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Impact of Commercial Yeasts on Phenolic Profile of Plavac Mali Wines from Croatia

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Abstract: Wine quality is influenced by the presence of over 500 different chemical compounds, with polyphenols having a crucial role in color intensity and tonality, astringency, mouthfeel, and overall impression formation, especially in red wine production. Their concentrations in wine can vary notably depending on the grape variety, the temperature and the length of maceration process, aging duration, and yeast selection. Therefore, in this work, the main goal was to determine the influence of five commercially available *Saccharomyces* yeasts provided from Lallemand, France and AEB, Italy, on the phenolic compound composition and chromatic parameters of Plavac mali wines produced from the grapes from coastal Dalmatia, grown at two different micro-locations. The achieved results pointed out the marked difference in individual polyphenol compound adsorption between tested yeasts. Fermol Super 16 was the one with the lowest and Lalvin D21 the strongest adsorption ability, regardless of vine growing location. These differences can be explained by the content of some anthocyanins (delphinidin and petunidin-3-*O*-glucoside) and gallic acid, and some flavan-3-ols. Tested strains also influenced wine color intensity, pointing out the possibility of modulating the style of a Plavac mali by the use of commercial yeasts.

Keywords: polyphenols; Plavac mali; yeasts; wine color

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1. Introduction

The sensory characteristics of wines are influenced by many factors, such as the type of grape variety, grape growing locations with their specific climatological and pedological conditions, the viticultural and winemaking techniques, and vintage year [1–4]. Wine quality is determined by several parameters, such as color intensity and tonality, aroma profile, taste complexity, astringency, mouthfeel, and overall impression. Among over 500 different chemical compounds influencing the parameters mentioned above, polyphenols have marked importance, especially in red wine production [5]. They are secondary metabolites present in berry skin, mainly extracted during the winemaking process and can be classified as flavonoids (anthocyanins, flavan-3-ols, flavonols) and non-flavonoids (phenolic acids, stilbenes) [6]. In wine, phenolic concentrations can vary notably depending on several factors, such as grape variety, the temperature and the length of the maceration process, aging duration, and yeast selection [7–9]. Yeast's influence on the polyphenol composition of wine was noted back in 2004 by Caridi et al. [10], showing interesting correlations between yeast strain and chromatic properties, phenolic profile, and the antioxidant power of wine. The modification of anthocyanin concentration during the fermentation process by yeasts was presented in work by Medina et al. [11], while Morata et al. [12] concluded that the strain used in red winemaking has a substantial influence on the formation of stable pigments, as well as the ability to adsorb color molecules by cell walls. According to Echeverigaray et al. [13], yeasts could be grouped as low, medium, and high anthocyanins adsorption strains. The influence of fermentative strains on the content of resveratrol

glucoside isomers (*trans* and *cis* piceid) was presented in work by Clare et al. [14], showing no significant difference but pointing out a close correlation between stilbenes and total phenolic extraction rate. The importance of yeast strain choice in optimizing Pinot noir wine phenolics was shown in work by Carew et al. [15]. Their research demonstrated a significant influence of yeast strain on the concentration and composition of wine tannin. The marked difference in total polyphenols concentrations and flavans and proanthocyanidins by using two different yeasts was presented by [10], indicating different absorption intensities between them. Recently, Samoticha et al. [16] compared the influence of two *S. cerevisiae* strains, one *S. bayanus* strain, and spontaneous fermentation on the phenolic profile of Aurora white wine. The achieved results pointed out the significant impact of yeast strain on flavan-3-ols and total phenolic profile with higher concentrations presented in *S. cerevisiae* fermented wines. The positive impact of autochthonous selected yeast strains on the concentration of Negroamaro and Primitivo wines' phenolic composition was published by Grieco et al. [17]. On the contrary, Sacchi et al. [18] cited few works where yeast strain did not greatly affect the phenolic composition, pointing out that in many of these studies, the lots were pressed at dryness, so the skin contact time between lots could be different. Among the red grape varieties cultivated in Croatia, Plavac mali is the most planted, covering 1426.62 ha [19]. Since Plavac mali is a late-ripening variety, and to obtain high-quality wines, it requires growing sites with long vegetation periods typical only for south parts of Dalmatia, especially dalmatian islands such as Hvar, Korčula, Vis, and peninsula Pelješac. Targeted profiling by ultra-high performance liquid chromatography of monovarietal wines produced in Croatia [20] singled out Plavac mali as the one with the most specific phenolic composition that could assure better varietal typicity definition and in this way strengthen their identity and position on the market. The main aim of this study was to investigate the influence of commercially available yeasts on the polyphenolic composition of Plavac mali wines produced from the grapes from costal Dalmatia, grown at two different micro locations. Additionally, the second aim was to define the impact of the geographical and pedological heterogeneity of these locations on the chemical composition of Plavac mali produced by different yeasts.

