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Effects of Inactive Yeast Biostimulants on Mechanical and Color Attributes of Wine Grape Cultivars

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Abstract: Background: Biostimulants naturally improve plant growth, stress tolerance, nutrient use efficiency and activate defenses by increasing protective metabolites (phenols, anthocyanins) in grapes. In viticulture, especially when using inactive yeasts, they modulate genetic expression, improve skin resistance, color and aroma profile of wine grapes in line with sustainable practices. Methods: Two wine grape cultivars, Merlot and Cabernet Sauvignon, were sprayed with the inactive yeast *Saccharomyces cerevisiae* in a single treatment in pre-veraison or in a double treatment in pre-veraison and veraison. Berry weight, must, total polyphenols, anthocyanins, mechanical and colorimetric properties were measured on fresh grapes. Results: Two-way ANOVA revealed that TA, pH and TPC were not affected, while mean berry weight and anthocyanin content varied by cultivar, treatment and interaction; TSS differed only by cultivar. Inactive yeasts reduced weight in the single treatment thesis but stabilised it in the double treatment one; anthocyanins decreased in Cabernet Sauvignon but increased in Merlot. Mechanical and colorimetric analyses showed cultivar-dependent responses, with significant improvements in elasticity, skin thickness and hue of berries, especially in Merlot when the treatment was applied twice. Conclusions: Inactive yeasts showed an effect on the weight of the berries, the anthocyanins, the mechanics and the color: Merlot significantly improved skin thickness, elasticity and hue. while Cabernet remained less reactive to treatments.

Keywords: biostimulants; wine grapes; mechanical traits; reflectance spectrum

1. Introduction

The growing attention on sustainable viticulture has encouraged the development of innovative strategies aimed at improving production quality while minimizing environmental impact. The quality and yield of grapevine production are increasingly being compromised by adverse climatic conditions associated with ongoing climate change [1]. Moreover, future climate scenarios are already affecting the timing and duration of grapevine phenological stages [2]. In this context, among the emerging technologies, biostimulants of natural origin are gaining a central role due to their potential to enhance plant development, fruit quality and resistance to abiotic and environmental stress [3, 4, 5], by improving water and nutrient use efficiency [6].

Biostimulants activate plant defence pathways and promote the synthesis of protective metabolites, improving parameters such as phenolic maturation, polyphenols, anthocyanins and stilbenes content in berries, similarly to what observed with MAMPs (microbe-associated molecular patterns) [7, 8, 9]. In particular, it is known that the use of biostimulants in viticulture induces variations in the expression of genes involved in the anthocyanin synthesis pathways, including the Myb family genes and all other genes active in both the early and late phases of biosynthesis, as well as the genes responsible for the transport of anthocyanins into the vacuole [10]. This leads to a direct increase in the anthocyanin content in the berries and the associated colorimetric parameters. These

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biostimulants align with sustainable and organic practices, helping reduce reliance on chemical fertilizers and pesticides, which negatively impact soil biodiversity [11]. Rich in organic nitrogen, amino acids and bioactive compounds, biostimulants improve nutrient availability and uptake [12].

Among the various classes of biostimulants currently used in agriculture Inactive Yeasts (IY) represent a promising tool for the viticulture sector [13, 14, 15]. Derived from fermentation by-products of Saccharomyces cerevisiae cells, IY are subjected to specific treatments that inactivate their vitality while preserving functional components such as mannoproteins, β-1,3- and β-1,6-glucans, chitin, lipids and sterols [7]. Inactive yeasts act as elicitors and retain all the properties of the biostimulants described above. They promote a balanced ripening of the grapes, stimulate the defense mechanisms of the vines and the functioning of the synthesis pathways of secondary metabolites, thus improving the aromatic profile and the volatile components of the grapes and consequently of the wines. [9, 16, 17, 18]. Besides, IY enhance grape quality by improving the mechanical properties of grape berry skins [19]. In particular, they promote skin hardening — as evidenced by increased skin break force — and skin thickening, which can improve the berries' resistance to physical damage and pathogen attacks. The observed increase in berry skin thickness, likely a defence mechanism triggered by IY may also influence the release of anthocyanins during the maceration process. It is important to note that within each grape cultivar, mechanical characteristics — especially skin hardness — are vintage-dependent and correlate with seasonal climatic indices [20]. Furthermore, the effects of IY on the mechanical properties of grape berries are closely linked to their colorimetric properties. Grape color serves as an indicator of anthocyanin content, which is influenced by the texture of the berries and the properties of the cell walls that affect the extraction of anthocyanins during winemaking [21]. Consequently, structural indices derived from mechanical analysis can provide valuable insights for the optimisation of maceration processes and, before that, to protect the grapes from pathogenic attacks when they are dried for the production of passito wines.

In literature, the most common time for the application of biostimulant treatments in vineyards corresponds to the beginning of the phenological phase of veraison, namely the vegetative period during which the accumulation of anthocyanins in skins starts and reaches its maximum around harvest [22]. Usually, two treatments with biostimulants are performed around veraison, the first one at the beginning and the second one approximately after two weeks [7, 23]. Pastore et al. [10] applied the second treatment at an advanced veraison stage, while in other researches the first treatment occurred during the bud burst stage and the next one immediately after flowering [15]. In other studies, treatments were carried out at three phenological stages: bud burst, full flowering and between the beginning of fruit set and the pea size [24]. This variation is due both to the commercial recommendations of the products and to the type of vineyard – whether intended for the production of table grapes or wine grapes – and whether the biostimulant is used alone or in combination with other biostimulants of different origins.

