed the differences between the measurements in field and in laboratory (Cerovic et al., 2008; BenGhozlen et al., 2010a,b; Bramley et al., 2011), or in field and in winery (Diago et al., 2013). No contribution was found about the comparison between different matrix conditions.

To improve knowledge of the real potentialities of Force A, we carried out a study to investigate the different fluorescence sensor responses when used to analyze anthocyanins content in grape berries under different conditions: in open field and in laboratory, on fresh and frozen berries. All measurements were conducted in duplicate, using the two instrument configurations, trying to figure out which configuration could better explain the natural field variability.

2. Materials and Methods

The study was carried out in a private vineyard, cv. Cannonau, in North Sardinia (Italy) in 2016, during the ripening period. The Multiplex3®, a fluorimetric sensor developed by Force-A (Orsay, France), was used for non-destructive optical measurements.

The instrument used for this study was described in detail in Ben Ghozlen et al. (2010a). Briefly, Multiplex3® has four LED-matrix light sources: 373 nm (Ultraviolet, UV), 470 nm (Blue, B), 516 nm (Green, G) and 635 nm (Red, R) and three synchronized detectors for fluorescence recording: yellow (YF), red (RF) and far-red (FRF). The LEDs pulse sequentially at 240 Hz with 45 ms per flash, then each field, hand-held, or laboratory measurement is composed by a set of 250 flashes of the four colors (UV, B, G, and R).

The sensor calculates automatically a set of chosen indices as combination of florescence signals, and stores on an SD card the mean and standard deviation of the 250 flashes, both for the signals either for indices.

In this work was used a mask with 8cm diameter surface (50 cm^2) and a distance of 10 cm between the detector and the sample.

For the subsequent analysis was chose the FERARI index that have been successfully correlated with the anthocyanin content in grapes (Cerovic et al. 2008; Ben Ghozlen et al. 2010b). The fluorescence index used in this work is defined as the logarithm of the far-red fluorescence excited by red led:

$$\mathbf{FERARI = \log (5000/FRF\_R)}$$

The study was carried out in three different steps:

- 12 bunches were measured in vineyard (Field Berries = BF),
- 12 samples (mean of 3 plate/bunch) were measured, the same day, in laboratory on Multiplex plate (Fresh Berries = BSH),
- 12 BSH samples (mean of 3 plate/bunch) were stored at -18° C and were measured, 36 h after, in laboratory on Multiplex plate (Berries Frozen = BZ)

In other hand, the same sample was measured three times: in open field (BF), on fresh matrix (BSH) and on frozen matrix (BZ) (Table 1).

Table 1: Experimental design adopted

Matrix	Physic state	Location	Sample (n°)	N° Multiplex plate
BF	Fresh	Field	12	-
BSH	Fresh	Laboratory	12	36
BZ	Frozen	Laboratory	12	36

For each step, the samples were investigated both with Configuration 2 (CFG 2) either with Configuration 4 (CFG 4). The CFG 2 and CFG 4 are typical for unripe fruit outdoors and mature fruit with low level of chlorophyll, respectively. The results of these configurations were statistically compared in order to indicate the best Multiplex setting for a low anthocyanin content variety as Cannonau.

After that, a spectrophotometry analysis was used to evaluated the amounts of total anthocyanins, according to Di Stefano and Cravero (1991) method, with ultraviolet absorption measured at 700 nm and 520 nm.

3. Results and Discussion

The statistical analysis has shown that, working on Cannonau variety, configuration CFG 2 is different from configuration CFG 4.

The relationship between the FERARI index and the total anthocyanins content (mg/L) for cv. Cannonau was explored through the Pearson coefficient. Determination coefficient was used to analyze the relation between the variables.
The comparison of the performances obtained with CFG 2 and CFG 4 on the different berries state conditions were reported in Table 2.
Even though Pearson correlation coefficients and coefficient of determination of BZ samples have showed the same results for both configurations, BF and BSH have revealed differences both in r than in R^2 coefficients. Particularly, the relationship between FERARI index and anthocyanins content obtained in BF, has shown the lowest values. That result display the strength relationship between the two variables considered from time to time, in BZ, in both configurations (r=0.89), and in BSH in CFG 4 (0.84). The weakest relation was detected on BF in CFG 2 (r = 0.64). Working on cv. Cannonau, Mercenaro et al. (2016) obtained similar results using the CFG 2.