2. Materials and Methods

2.1. Yeast Strains

The commercial *S. cerevisiae* strains were provided from Lallemand, France, and AEB, Italy, as active dry yeasts. All yeast strains were precultured in the same grape must at 25 °C for 72 h. Lalvin ICV D21 was selected for fermenting red wines with stable color, mid-palate tannin, and unlike most wine yeasts contributing both higher acidity and positive polyphenol-reactive polysaccharides. Fermol Super 16 (S16) is a multipurpose yeast for structured red wines, displaying high fermentative activity even under difficult conditions, ideal for obtaining structured red wines. Fermol Power (P) is a multipurpose yeast that optimally takes advantage of nitrogen availability and can keep a high metabolic activity even under critical conditions. Fermol Grand Rouge (GR) possesses excellent technological characteristics, producing red wines with a good tannic structure suitable for aging. Fermol Premier Cru (PC) is a yeast selected to produce structured and complex wines suitable for aging. The main yeast characteristics are listed in Table 1. Each yeast strain was added at approximately 1×10^7 cells/mL, and fermentations were carried out at 20 °C according to the manufacturer's instructions. The cell concentrations (hemocytometry) and viability (methylene blue staining) were determined under a light microscope [21].

Table 1. Main characteristics of used yeast strains.

	Lalvin ICV D21	Fermol Power	Fermol Grand Rouge	Fermol Premier Cru	Fermol Super 16
Yeast	S. cerevisiae	S. cerevisiae	S. cerevisiae	S. cerevisiae	S. cerevisiae
Temperature range	16–30 °C	low temperatures	up to 30 °C	18–34 °C	up to 34 °C
Fermentation speed	moderate	normal	normal	normal	normal
SO_2 production	low	low	low	low	low
Alcohol tolerance	high (16 vol%)	high (14 vol%)	high (15 vol%)	high	high (18 vol%)
Nitrogen needs	medium	low	medium	medium	medium

2.2. Vineyard Locations

The grape used for vinification in this research was produced in two vineyards located in the wine region Dalmatia (Figure 1). One of the vineyards is located on the Island of Korčula and the vineyard is characterized by soil obtained by carst reclamation with a high share of stone fraction, and it has a south orientation close to the coast on a slope of medium steepness. The vineyard on the Island of Vis is located on a site with deep sandy soil and is isolated from any direct influence of the sea, and is on a flat surface. Both locations were characterized with similar temperatures and precipitation in 2019, reaching average monthly temperatures in summer months from 25.4 °C in June to high as 27.4 °C in August.