In this study, we investigated the efficacy of IY on different wine grape cultivars through mechanical tests (compression, penetration and skin thickness) and color analysis (CIELab coordinates). This research is in line with the perspective of more sustainable viticulture, which aims to improve wine production through optimized agronomic practices.

2. Materials and Methods

Field Trial

In 2024, samples of grapes of Merlot and Cabernet Sauvignon cultivars were harvested from two contiguous vineyards in the experimental farm of the CREA Viticulture and Enology Research Center in the Arezzo – Tuscany (Italy) (43° 28′ 28.68′′ N – 11° 48′ 46.95′ E, 250 m a.s.l.) when the technological ripeness was optimal for the production of their wines. The vines were grafted onto Kober 5BB in 2013 with planting distances of 3.00 m x 0.90 m, trained in spurred cordon with a number of 20 buds per plant in a sandy loam soil from a non-irrigated, terraced river origin.

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For the experimental trial, a biostimulant formulation based on Inactive Yeast (IY) *Saccharomyces cerevisiae* was administered. Two theses were defined: a thesis subjected to only one treatment in the pre-veraison at DOY 186-190 (T1), while a second thesis has foreseen a first treatment in pre-veraison followed by a second one in veraison at DOY 205-212 (T2). The two theses T1 (one treatment) and T2 (two treatments) were compared with an untreated control thesis (test). Each thesis consisted of 3 replicates with 6 vines in each replicate in a completely randomised block design. Each treatment was applied at a dose of 0.8 kg/ha, distributing a water volume of about 600-1000 litres/ha depending on the development of the plant canopy and spraying all above-ground parts avoiding dripping.

Measurements

At harvest, twelve bunches were randomly harvested and for each experimental replication and thesis. A total of about 200 berries were pooled from each replicate. From this pool, approximately 30 intact berries without skin defects and with complete stems were then selected for each of the three replicates and for each thesis. Medium Berry Weight (MBW), Total Soluble Solids (TSS), Titratable Acidity (TA) and pH were determined according to the OIV official methods.

Mechanical and colorimetric properties (CIELab coordinates) were carried out on the fresh berries. The remaining part of the berries was frozen to allow the analysis of the Total Polyphenols Content (TPC) and Anthocyanins content (ANT).

The mechanical properties of the berries were tested using the Texture Analyser mod.BT1-FR0.5TND14 from Zwick/Roell (Zwick GmbH & Co.Gk – August-Nagel-Straße 11), equipped with a compression load cell with a nominal force of 500 N. The data were recorded with the software TESTXPERT II V. 3.31 in a Windows environment at 500 Hz. The selection of some of the operating conditions of the device for performing the various tests was based as reported by Letaief et al. [25].

Color was measured on 30 whole berries using a CM-5 chromameter (Konica Minolta, Chiyoda, Tokyo, Japan) based on the CIELab color system based on a three-dimensional space defined by: L* axis (lightness), which ranges from 0 (black) to 100 (white), a* axis, representing the red-green spectrum with positive values indicating red and negative values indicating green; and the b* axis, corresponding to the yellow-blue spectrum, where positive values indicate yellow and negative values indicate blue. Furthermore, for each berry the reflectance between the wavelengths 360 nm and 740 nm was recorded.

The mechanical properties of 30 berries per cultivar were measured by a double compression test with a flat cylindrical steel probe with a diameter of 20 mm, up to a deformation of 20% of the original volume of the berry. The waiting time between the first and second compression was 2 s., while the lowering speed of the crossbar was set to 1 mm/s.. The following parameters were measured:

- Hardness (N): maximum force recorded during the first compression cycle;
- Cohesiveness (adim.): Measurement of the strength of the internal bonds that allow the berry to "reform" its structure;
- Springiness (mm): height regained by the berry between the end of the first cycle and the beginning of the second;
- Gumminess (N): energy required to dissolve the berry so that it resembles a semi-solid, deglutible food;
- Chewiness (mJ): Energy required to chew the berry until it is ready to deglutition;
- Resilience (adim.): Ability of the berry to return to its original position after being squeezed.

The penetration test of the grape skin was assessed by placing berries in an equatorial position on a perforated metal platform and a probe with a diameter of approximately 2 mm was lowered at a speed of 1 mm/s until it penetrated the berry skin and reached a depth of 2 mm beyond the surface [25]. The puncture resistance of the skin was recorded in the form of a diagram and processed using MATLAB software (version R2019b). The calculated parameters include:

- Maximum breaking force (FB – Force Break): expressed in Newtons (N), represents the force required to break the skin;

 doi:10.20944/preprints202505.2092.v1

- Energy required for perforation (EB – Energy Break): calculated as the area under the time-deformation curve, between the start of the test (zero force or trigger point, i.e., the point at which the probe touches the grape) and the complete breaking point of the skin (yield point).