Table 2: Pearson correlation coefficients ($p \leq 0.05$) and coefficient of determination R^2($p \leq 0.05$) between FERARI index and total anthocyanins content in BF, BSH and BZ for CFG 2 and CFG 4, respectively.

statistic	CFG 2			CFG 4		
	BF	BSH	BZ	BF	BSH	BZ
r	0.64	0.70	0.89	0.79	0.84	0.89
R^2	0.42	0.50	0.80	0.62	0.71	0.80

As regards the measure of goodness of the representation of the data using the regression line, the worst result was shown by BF in CFG 2 (R^2=0.42). At the opposite, the BZ samples have revealed the best performance (R^2=0.80), both in CFG 2 either in CFG 4.
These relationships between the color berries skin and the chemical analysis can also be seen in Figure 1, Figure 2, and Figure 3, in which were shown the distribution of the measured FERARI values and the total anthocyanin content extracted in BF, BSH and BZ.

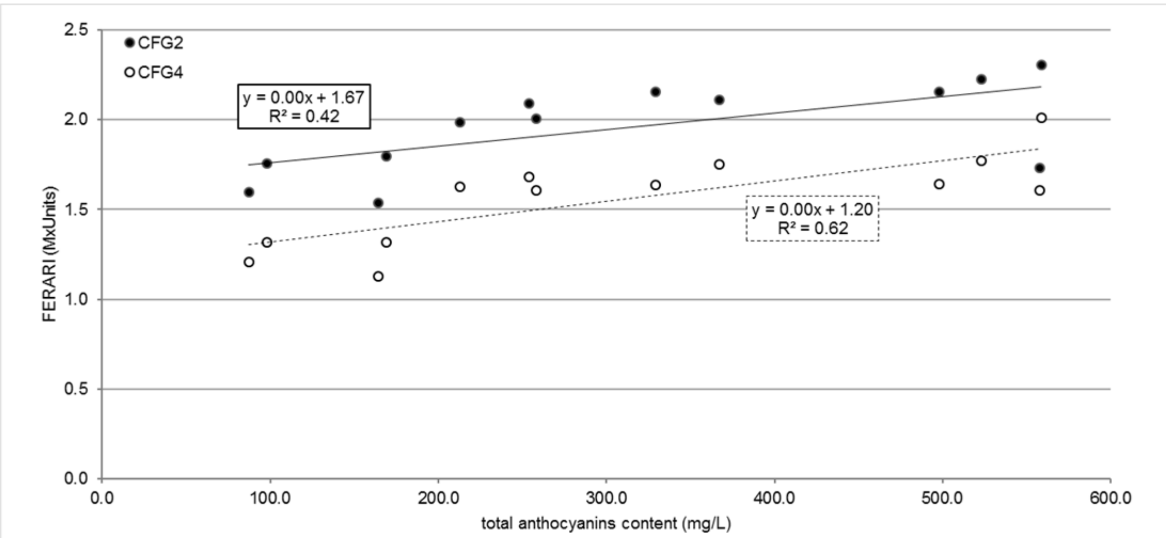

*Figure 1: Regressions between FERARI index of BF and total anthocyanins content in cv. Cannonau berries with CFG 2 and CFG 4, respectively. (*p ≤ 0.05)*

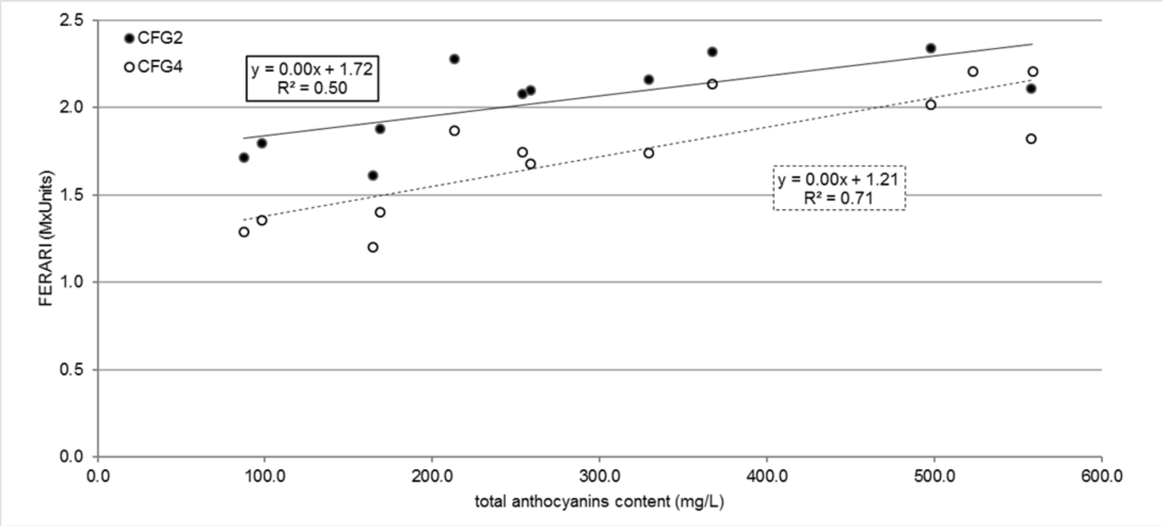

*Figure 2: Regressions between FERARI index of BSH and total anthocyanins content in cv. Cannonau berries with CFG 2 and CFG 4, respectively. (*p ≤ 0.05)*