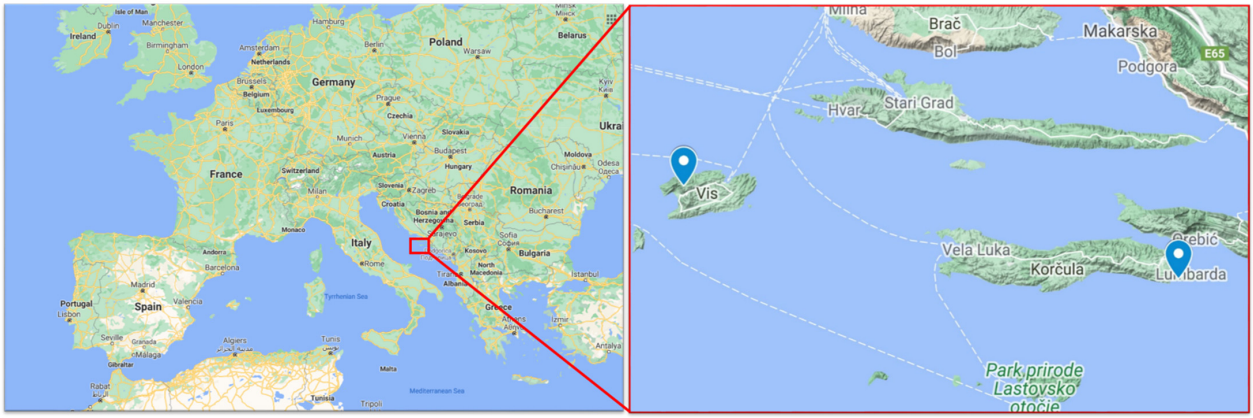

Figure 1. Location of the two vineyards on Dalmatian islands Korčula and Vis.

2.3. Fermentation Trials

In 2019, 500 kg of Plavac mali grapes from two locations (Vis, Korčula) was harvested, destemmed, crushed, and distributed evenly into 45 L stainless steel fermenters; each experimental variant was reproduced in three replicates (n = 3). The basic chemical composition of the grapes was, for Plavac mali (Vis): initial sugar 245 g/L; total acidity 7.15 g/L as tartaric acid, and pH 3.40, and for Plavac mali (Korčula): initial sugar 234 g/L; total acidity 6.05 g/L as tartaric acid, and pH 3.50. In all variants, the addition of sulfur dioxide (SO_2) in a concentration of 50 mg/L and inoculation by commercial *S. cerevisiae* strains was conducted. The caceration process lasted for 7 days at 20 °C, and during that period, mash aeration and cap management were carried out by mechanical mixing. By the end of the maceration process, wines were devatted from the pomace and the solid pulp left behind was pressed by the use of a hydropress (Lancman VS-A 80). Free run wines and pressed wines were mixed together. The course of fermentation was monitored by the sugar consumption, and it was considered complete when the residual sugar concentrations

were under 4.0 g/L. In all variants, fermentation started 24 h after inoculation and lasted between 12–14 days. In that period, kinetic fermentation, monitored by the decomposition of sugars, showed no marked difference. The final wines were bottled in 750 mL glass bottles with screw caps and transported to the laboratory of the Department of Viticulture and Enology, Faculty of Agriculture University of Zagreb for chemical analysis.

2.4. Physicochemical Analysis

The basic wine parameters, including alcohol content (%, v/v), pH values, total and volatile acidity, were quantified by applying methods recommended by the International Organization of Vine and Wine [22].

2.5. Organic Acids Analysis

The analysis of individual acids (malic and lactic acid) was carried out by an HPLC system Agilent Series 1100 equipped with a diode array detector (Agilent, Palo Alto, CA, USA). In brief, the determination was performed isocratically with a flow rate set to 0.6 mL min^{-1} with 0.065% phosphoric acid (p.a. Merck, Darmstadt, Germany) as a mobile phase. The Column Aminex HPX-87H 300 mm × 7.8 mm i.d (Bio-Rad Laboratories, Hercules, CA, USA) was heated at 65 °C, while the detector was set to 210 nm.