Skin thickness (Th) was measured by accurately removing berry skin from the lateral surface using a scalpel and a skin fragments of 25 cm2 was blot with absorbent paper. The prepared skin sample was placed on the metal plate of the device and stretched well avoiding wrinkles. A cylinder with a flat base and a diameter of 2 mm was used to measure skin thickness by means of a descending rate of 0.2 mm/s.. An instrumental release threshold of 0.05 N was set in order to let the probe to fully adhere to the skin sample before data acquisition, to reduce or eliminate the so-called tail effect due to the displacement of the contact point [26]. After the position of the probe was calibrated, the thickness of the skin was calculated by graphical processing using MATLAB software (version R2019b) as the distance between the contact point of the probe with the grape skin (trigger) and the base of the platform during a compression test.

The total phenolic content (TPF) and Anthocyanin content (ANT) of the skins from 120 frozen berries were measured. Berries were peeled and the skins were weighted, dried at 37°C and powdered. 0.5 g of samples powder was incubated overnight in 10 mL of 70% ethanol and 1% hydrochloric acid. Subsequently, the sample extracts were filtered through a 0.45 µm syringe cellulose filter and stored at −20 °C until further analysis. TPC was determined by employing the Folin-Ciocalteu colorimetric method, as delineated by Waterhouse [27]. The reaction mixture was prepared with 1 mL of water, 0.02 mL of sample extract, 0.2 mL of the Folin-Ciocalteu reagent, and 0.8 mL of a 10% sodium carbonate solution. Absorbance was measured at 760 nm following a 90-minute incubation period at room temperature with a spectrophotometer Agilent 8453 (Agilent Technologies, Santa Clara, California). The results were expressed as milligrams of gallic acid equivalent per gram of dry weight, based on a gallic acid calibration curve (50 to 500 mg/L with R2 = 0.998).

ANT was determined using a protocol based on the differential pH method proposed by Lee et al. [28]. Appropriate dilutions of grape extract were mixed with buffers of 0.025 M potassium chloride (pH 1) or 0.4 M sodium acetate (pH 4.5). Absorbance was measured at 520 and 700 nm using the Agilent 8453 spectrophotometric system (Agilent Technologies, Santa Clara, CA). The results were expressed in milligrams of cyanidin-3-glucoside equivalents per gram of dried grape skin (mg Cy/g skin).

Statistical Analysis

The normal distribution of data and their variance were checked by Shapiro -Wilk test and Levene test. Outliers were evidenced by Grubb test. Data were subjected to a two-way ANOVA, in order to verify any significative interaction between the two factors Cultivar × Treatments. Subsequently, a multiple pairwise comparisons by Tukey test post-hoc test was applied to separate means of each variable in both cultivars at a significance level of $\alpha = 0.05$. Finally, mechanical and color parameters were separately subjected to Principal Component Analysis to verify the effect of IY treatments on the variables on the two cultivars. Statistical analysis was conducted by means of Statgraphics Centurion XV version 15 (The Plains, Virginia).

3. Results

Table 1 shows the results of the two-way ANOVA on must and qualitative traits of Merlot and Cabernet S. berries treated with Inactive Yeasts (IY) in pre-veraison (T1) and veraison (T2). Tritatable Acidity (TA), pH and Total Polyphenolic Content (TPC) were neither statistically influenced by the factor Cultivar nor by the factor Treatment nor by the interaction between the two factors. On the contrary, MBW and ANT were influenced by both factors and their interaction, while TSS showed variations only in relation to the factor Cultivar. Given the evident effect of the Cultivar on the parameters considered, we further investigated how the individual variables within each cultivar behaved as a function of treatment level. The table contains the pairwise comparisons of the mean

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values for the analysed variables within each cultivar, which were determined using the Tukey test. In relation to MBW, in both cultivars a loss of berry weight was observed in the T1 thesis, while in the T2 the weight was almost unchanged compared to the control thesis Test. Contrasting results were instead obtained for ANT, which decreased significantly for Cabernet Sauvignon. in T2, while it increased significantly for Merlot, especially in T2. On the other hand, TPCs in Cabernet Sauvignon. were almost unchanged despite the theses, while in Merlot their increase was evident in particular in thesis T1, although T2 also showed an increase, although not significant compared to Test.

Table 2 reports the two-way ANOVA and the Tukey test on mechanical properties. Hardness (H), Chewiness (Ch), Gumminess (G), Force Break (FB), Energy Break (EB) and Skin Thickness (Th) were cultivar dependent, while no statistically significant differences were observed for Cohesiveness (Co), Elasticity (E) and Resilience (R). The effects of the Treatments with IY on the mechanical variables partly overlapped with those of the cultivars, although some discrepancies can be observed in E in particular, which appeared to be highly influenced by the factor Treatment. Moreover, E and Th were influenced by a high interaction between the two factors, suggesting the efficacy of IY on both cultivars in enhancing E and promoting skin thickness (Th). As previously reported, we checked how each singular cultivar behaved as a function of treatment level. In Cabernet Sauvignon the application of IY had an effect on H, E and Th. H and E, in particular, appeared to be compromised in the theses underwent a single treatment, while the thesis with a double IY application kept H unchanged with respect to Test thesis, while E showed a significant increase, which was even higher than in the untreated thesis. of particular interest is FB, which increased after IY treatment, especially for theses with double application T2.

Table 1. Two way ANOVA and means separation by Tukey test of must and qualitative traits of two wine grape cultivars treated with one (T1) or two (T2) Inactive Yeasts applications vs Test (Control) and their interaction.

