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BRIEF RESEARCH ARTICLE

Image-based Appraisal of Woody Starch Reserves in Grapevine

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Abstract Maintaining stable yield in a climate change scenario requires the implementation of adaptive strategies for vine and vineyard management. Knowing the level of starch stored in perennial vine organs may help to reduce the risk of environmental constraints. Therefore, the development of decision support tools is important to assist winegrowers. The aim of this work was to test the possibility of estimating the starch content in grapevine woody tissues using digital imaging analysis. A colorimetric index (CI) based on red (*R*), green (*G*), and blue (*B*) mean pixel values was proposed and compared with an existing spectrophotometric index (SI). Shoots of three grapevine cultivars were collected during the 2020 dormant season and forced to produce new rooted cuttings. From these cuttings, a total of 73 internodes were sampled and their woody sections stained with Lugol's iodine solution and CI and SI determined. In the three varieties, the CI well correlated with the SI (Spearman's test ranged from − 0.84 to − 0.93, $p < 0.0001$). The resulting CI-based model developed can accurately ($R^2 = 0.84$) predict the SI confirming it is a reliable method for the digital determination of woody reserves in grapevine, supporting management decisions in viticulture.

Keywords Colorimetric index · Decision support system · Lugol's iodine solution · Organic carbon reserves · RGB · Staining

Introduction

Grapevine is a deciduous perennial species cultivated in a wide range of climatic conditions affecting phenological stages including the start and duration of the growing season, harvest, and leaf senescence time [9, 14]. In addition, the internal cycle of the vine, as influenced by climatic conditions and extreme events (e.g., heat waves, prolonged drought), further impact the timing of phenological stages [21].

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Grapevine stores organic carbon reserves through starch accumulation in woody tissues (roots, trunk, branches, and canes), which are fundamental for vegetative and reproductive growth during the following spring [4, 15, 22, 27, 32, 33].

The annual dynamic of woody starch reserve concentrations follows a known pattern with a maximum level in winter which progressively declines toward the minimum in mid-summer; after that, the level of reserves recovers toward the maximum [30].

During the growing season, vines could be exposed to water deficit and thermal stresses, potentially decreasing the current- and next-year yield and also influencing the reserves cycle [5, 18]. Furthermore, mild winters, which are increasingly occurring due to climate changes, induce an early relief of dormancy and, in turn, an anticipated mobilization of reserves and vine growth, increasing the frost susceptibility of vines in springtime [3, 7, 19–21].

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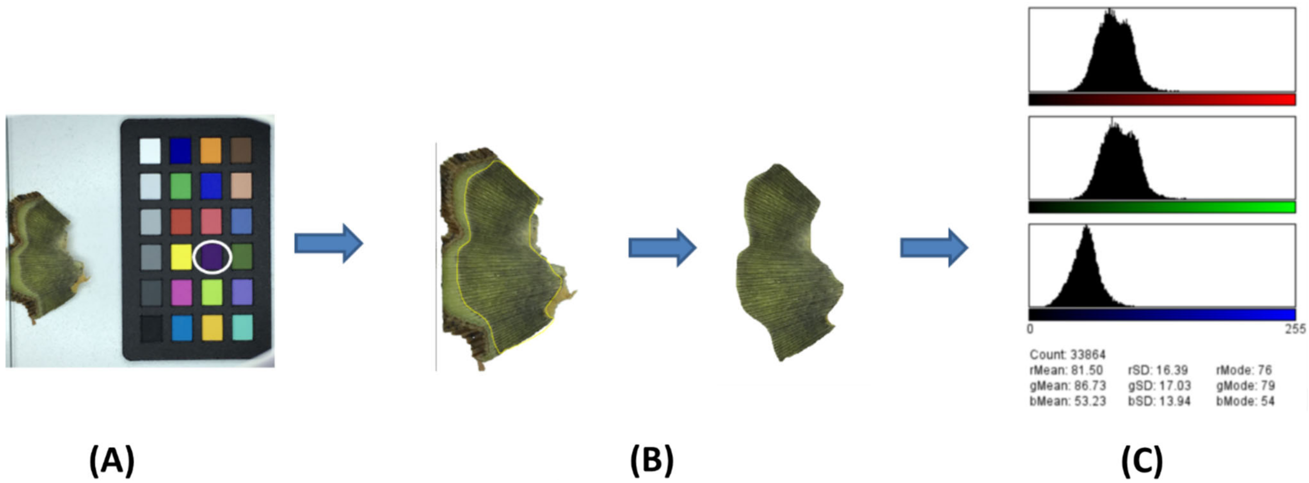