2.6. Polyphenol Compounds Determination

The wine samples were filtered with a Phenex-PTFE (polytetrafluorethylene) 0.20 µm syringe filter (Phenomenex, Tor-rance, USA), and analyzed by HPLC. The separation, identification, and quantification of flavonoids from grape skin extracts were performed on an Agilent 1100 Series system (Agilent, Germany), equipped with an auto sampler, column thermostat, diode array detector (DAD), fluorescence detector (FLD) and coupled to an Agilent Chem Station data-processing station. The separation was performed with a reversed-phase column Luna Phenyl-Hexyl (4.6 mm × 250 mm; 5 µm particle (Phenomenex, Torrance, CA, USA)), with Phenyl guard column (4.0 mm × 3.0 mm) heated at 50 °C. The solvents were water:phosphoric acid (99.5:0.5, v/v, eluent A) and acetonitrile:water:phosphoric acid; 50:49.5:0.5, $v/v/v$, eluent B), and the flow rate was 0.9 mL/min. The linear gradient for eluent B was: 0 min, 0%; 7 min, 20%; 35 min, 40%; 40 min, 40%; 45 min, 80%; 50 min, 100%; 60 min 0%. The injection volume for all samples was 20 µL. The diode array detector was set to an acquisition range of 200–700 nm. Hydroxybenzoic acids were detected at 280 nm, hydroxycinnamic acid at 320 nm, flavonols at 360 nm, and anthocyanins at 518 nm using DAD while flavan-3-ols were detected at λ_{ex} = 225 nm and λ_{ex} = 320 nm using FLD. The identification of individual phenolic compounds was performed by matching the retention time of each chromatographic peak with external standards and DAD spectrum. Individual phenolic compound peaks were quantified using the calibration curve of the corresponding standard compound, which was based on the peak area. As a standard, the following compounds were used: delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, quercetin-3-*O*-glucoside, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, *trans*-piceid, gallic acid, syringic acid, protocatechuic acid, epigallocatechin gallate, epicatechin-gallate, gallocatechin, epigallocatechin, procyanidins B1, B2, and B3, catechin and epicatechin (Extrasynthese, Genay cedex, France); caftaric acid, quercetin and kaempferol (Sigma-Aldrich, St. Louis, MO, USA) [23].

2.7. Color Parameters

The color intensity, hue/tint/tonality and pigments were analyzed by the direct measurement of wine absorbance at 420, 520, and 620 nm by using a Specord 400 spectrophotometer (Analytik Jena, Jena, Germany). The color intensity (CI), color tint/tonality/hue (T), and the proportion of yellow (% Ye), red (% Rd), and blue (% Bl) pigments were calculated as follows: CI = Abs 420 + Abs 520 + Abs 620; T = Abs 420/Abs 520; % Ye = (Abs 420/CI) × 100, % Rd = (Abs 520/CI) × 100, % Bl = (Abs 620/CI) × 100 [24].

2.8. Statistical Analysis

ANOVA was used to test the significance of the effects of the grapevine source (location) and yeast strains as well as their interaction for all parameters analyzed. In the case of significant results obtained by ANOVA, the means were compared using Duncan's multiple range test. To evaluate the total variability in polyphenolic profiles of wines from two locations and five different yeast strains used in alcoholic fermentation, principle component analysis (PCA) was performed, and variables and observation scores for the first two canonical factors were used to create scatter plots to explain multivariate differences among samples. All of the analyses were carried out using XLSTAT software v.2020.3.1. (Addinsoft, New York, NY, USA). The results were statistically assessed with an analysis of variance and Duncan's multiple range test to identify significant differences ($p < 0.05$). Multivariate analysis was carried out with XLSTAT software v.2020.3.1. (Addinsoft, New York, NY, USA).