	Factors			Cabernet Sauvignon			Merlot		
	Cultivar	Treatment	Interaction	Test	T1	T2	Test	T1	T2
MBW (g)	***	***	*	0.78ab	0.73b	0.85a	1.17a	0.95b	1.04ab
TSS (°Brix)	***	n.s.	n.s.	25.0	24.5	25.2	26.9	26.9	25.9
TA (g/L)	n.s.	n.s.	n.s.	4.35	4.62	4.57	3.93	3.99	4.13
pH	n.s.	n.s.	n.s.	3.60	3.52	3.55	3.65	3.62	3.62
TPC (mg GAE/g)	n.s.	n.s.	n.s.	45.88	44.14	42.42	37.87b	40.92a	39.84ab
ANT (mg Cy/g skin)	*	*	*	16.35ab	18.02a	14.79b	16.36b	17.30ab	18.35a

MBW = Medium Berry Weight; TSS = Total Soluble Solids; TA = Tritatable Acidity; TPF = Total Polyphenols Content; ANT = Anthocyanins; * = P≤0.05; ** = P≤0.01; *** = P≤0.001; n.s. = not significant. Different letters indicate significative difference at P < 0.05.

In Merlot Ch and E seemed to be negatively affected by the treatment both in T1 and T2, while the treatments did not show to have any effect on the other parameters. Interestingly, Th was improved in T1 thesis, while returning to values similar to untreated thesis in T2.

Table 2. Two way ANOVA and means separation by Tukey test of mechanical parameters of two wine grape cultivars treated with one (T1) or two (T2) Inactive Yeasts applications vs Test (Control) and their interaction.

	Factors			Cabernet Sauvignon			Merlot		
	Cultivar	Treatment	Interaction	Test	T1	T2	Test	T1	T2
H (N)	***	*	n.s.	3.88a	3.48b	3.85ab	4.59	3.97	3.93
Ch (mJ)	*	*	n.s.	2.69	2.21	2.95	3.93a	2.62b	3.22ab
Co	n.s.	n.s.	n.s.	0.47	0.43	0.5	0.48	0.45	0.49
E (mm)	n.s.	***	***	1.47b	1.27c	1.66a	1.65a	1.36b	1.34b
G (N)	*	*	n.s.	1.83	1.59	1.89	2.31	1.86	2.05
R	n.s.	n.s.	n.s.	0.35	0.34	0.34	0.35	0.34	0.35
FB (N)	***	**	n.s.	0.74b	0.83ab	0.84a	1.16	1.18	1.3
EB (mJ)	***	*	n.s.	0.62	0.73	0.73	1.22	1.24	1.41
Th (mm)	***	***	*	0.18	0.19	0.18	0.20b	0.30a	0.22b

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H = Hardness; Ch = Chewiness; Co: Coesiveness; E = Elasticity; G = Gumminess; R = Resilience; FB = Force Break; EB = Energy Break; Th = Skin Thickness; * = P≤0.05; ** = P≤0.01; *** = P≤0.001; n.s. = not significant. Different letters indicate significative difference at P < 0.05.

Finally, Table 3 reports the two-way ANOVA and Tukey test on CIELab coordinates. They resulted statistically different between the cultivars, with the exception of Chroma (C*), which in turn was influenced only by the treatment. Significant interactions were observed both for C* and Hue angle (h). From the analysis of the effects of the treatments on the single cultivar, they had no effect on the components Lightness (L*), red/green scale (a*) and yellow/blu scale (b*), while C* decreased significantly, especially in the theses subjected to double application T2. At the same time, h*, which remained unchanged between Merlot theses, seemed to be positively influenced to some extent by the treatments themselves

Table 3. Two way ANOVA and means separation by Tukey test of CIELAB coordinates of two wine grape cultivars treated with one (T1) or two (T2) Inactive Yeasts applications vs Test (Control) and their interaction.

	Factors			Cabernet Sauvignon			Merlot		
	Cultivar	Treatment	Interaction	Test	T1	T2	Test	T1	T2
L*	***	n.s.	n.s.	35.53	35.48	33.64	31.19	32.02	31.53
a*	***	n.s.	n.s.	-0.78	-0.77	-0.61	-0.07	0.04	0.02
b*	***	n.s.	n.s.	-4.54	-4.49	-4.15	-3.03	-3.29	-2.93
C*	n.s.	**	***	3.67a	2.00c	2.98b	2.75ab	3.19a	2.35b
h*	*	n.s.	**	266.35b	271.71a	268.69ab	270.4	269.1	274.0

L* = Lightness; a* = red/green scale; b* = yellow/blu scale; C* = Chroma; h* = Hue angle; * = P≤0.05; ** = P≤0.01; *** = P≤0.001; n.s. = not significant. Different letters indicate significative difference at P < 0.05.

With regard to PCA, the mechanical and colorimetric parameters were analyzed separately (Figures 1 and 2). Figure 1 shows the distribution of the combined mechanical profiles, from which it is evident that the first two principal components account for 79.8% of the total variance. In particular, PC1 is predominantly characterized by H, G and Ch, while FB and EB are positively correlated in almost the same way on both PC1 and PC2. Similarly, the variables R, Co and E are positively correlated with PC1 but negatively correlated with PC2. Meanwhile, Th is the only variable that correlates strongly and positively with PC2. All these results illustrate the different behaviour of Merlot and Cabernet Sauvignon cultivars in response to the different IY treatments.