Fig. 1 Workflow of the image analysis: **A** imaging of the woody section, **B** segmentation of the woody object and of the Lugol stained area (edged in yellow), **C** extraction of the R, G, and B mean values. The R, G, and B values of the "purple" square (white circle in the color checker card) were used as references in Eq. 2 (color figure online)

The knowledge of starch levels in woody tissues would support some adaptive management practices (e.g., the timing of winter pruning, canopy, and soil management), minimizing yield, and vegetative growth oscillations [10, 21]. Therefore, reliable, high-throughput and fast methods for woody starch estimations are desirable [6]. Recently, some methods based on NIR and reflectance spectroscopy have been released for starch estimation in grapevine woody tissues [6, 25]. Nowadays, image-based approaches are emerging to easily identify specific plant traits (e.g., plant water status, sugar accumulation) [2, 24], however, the use of imaging for starch reserves appraisal has not been adequately explored.

Therefore, considering that starch might be traced through colorimetric indicators to extract information (e.g., ripening stage in apple fruit [8]) and that the reflectance spectroscopy method accurately predicts starch levels [25], this work examined the possibility of set-up an image-based colorimetric index consistent with the spectrophotometric one, creating the basis for a comprehensive method linking starch reserves quantification to imaging.

Materials and Methods

The experiment was carried out in the 2020 dormant season, about 20 one-year shoots of Primitivo, Negroamaro, and Aglianico grapevine cultivars were collected in commercial vineyards (South Italy, Basilicata, and Apulia Region). These cultivars were selected because of their different ripening time. The cultivar Aglianico is a late ripening variety (end of October) in one of the most cultivated in Apulia, Basilicata, and Campanian area; Primitivo is an early ripening variety (late August-early September), and Negroamaro is an intermediate ripening variety (late September-early October). These two latter varieties are mainly cultivated in Apulia and Basilicata [11].

At the sampling time, a group of shoots (about 30% of total) were immediately used for woody section preparation and analysis (see below). The remaining shoots were forced, at room temperature, to produce rooted cuttings by immersing their base in tap water. To keep the cuttings in good health, we replaced the water daily, and at the same time, we washed the base of the cutting with running water. Cuttings progressively and differentially sprouted out, generating new roots, and growing shoots during the following three months. At the end of this period, the shoots were used for woody section preparation and analysis as follow.

From the mid part of each shoot, a three-bud-long segment was collected and used to prepare a woody section for paired colorimetric and spectrophotometric analysis. For each segment, an internode was used to prepare a thin woody section of about 4 cm^2 area using a penknife for a total of 73 sections to be used for the colorimetric and spectrophotometric analysis [25]. The sections were manually stained with Lugol's iodine solution. The reflectance spectrum of each section was analyzed using a Jaz system spectrophotometer (Ocean Optics, B.V., Dunedin, USA) as described in [25] before (t_0) Lugol's iodine solution application and after its reaction (t_1). The Spectrophotometric index (SI) was calculated following the formula:

$$\text{Spectrophotometric index (SI)} = \frac{R_{t1(900)}}{R_{t1(555)}} - \frac{R_{t0(900)}}{R_{t0(555)}} \quad (1)$$

where R corresponds to the reflectance value at 555 and 900 nm wavelengths of the spectrum measured on woody tissues at time t_0 and t_1.