3. Results and Discussion

3.1. Physicochemical Composition

The results of the basic physicochemical analysis of wines are presented in Table 2, showing the significant impact of yeast strain in dry extract, total acidity as well as malic and succinic acid. Our results confirmed the ability of Lalvin ICV D21 to contribute higher acidity that could be connected with significantly higher concentrations of succinic and malic acid in Plavac mali wines, from both locations. Yeast influence in succinic acid production is well documented in published works [25–27], while concentration differences between them are mainly determined by grape must composition and vinification process properties. From the results presented in Table 3, we can see how strong the influence of grape growing location was, with significantly higher concentrations of almost all analyzed parameters in the Plavac mali wines from Vis. The interactions between grapevine source location and yeast strains used for fermentation was not significant for all physicochemical parameters. The presence of (+)-catechin in synthetic grape juice reduced the production of acetic acid and increased the production of succinic acid during fermentation [28]. Looking at our results (Table 4), the presence of (+)-catechin in Plavac mali wines produced by Lalvin ICV D21 was not significantly the highest, but the concentration of procyanidin B1 was. Therefore, we can assume that flavan-3-ols in general can have a positive impact in succinic acid production, which is a possibility already mentioned by other authors [25]. According to Chidi et al. [27] succinic acid levels were significantly higher under aerobic conditions (up to 3.81 g/L) compared to anaerobic (up to 0.61), with Anchor VIN13 yeast being the highest producer. Generally speaking, the average succinic acid level range is between 0.5 and 1.5 g/L [29].

Table 2. Physicochemical properties of Plavac mali wines.

Compounds	Vis					Korčula				
	S16	**GR**	**P**	**PC**	**D21**	**S16**	**GR**	**P**	**PC**	**D21**
Alcohol (%, *v*/*v*)	13.9	14.1	14.4	14.2	14.1	13.8	13.8	13.6	13.9	13.5
Dry extract (g/L)	29.8 ^{b}	30.5 ^{ab}	31.1 ^{a}	29.2 ^{b}	30.4 ^{ab}	26.5 ^{b}	27.7 ^{a}	26.4 ^{b}	25.6 ^{b}	26.9 ^{b}
Reducing sugars (g/L)	3.5	3.2	3.2	3.8	3.9	3.1	3.8	3.5	3.3	3.1
Total acidity * (g/L)	6.5 ^{b}	6.9 ^{ab}	7.0 ^{a}	6.4 ^{b}	7.0 ^{a}	5.2 ^{b}	5.8 ^{a}	5.3 ^{b}	5.4 ^{b}	5.7 ^{a}
Volatile acidity ** (g/L)	0.40	0.45	0.44	0.48	0.37	0.34	0.36	0.38	0.32	0.30
pH	3.52	3.48	3.52	3.51	3.51	3.72	3.57	3.63	3.59	3.65
Malic acid (g/L)	1.01 ^{b}	1.13 ^{ab}	1.13 ^{a}	0.93 ^{ab}	1.31 ^{a}	0.64 ^{b}	0.69 ^{b}	0.66 ^{b}	0.75 ^{b}	0.95 ^{a}
Succinic acid (g/L)	0.85 ^{b}	0.86 ^{b}	0.92 ^{a}	0.82 ^{b}	0.99 ^{a}	0.64 ^{c}	0.74 ^{b}	0.85 ^{a}	0.75 ^{b}	0.88 ^{a}

* tartaric acid and ** acetic acid equivalents. Concentrations expressed as mean values (n = 3). Means with different superscript letters, for each location separately, in the same row differ significantly ($p \leq 0.05$).

Table 3. Physicochemical properties of Plavac mali wines according to grape growing location.

Location	Alcohol (%, *v*/*v*)	Dry Extract (g/L)	Reducing Sugars (g/L)	Total Acidity * (g/L)	Volatile Acidity ** (g/L)	pH	Malic Acid (g/L)	Succinic Acid (g/L)
VIS	14.14 ^{a}	30.20 ^{a}	3.52 ^{a}	6.76 ^{a}	0.43 ^{a}	3.51 ^{b}	1.10 ^{a}	0.89 ^{a}
KORČULA	13.72 ^{b}	26.62 ^{b}	3.36 ^{a}	5.48 ^{b}	0.32 ^{b}	3.63 ^{a}	0.74 ^{b}	0.77 ^{b}

* tartaric acid and ** acetic acid equivalents. Concentrations expressed as mean values (n = 5). Means with different superscript letters, for each location separately, in the same row differ significantly ($p \leq 0.05$).