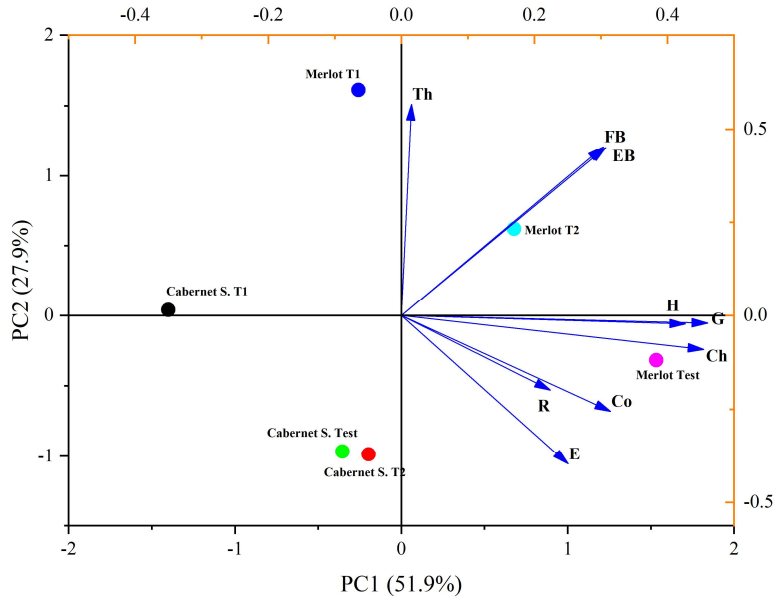

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Figure 1. Principal Componet Analysis based on texturometric parameters of two wine grape cultivars, Cabernet S. and Merlot, subjected to one (T1) and two (T2) foliar spray treatments with Inactive Yeasts at veraison compared with control thesis (Test). H = Hardness; Ch = Chewiness; Co: Coesiveness; E = Elasticity; G = Gumminess; R = Resilience; FB = Force Break; EB = Energy Break; Th = Skin Thickness.

On the one hand, Merlot exhibited better mechanical properties per se compared to Cabernet Sauvignon, regardless of the IY treatments. Moreover, Merlot seems to benefit from the IY treatments, especially in terms of Th, with a more pronounced effect at T1 and a less intense effect at T2, which nevertheless seems to confer greater resistance to skin penetration. On the other hand, as the ANOVA results in Table 1 already show, Cabernet Sauvignon seems to benefit much less from the IY treatments. For this cultivar, a second treatment (T2) seems to be completely ineffective in improving mechanical parameters, as shown by the PCA, where T2 and the Test control almost overlap, although some discrete properties in E are preserved.

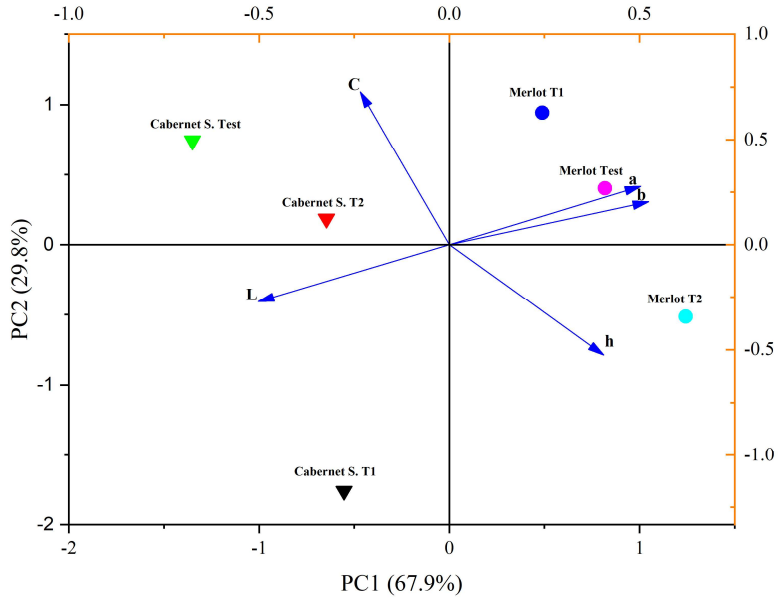

Figure 2. Principal Componet Analysis based on color components of two wine grape cultivars, Cabernet S. and Merlot, subjected to one (T1) and two (T2) foliar spray treatments with Inactive Yeasts at veraison compared with control thesis (Test). L* = lightness; a* = red/green scale; b* = yellow/blu scale; C* = Chroma; h* = Hue angle.

Referring to Figure 2, which shows the PCA of the CIELab coordinates together with the parameters C and h, the different responses of the two cultivars to the IY treatments are confirmed. In general, the two principal components almost completely explain the variability examined. PC1 is positively characterized by a and b and negatively (i.e., in the opposite way) by L. C, on the other hand, seems to be more strongly and positively correlated with PC2.

Overall, Merlot shows a stronger correlation with the parameters a, b and h. The hue (h) seems to benefit the most from T2, as the berries show a tendency towards a bluish hue, in contrast to T1 and the Test control, which look similar and show predominantly reddish hues. Conversely, with respect to C, T1 seems to confer greater color brilliance than T2. It is noteworthy that lightness (L) is generally more strongly associated with Cabernet Sauvignon, regardless of IY treatment. For Cabernet Sauvignon, T1 appears to favor an increase in hue (h), which is then lost at T2, where the profile is again similar to that of the Test control, as previously observed for mechanical properties. The positioning of Cabernet Sauvignon profiles in PCA space, opposite to the direction of the a and b indices, generally indicates that Cabernet Sauvignon berries tend to be lighter, with a color tilt towards blue/green, while Merlot shows a tendency towards yellow/red hues.