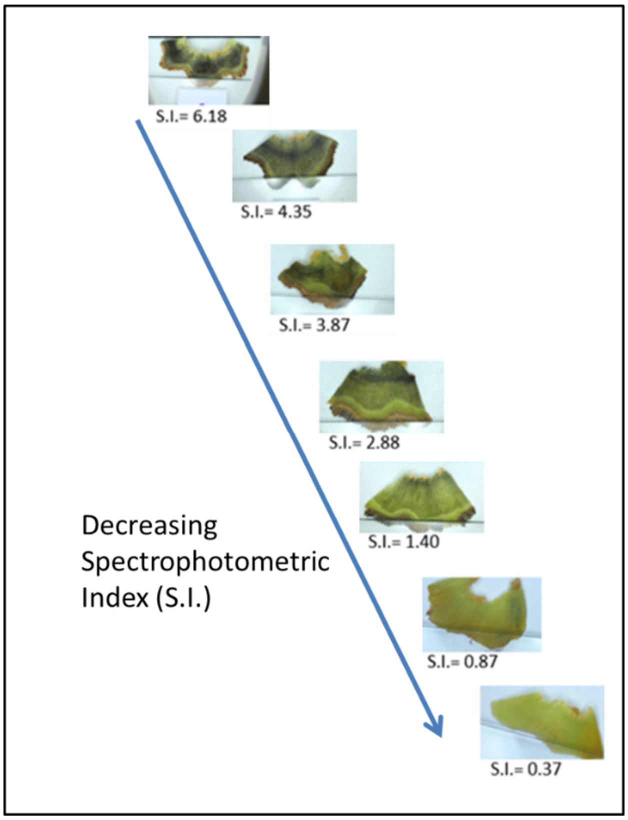

Fig. 2 Images of some sections pictured after the reaction of the Lugol's iodine indicator solution along with their corresponding spectrophotometric index. Note that sections unstained after the Lugol's reaction had the lowest SI because of the conceivable lack of starch

Three spectra readings per section were collected at different points of the section before the Lugol's solution application (t_0) and averaged. Then, after Lugol's solution application and reaction (if any) three single spectra were measured per section. The three spectra values per section were used as input in Eq. 1, and the same mean spectrum value measured at t_0 was used to calculate the spectrophotometric index. The three SI values calculated per each section were then averaged and assigned to the section.

After the reflectance measurement following the Lugol's reaction, each sample was pictured using a tablet (iPad Pro, Apple, equipped with a 12 Mpixel RGB camera) positioned at about 15 cm distance from the sample. A reference color chart (ColorChecker Classic Nano, x-rite PANTONE®, 24 × 40 mm) was included in each photograph for white balance (Fig. 1). To avoid shades, a ring-shaped lamp was used to illuminate the sample from the top. Images were processed using the open-source software ImageJ [23].

In each image, the area of the woody section corresponding to the parenchyma rays (see the yellow-edged part of the section in Fig. 1) was segmented and processed to extract the red (R), green (G), and blue (B) mean values. The R, G, and B coordinates of the "Purple" painted square of the ColorChecker (see white circle in Fig. 1a) were used as reference. The Colorimetric index (CI) was calculated using the formula:

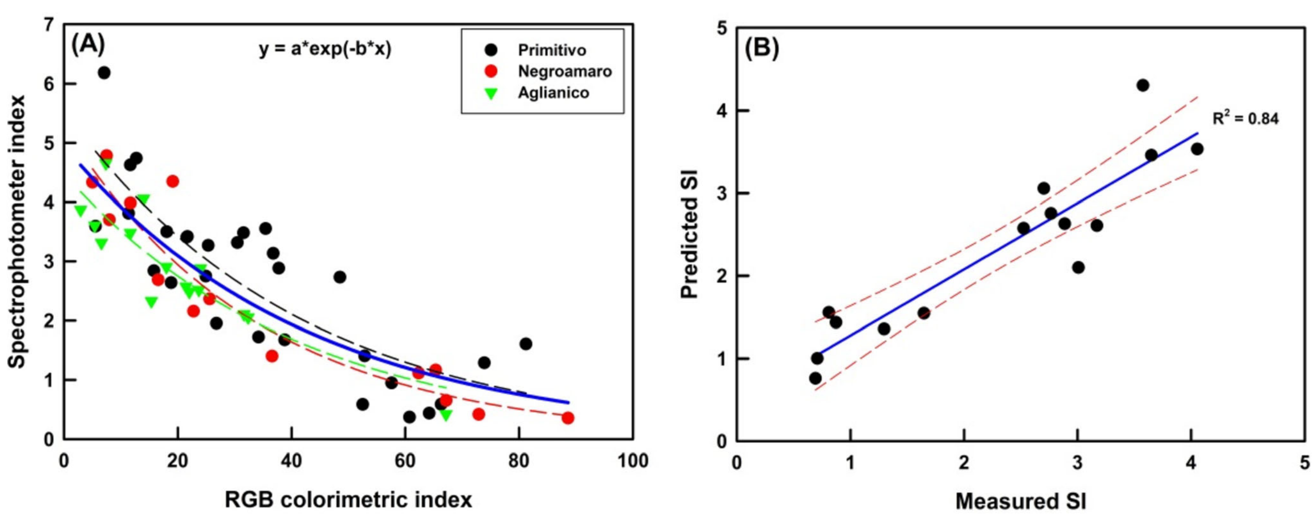

Fig. 3 **A** scatterplot of RGB colorimetric vs. spectrophotometric index determined in woody sections. Note that the dashed fitting curves relate to the model separately parametrized over each variety. The bold continuous fitting curve relates to the model parametrized over the pooled data of all varieties. The parameters of the fitting models are reported in Table 1. **B** Correlation between measured and predicted spectrophotometric index (SI). The measured SI data were the test fraction of the dataset not used for model training. Dashed lines in panel B represent the 95% confidence interval around the linear model (continuous line) (color figure online)

Table 1 Values of the parameters (± SE) of the exponential decay fitting model [$y = a*\exp(-b*x)$] retrieved per each cultivar and values of ρ (Spearman's rank order correlation coefficient)

Variety modelled	*a*	*b*	*ρ*	*p*
Primitivo	5.5558 ± 0.5122	0.0242 ± 0.0037	− 0.84	< 0.0001
Negroamaro	5.2785 ± 0.4310	0.0292 ± 0.0046	− 0.95	< 0.0001
Aglianico	4.4847 ± 0.3222	0.0245 ± 0.0044	− 0.89	< 0.0001
All	4.9520 ± 0.2646	0.0235 ± 0.0025	− 0.86	< 0.0001

The number of observations (n) was 29, 14, and 15 in Primitivo, Negroamaro, and Aglianico, respectively

Table 2 Summary of the ANOVA test performed over the three cultivar groups of predicted SI data which were estimated using the decay fitting model parametrized in each cultivar

	Df	Sum Sq	Mean Sq	*F* value	Pr(> *F*)
Cultivar	2	1.25	0.6235	0.322	0.726
Residuals	57	110.34	1.9358		

$$\text{Colorimetric index (CI)} = \left(\frac{R+G+B}{3}\right)_{\text{sample}} - \left(\frac{R+G+B}{3}\right)_{\text{purplereference}} \quad (2)$$

All the dataset ($n = 73$) was randomly split in a training (70%) and test (30%) dataset. The training was used for pattern recognition between RGB-based CI (covariate) and SI (response variable) and for the model parametrization.

In order to test the suitability of an unique model across the various cultivars, the SI and CI data pertaining each cultivar were plotted separately and iteratively fitted using the exponential decay model [$y = a*\exp(-b*x)$] which was parameterized, minimizing the squared residuals (measured values——fitted values).