Table 4. Phenolic profile of Plavac mali wines.

Compounds (mg/L)	Vis					Korčula					Vis	Korčula
	S16	GR	P	PC	D21	S16	GR	P	PC	D21	$\bar{x}$	$\bar{x}$
Delphinidin-3-*O*-glucoside	32.63 ^{a}	24.64 ^{b}	30.55 ^{a}	25.47 ^{b}	25.84 ^{b}	18.95 ^{a}	11.41 ^{c}	14.15 ^{b}	7.75 ^{d}	6.78 ^{e}	27.82 ^{a}	11.81 ^{b}
Cyanidin-3-*O*-glucoside	1.46 ^{bc}	1.35 ^{c}	1.84 ^{a}	0.85 ^{d}	1.53 ^{b}	0.22 ^{a}	0.11 ^{b}	0.13 ^{b}	0.24 ^{a}	0.14 ^{b}	1.41 ^{a}	0.17 ^{b}
Petunidin-3-*O*-glucoside	17.90 ^{a}	13.52 ^{d}	16.67 ^{b}	16.26 ^{b}	14.85 ^{c}	17.01 ^{a}	10.42 ^{c}	12.38 ^{b}	7.82 ^{d}	7.76 ^{d}	15.84 ^{a}	11.08 ^{b}
Peonidin-3-*O*-glucoside	9.64 ^{b}	8.61 ^{c}	11.18 ^{a}	7.45 ^{d}	9.98 ^{b}	4.45 ^{a}	3.01 ^{b}	4.17 ^{a}	2.30 ^{c}	2.12 ^{c}	9.37 ^{a}	3.21 ^{b}
Malvidin-3-*O*-glucoside	225.96 ^{a}	196.05 ^{c}	223.06 ^{a}	226.07 ^{a}	214.08 ^{b}	349.16 ^{a}	239.70 ^{c}	298.64 ^{b}	210.74 ^{d}	215.70 ^{d}	217.04 ^{b}	262.79 ^{a}
Σ Anthocyanins	287.58 ^{a}	244.16 ^{d}	283.29 ^{a}	276.10 ^{b}	266.27 ^{c}	389.78 ^{a}	264.63 ^{c}	329.46 ^{b}	228.83 ^{d}	232.48 ^{d}	271.48 ^{a}	289.04 ^{a}
Quercetin-3-*O*-glucoside	7.86 ^{a}	5.44 ^{d}	7.14 ^{b}	7.02 ^{b}	6.12 ^{c}	7.16 ^{b}	5.44 ^{c}	7.95 ^{a}	4.08 ^{d}	3.27 ^{e}	6.72 ^{a}	5.58 ^{b}
Quercetin	3.07 ^{a}	1.77 ^{d}	2.43 ^{b}	2.57 ^{b}	2.23 ^{c}	0.96 ^{a}	0.67 ^{a}	0.79 ^{a}	0.69 ^{a}	0.75 ^{a}	2.41^{a}	0.77 ^{b}
Kaempferol	0.33 ^{a}	0.28 ^{a}	0.32 ^{a}	0.28 ^{a}	0.30 ^{a}	0.24 ^{a}	0.18 ^{b}	0.23 ^{a}	0.19 ^{b}	0.20 ^{ab}	0.30 ^{a}	0.21 ^{b}
Σ Flavonols	11.25 ^{a}	7.49 ^{d}	9.89 ^{b}	9.87 ^{b}	8.64 ^{c}	8.34 ^{b}	6.29 ^{c}	8.97 ^{a}	4.95 ^{d}	4.22 ^{e}	9.42 ^{a}	6.55 ^{b}
trans-caftaric acid	33.59 ^{a}	30.24 ^{c}	31.98 ^{b}	33.75 ^{a}	31.13 ^{bc}	31.94 ^{ab}	28.66 ^{bc}	32.49 ^{a}	25.17 ^{c}	18.11 ^{d}	32.14 ^{a}	27.27 ^{b}
Caffeic acid	3.80 ^{ab}	3.28 ^{c}	4.23 ^{a}	3.44 ^{bc}	3.91 ^{ab}	1.63 ^{b}	1.56 ^{b}	1.85 ^{a}	1.54 ^{b}	1.55 ^{b}	3.73 ^{a}	1.63 ^{b}
trans-coutaric acid	5.12 ^{b}	4.42 ^{c}	5.11 ^{b}	5.57 ^{a}	5.09 ^{b}	5.36 ^{b}	4.65 ^{c}	6.38 ^{a}	4.01 ^{d}	2.86 ^{e}	5.06 ^{a}	4.65 ^{b}