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To further investigate the color differences between the two cultivars induced by two IY treatments, an analysis of the individual colorimetric spectra in the wavelength range between 360 nm and 740 nm was performed based on the reflectance measured by the colorimeter.

Figure 3 shows the spectral behavior of the three profiles in Cabernet Sauvignon, where significant differences are observed from 480 nm up to 740 nm in a continuous manner, while in the range between 369 nm and 470 nm — covering the ultraviolet, violet and part of the blue range — no significant differences are observed. These differences occurred mainly in the green, yellow, orange and partially in the red region of the spectrum. Overall, Figure 3 shows an almost identical spectral behavior between the Test control and T1, especially between 480 nm and 670 nm, with differences that are present between 680 nm and 740 nm, but tend to weaken. As can also be observed in the PCA of the CIELAB parameters (Figure 2), there is a greater variability in color between the different treatments for Cabernet Sauvignon than for Merlot. In Merlot (Figure 4), the three profiles are very similar, at least up to 660 nm, with statistically significant differences only occurring between 670 nm and 740 nm.

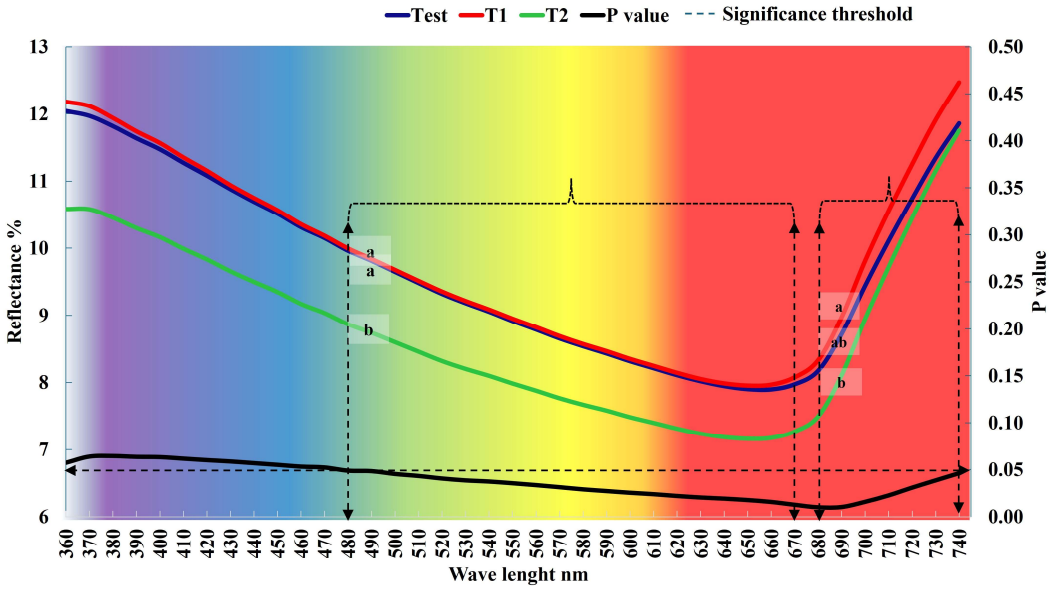

Figure 3. Reflectance wavelength of Cabernet Sauvignon berries subjected to one (T1) foliar spray treatment with Inactive Yeasts in pre-veraison or two treatments (T2) in pre-veraison and veraison compared with control thesis (Test).

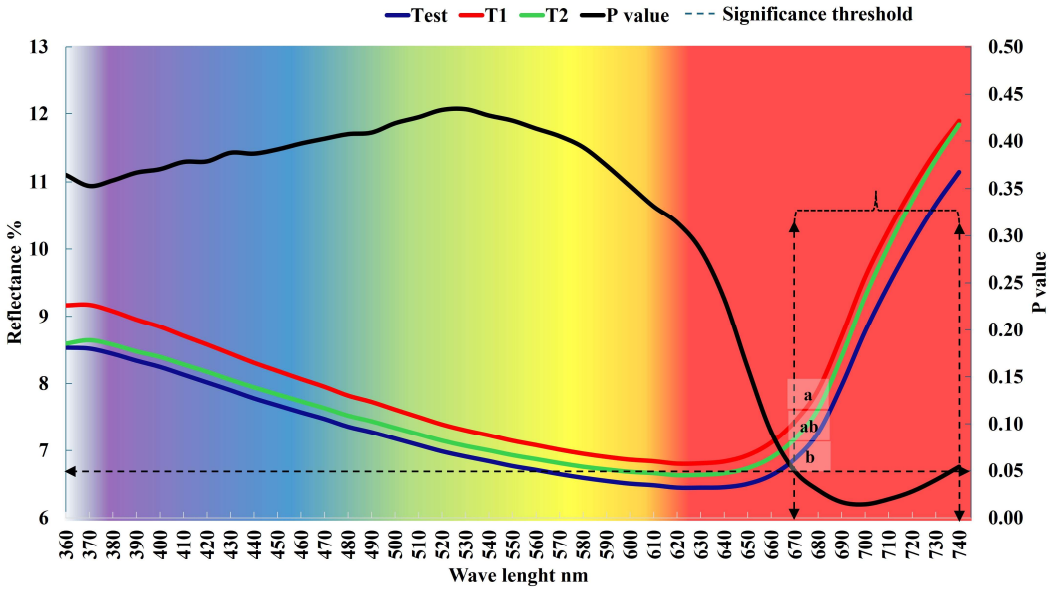

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Figure 4. Reflectance wavelength of Merlot berries subjected to one (T1) foliar spray treatment with Inactive Yeasts in pre-veraison or two treatments (T2) in pre-veraison and veraison compared with control thesis (Test).