Following this, a synthetic dataset ($n = 20$) covering the range of CI data across the various cultivars was randomly sampled. Thereafter, each fitting model was used over the same benchmark dataset to produce three populations of predicted SI values. These three populations were then subjected to an ANOVA test. The unique model was validated using the test dataset and its accuracy was evaluated through the coefficient of determination (R^2).

Results and Discussion

In this study, the shoots were sampled during the winter season when the amount of starch stored in the woody organs would be at the maximum [17, 29]. The overall dataset of the SI values is consistent with values reported by [25, 26]. Furthermore, the range of our SI values are even larger than that published in that papers, which would improve the generalization of a predicting model.

In addition, the mean (± SE) spectrophotometric index (SI) measured before forcing was comparable across the cultivar ($p = 0.08$) being 3.21 ± 0.30 in Aglianico, 3.13 ± 0.31 in Negroamaro, and 3.52 ± 0.30 in Primitivo. At the end of the forcing period, the SI recorded was significantly ($p < 0.001$) lower than that measured before forcing and reached 1.35 ± 0.54 in Aglianico, 0.67 ± 0.42 in Negroamaro, and 0.64 ± 0.42 in Primitivo. These last values measured in Aglianico significantly ($p < 0.001$) differed from those recorded in Primitivo and Negroamaro.

The blue-purple color of tissues stained with Lugol's iodine solution (wood sections in this study) was the result of the starch-iodine complex formation, and its topology is putatively related to the starch concentration [1, 12, 13, 16, 25, 31].

The iodine test is commonly used to assess the optimal harvest time in apples and pears [8, 28]. In grapevine, the starch-iodine complex was used by [32] to assess starch localization in roots and canes from dormancy to pea berry size phenological stage.

In this research, the variation of mean R, G, and B pixel values of the area corresponding to the parenchyma rays was examined as the distance from the mean R, G, and B of the reference standard color (see Eq. 2). Figure 2 shows some stained sections with a variable fraction of "blue-purple" induced by the Lugol and the corresponding SI values.

The RGB-image colorimetric index proposed, for the three varieties, was in good agreement with that measured with the spectrophotometric one (Fig. 3A, and Table 1), suggesting the color darkening of the woody section due to the Lugol's iodine reaction is related to its spectral modifications. The resulting values of the parameters of the three models are reported in Table 1. The three exponential decay models have shown significant ($p < 0.0001$) Spearman's rank order correlations of − 0.84, − 0.95, and − 0.89, respectively, for Primitivo, Negroamaro, and Aglianico.

It could be noted that the parameters were comparable anticipating that the related models would also be comparable. The value of the correlation coefficient of − 0.86 ($p < 0.0001$) of the model parametrized over the training data of all cultivars was also comparable to that of other models. In addition, the summary of the ANOVA test (Table 2) over the SI data predicted using the models separately parametrized in each cultivar (not shown), confirms data belongs the same population ($p = 0.726$). Hence, results suggest that a unique model can be used over the various cultivars.

That unique model was validated on the test RGB dataset to predict the SI values. Figure 3B shows that the correlation between measured SI and predicted SI was able to explain up to 84% of the variance of the test data confirming its goodness of fit. Results are difficult to discuss more deeply because of poor data existing on this specific point.

Conclusions

This paper developed an image-based colorimetric index potentially suitable for the estimation of starch reserves in the wood of grapevine. Although efforts are required to link analytical starch concentrations and the RGB-based index proposed, this study revealed that the use of imaging techniques is promising to develop new protocols to aid affordable and fast determination of reserves supporting adaptive management practices within a digital viticulture domain.

Author Contributions VN, LR, and GM contributed to the conceptualization; GM and LR were involved in the formal analysis; LR and DGD contributed to writing—original draft preparation; DGD, VN, GG, GM, and LR were involved in writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Delcarations

Conflict of interest The authors declare no conflict of interest.

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