trans-coumaric acid	0.89 ^{c}	1.82 ^{a}	1.75 ^{a}	1.10 ^{b}	1.84 ^{a}	0.32 ^{c}	0.75 ^{a}	0.53 ^{b}	0.43 ^{bc}	0.87 ^{a}	1.48 ^{a}	0.58 ^{b}
Ferulic acid	0.32 ^{c}	0.54 ^{a}	0.48 ^{ab}	0.44 ^{b}	0.57 ^{a}	0.19 ^{b}	0.29 ^{b}	0.21 ^{b}	0.20 ^{b}	0.57 ^{a}	0.47 ^{a}	0.29 ^{b}
Gallic acid	21.59 ^{a}	17.89 ^{b}	17.67 ^{b}	16.34 ^{b}	16.00 ^{b}	29.03 ^{a}	27.21 ^{ab}	25.32 ^{b}	21.96 ^{c}	20.92 ^{c}	17.90 ^{b}	24.89 ^{a}
Syringic acid	2.44 ^{a}	2.12 ^{ab}	1.86 ^{b}	2.17 ^{ab}	1.98 ^{b}	3.87 ^{a}	3.34 ^{bc}	3.55 ^{b}	3.20 ^{c}	3.41 ^{bc}	2.11 ^{b}	3.47 ^{a}
Σ Phenolic acids	67.74 ^{a}	60.32 ^{b}	63.08 ^{b}	62.79 ^{b}	60.50 ^{b}	72.32 ^{a}	66.45 ^{b}	70.32 ^{a}	56.50 ^{c}	48.27 ^{d}	62.88 ^{a}	62.77 ^{a}
(+)-Gallocatechin	1.65 ^{a}	1.49 ^{b}	1.54 ^{ab}	1.44 ^{b}	1.57 ^{ab}	1.16 ^{c}	1.17 ^{c}	1.57 ^{a}	1.34 ^{b}	0.96 ^{d}	1.53 ^{a}	1.24 ^{b}
Procyanidin B1	70.04 ^{c}	59.14 ^{e}	101.70 ^{b}	66.96 ^{d}	128.73 ^{a}	74.55 ^{b}	57.87 ^{c}	49.75 ^{d}	57.79 ^{c}	92.75 ^{a}	71.31 ^{a}	66.54 ^{b}
(-)-Epigallocatechin	20.67 ^{a}	17.53 ^{c}	17.38 ^{c}	19.17 ^{b}	13.60 ^{d}	14.67 ^{a}	11.90 ^{bc}	12.58 ^{b}	10.85 ^{cd}	9.42 ^{d}	17.67 ^{a}	11.88 ^{b}
Procyanidin B3	2.16 ^{a}	2.06 ^{a}	1.94 ^{a}	2.08 ^{a}	2.17 ^{a}	2.22 ^{a}	1.87 ^{b}	1.84 ^{b}	1.44 ^{c}	1.39 ^{c}	2.08 ^{a}	1.75 ^{b}
(+)-Catechin	29.97 ^{a}	23.71 ^{c}	24.45 ^{c}	26.76 ^{b}	22.43 ^{c}	16.08 ^{a}	13.72 ^{b}	13.87 ^{b}	10.40 ^{c}	9.45 ^{c}	25.46 ^{a}	12.70 ^{b}
Procyanidin B4	4.12 ^{a}	3.71 ^{b}	3.74 ^{b}	4.14 ^{a}	3.60 ^{b}	3.68 ^{a}	3.08 ^{b}	3.14 ^{b}	2.59 ^{c}	2.40 ^{c}	3.86 ^{a}	2.98 ^{b}
Procyanidin B2	7.94 ^{a}	6.14 ^{c}	6.65 ^{b}	6.55 ^{b}	5.58 ^{d}	6.67 ^{a}	6.31 ^{b}	5.80 ^{c}	4.68 ^{d}	4.38 ^{e}	6.57 ^{a}	5.57 ^{b}
(-)-Epicatechin	18.70 ^{a}	14.63 ^{c}	16.58 ^{b}	16.10 ^{b}	13.57 ^{d}	10.95 ^{a}	10.80 ^{a}	7.90 ^{b}	7.01 ^{c}	6.83 ^{c}	15.92 ^{a}	8.69 ^{b}
Σ Flavan-3-ols	155.24 ^{c}	128.39 ^{e}	173.95 ^{b}	143.19 ^{d}	191.24 ^{a}	129.96 ^{a}	106.70 ^{b}	96.45 ^{c}	96.09 ^{c}	127.56 ^{a}	158.40 ^{a}	111.35 ^{b}
trans-piceid	11.06 ^{ab}	9.51 ^{c}	12.29 ^{a}	9.90 ^{bc}	10.57 ^{bc}	3.94 ^{b}	3.61 ^{c}	4.49 ^{a}	2.74 ^{d}	2.12 ^{e}	10.67 ^{a}	3.38 ^{b}