4. Discussion

The results indicate that both the grape cultivar and the IY treatment significantly influenced a range of mechanical, colorimetric, and physicochemical parameters in Cabernet Sauvignon and Merlot. Overall, while inherent cultivar differences accounted for many traits, the application of IY treatments produced measurable changes that differed between the two cultivars. Moreover, multivariate analyses provided an integrated perspective by revealing how clusters of variables jointly explained variability, thereby highlighting contrasting patterns between cultivars and among treatment levels.

In relation to must traits, TSS, pH and TA were not affected by IY treatments in both the cultivars considered, consistent with other studies [7, 8, 17, 20], but contrasting with Villangò et al. [23] who reported that foliar spraying significantly affected grape titratable acidity and pH in Syrah, although these effects are often influenced by vintage.

The ANOVA clearly showed that grape cultivar exerted a strong influence on many measured parameters. For instance, significant differences in mechanical variables—such as H, Ch, G, FB, EB and Th, underscored the intrinsic differences between Cabernet Sauvignon and Merlot.

With respect to berry texture, differences between the cultivars are also apparent. Merlot maintained a consistent hardness (H), whereas Cabernet Sauvignon exhibited a drop in hardness at T1 followed by partial recovery at T2, although overall H showed a declining trend post-treatment. This suggests that the parameters of hardness and skin thickness (Th) do not necessarily correlate directly [25].

Furthermore, no significant treatment effects were observed on most other mechanical properties—with the notable exception of elasticity (E), which improved in Cabernet Sauvignon at T2, while Merlot experienced a decrease in E after T1. Similar trends were noted for firmness parameters FB and EB, which varied only modestly overall. However, when comparing the two cultivars directly, significant differences in FB and EB emerged—likely due to differences in total soluble solids (TSS), resulting in higher FB and EB in Merlot [25]. FB and EB have also been proposed as useful indicators of anthocyanin extractability in wine grapes [22].

When examining the grape skin characteristics, the IY treatment did not affect Th in Cabernet Sauvignon, which remained unchanged, whereas in Merlot it increased significantly at T1 and then returned to levels similar to the control at T2. This suggests that a second treatment may be unnecessary. In Merlot, the second treatment (T2) was associated with a decrease in both anthocyanins and polyphenols that paralleled the reduction in Th. In Cabernet Sauvignon, despite unaltered Th between treatments, we observed a non-significant reduction in TPF and a transient increase in anthocyanins at T1 that diminished again by T2. Other studies [29] on different wine grapes indicated that not all the cultivars respond in the same way to biostimulants treatments, and while Barbera showed no treatment effect during maceration, Nebbiolo exhibited a higher anthocyanin content. The different behaviour of cultivars could be explained by the vine's interaction with pathogens and its capacity to recognize the yeasts in the foliar spray—thereby activating specific defense mechanisms [30] and stimulating secondary metabolism for enhanced phenolic synthesis [31]. Moreover, contrasting responses between cultivars may be attributed to a dose-dependent effect. Some researchers [7] noted that the increase in total anthocyanins was not uniform across different concentrations of inactive yeast, implying a cultivar dependence [20]. In our study, the treatment improved total polyphenols and anthocyanins in Merlot predominantly at T1, while in Cabernet Sauvignon the polyphenol content remained stable and anthocyanins only showed a transient increase at T1.

Regarding the optimal timing of the foliar IY application, the nature and composition of the biostimulants used in viticulture and reported in literature does not always refer to *Saccharomyces*

 doi:10.20944/preprints202505.2092.v1

cerevisiae. In many cases the composition of biostimulants used is not known, although the treatment period and the number of treatments appear to be a crucial aspect.

Jindo et al. [6] report different types of treatments with biostimulants in viticulture, of which often neither the details of the product used nor the bioactive substance are known. The only paper reporting the foliar spray of *Saccharomyces cerevisiae* is the one proposed by Isçı et al. [32], who operated in greenhouse on Vitis champini, yielding increased rooting. However, although several works are reported in which they range from a single intervention to multiple interventions with biostimulants during the vegetative cycle, all the reported research has a common factor represented by the veraison phase, which appears to be the most suitable for carrying out the treatments, particularly under cooler or less optimal vintage conditions [23]. Other researchers [33] tried different applications of Ascophyllum nodosum starting from the end of dormancy, through blooming, fruit set, and veraison, yielding higher microelements intake in table grape, while in other cases [34] foliar application of Ascophyllum was performed at veraison, improving fruit color in Sangiovese. The effect of biostimulants on berry color appear once again related to the cultivar and the multivariate analysis of this research further supports this aspect. In Merlot, the clustering along color coordinates (a, b, and hue, h) indicates higher color saturation and a pronounced hue shift, particularly after the second treatment (T2) where a bluish tint appears. In contrast, for Cabernet Sauvignon, the PCA shows that the T2 profile largely overlaps with the control group, suggesting that additional or alternative treatments might be needed to achieve improvements similar to those observed in Merlot.