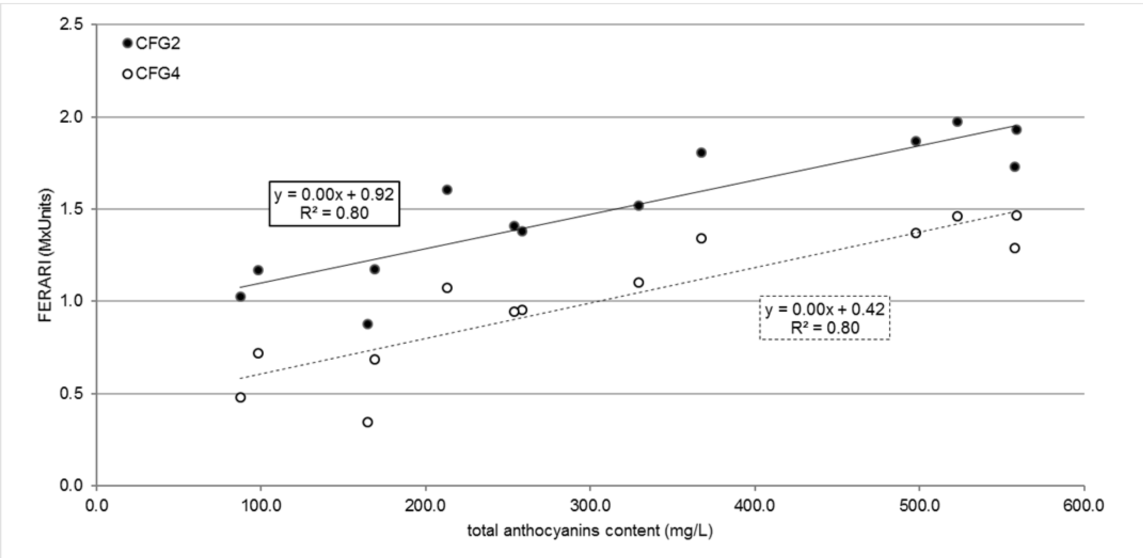

Figure 3: Regressions between FERARI index of BZ and total anthocyanins content in cv. Cannonau berries with CFG 2 and CFG 4, respectively. (p ≤ 0.05)

As observed, in whole cases the CFG 2 have displayed values higher than CFG 4.
Despite the berries conditions, that is fresh or frozen, several authors have reported positive correlation between FERARI and anthocyanins content using different model, such as linear equations (Ben Ghozlen et al., 2010a) or second level polynomial ones (Baluja et al., 2011), obtained from observations of French cultivars (Pinot Noir and Meunier) or exponential function for Graciano and Tempranillo variety in Spain (Diago et al., 2013).
In Sardinia, Gambella et al. (2016) have proposed a logarithmic curve for Cagnulari and Monica varieties. The multiple regression method was also adopted in Valentini et al. (2010) to describe the relationship between Multiplex indices and total anthocyanins content in Sangiovese berries.
Differences between field and laboratory measurements on fresh berries were similarly underlined in Cerovic et al., (2008), Ben Ghozlen et al., (2010a,b), Bramley et al.,(2011), and in Diago et al., (2013).
The discrepancies between the values obtained in open field and in laboratory could be explained by the distance between the instrument and the sample and the spatial distribution of berries.

Actually, whereas the measurement in laboratory is quite stable, in open field the environmental factor may affect the final results (the bunch don't fill all the 50 cm^2 area of measurement) (Ben Ghozlen et al., 2010a).

4. Conclusions

Several studies have analysed the relationship between FERARI index and the anthocyanins content, in this work was examined how the berries conditions, fresh or frozen in open field or in laboratory, may influence this link.

Despite the agronomic knowledge in relation to the analysis on fresh matrix, preferably in field, in this study was demonstrated that the best correlations and fitting on linear model for cv. Cannonau was obtained with the Multiplex CFG 4 on frozen berries (BF).

Results of this study confirm the potential usefulness of proximal sensing as non-invasive and rapid technique to assess key attributes of berries composition. In fact, the anthocyanin concentrations can be determined in a non-destructive way in fresh and frozen berries, after the calibration and validation of the relationship between the instrumental signal considered and the chemical analysis.

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