The foliar spray also influenced differently pigment accumulation in the two cultivars. In general, increasing treatment intensity tended to reduce color saturation in both cultivars, which may be associated with modifications in ripening or alterations in anthocyanin structure [23]. Colorimetric analysis reinforces that treatment effects are cultivar-dependent. Specifically, the PCA of CIELAB coordinates indicates that lightness (L) is more strongly associated with Cabernet Sauvignon, while color intensity (C) and hue (h) shift markedly following IY treatments.

Furthermore, in Cabernet Sauvignon, supplementation with two types of IY did not result in significant changes in the colorimetric parameters L*, a* and b* [35, 36]. The significant differences observed in hue (h) in Cabernet Sauvignon—but not in Merlot—might be due to different ripening states or cultivar responses attributable to differential gene activation, especially since berry color variation is closely linked to genetic variation at the VvmybA1 gene [37].

Spectral reflectance data collected across the 360–740 nm range further illustrate the effects of IY treatments. In Cabernet Sauvignon, significant differences in the spectral range between 480 and 740 nm among treatments suggest that IY application alters the pigment profile—particularly within the green to red wavelengths. Normally, the minimum of reflection in black wine grape berries, mainly related to chlorophyll absorption, is observed around 680 nm, with values less than 5% of radiation until ~700 nm [38]. In our case the two cultivars Cabernet Sauvignon and Merlot showed their lower reflectance, regardless of treatments, at 650 nm (around 7%) and 620 nm (around 6%), respectively. In Cabernet Sauvignon the similarity between the control and T1 in the central wavelengths implies that the most substantial changes occur at the spectral extremes, likely reflecting ripening-related pigment modifications. By contrast, in Merlot the spectral behaviour remains largely homogeneous up to 660 nm, with significant differences emerging only beyond this range. This relative stability at lower wavelengths may be due to a more stable skin structure or a pigment composition that is less affected by the treatment. A more detailed examination of the light spectra reveals further differences in pigment accumulation between 400 and 700 nm following treatment, as also reported by Gutierrez-Gamboa [18]. In our study, Cabernet Sauvignon appears to benefit more from the T2 treatment in terms of pigment accumulation, compared to T1 and the control, across the entire spectral range. In Merlot, however, differences in reflectance caused by T2 are evident only for wavelengths above 670 nm—corresponding to the red part of the reflectance spectrum, indicating that the treatment has a greater effect on chlorophyll than on anthocyanins. Additionally, analysis of the a and b components of the CIELab coordinates shows that Merlot exhibits a higher red index (a), whereas Cabernet

 doi:10.20944/preprints202505.2092.v1

Sauvignon, with more negative b values, expresses deeper shades in the violet-blue region (approximately 380–490 nm).

In this broader context, and in light of the increasingly frequent and intense abiotic stress events associated with climate change, the use of biostimulants is expected to become not only a promising strategy but also a fundamental paradigm for reducing reliance on synthetic chemical inputs in viticulture. The transition towards more sustainable, resilient, and environmentally friendly production systems necessitates agronomic practices that enhance the natural interactions between plants and their environment. However, to ensure optimal efficacy, it is essential to deepen our understanding of the physiological and biochemical mechanisms they trigger. A comprehensive understanding of the metabolic responses elicited by biostimulants is essential for optimising the timing, method, and frequency of application, thereby maximising benefits in terms of yield, fruit quality, and input reduction. In this regard, the integration of multidisciplinary tools – ranging from functional genomics to advanced phenotyping – offers promising opportunities to establish reliable, targeted application protocols aligned with the overarching goal of promoting climate-resilient and sustainable viticultural practices.

5. Conclusions

The differing responses observed between Cabernet Sauvignon and Merlot underscore the necessity for cultivar-specific optimisation in the context of yeast-based biostimulant treatments. The findings of this study imply that a universally applicable methodology may not be efficacious in enhancing mechanical and colorimetric quality traits in grape cultivation.

Beyond the realm of quality improvement, the importance of multivariate analyses emerged as a pivotal element, in discerning subtle interrelationships among various quality parameters. This holistic perspective, deemed essential for comprehending the immediate impacts of IY treatments, as well as for formulating agronomic practices that balance yield with improved sensory and nutritional profiles, is a crucial facet of the study.

Given the complex interactions between cultivar characteristics and treatment regimens, future studies should concentrate on the refinement of treatment parameters by means of a systematic variation of concentrations, timings, and frequencies, with a view to achieving optimal qualitative gains.

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Abbreviations

The following abbreviations are used in this manuscript:

IY	Inactive Yeast
MBW	Medium Berry Weight
TSS	Total Soluble Solids
TA	Tritatable Acidity
TPF	Total Polyphenols Content
ANT	Anthocyanins
H	Hardness
Ch	Chewiness
Co	Coesiveness
E	Elasticity
G	Gumminess
R	Resilience
FB	Force Break
EB	Energy Break
Th	Skin Thickness
L*	Lightness
a*	red/green scale
b*	yellow/blu scale
C*	Chroma
h*	Hue angle

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