Concentrations expressed as mean values (n = 3). Means with different superscript letters, for each location separately, in the same row differ significantly ($p \leq 0.05$).

3.2. Phenolic Profile of Plavac Mali Wines

The phenolic compound composition of Plavac mali wines from two locations is reported in Table 4, consisting of twenty four compounds belonging to the chemical classes of anthocyanins, phenolic acids, flavan-3-ols, flavonols and stilbenes. The achieved results indicated significant differences in the phenolic compound levels between Plavac mali wines produced with different yeast strains.

3.2.1. Anthocyanins

The red grape anthocyanin profile is genetically determined, and so its typicality has been used as a chemotaxonomic marker [30] and also for red wine authentication [20,31]. The main anthocyanins present in wines are monoglucosylate followed by acetyl, coumaryl and caffeoyl esters of cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and petunidin-3-*O*-glucoside [6]. Investigating the phenolic composition of monovarietal red wines from Croatia, Lukic et al. [20] noted significantly lower levels of individual anthocyanins in Plavac mali compared to Teran wines. Anthocyanin concentrations in our wines were much higher, especially with malvidin- and delphinidin-3-*O*-glucoside, showing a strong influence of vineyard location, which is in accordance with previously published data [32,33]. The influence of yeast strains was noted, especially in the anthocyanin profile of wines fermented with Fermol Super 16 (S16) and Fermol Power (P), which were ones which significantly had the highest concentrations of delphinidin and petunidin-3-*O*-glucoside. In work by Medina et al. [11], these two compounds were pointed out as the ones with the most pronounced losses related to the adsorption intensity and affinity to be adsorbed by the yeast cell walls because of their polarity. Therefore, we can assume that the cell structure of the above-mentioned yeast strains differed due to the dissimilar compositions of their cell walls, probably because of the different contents of polar groups exposed on cell wall surfaces in comparison with other three yeasts used. Similar considerations can be found in work by [8,15,34], while Morata et al. [34] detected strain-related anthocyanin adsorption differentiation ranging from 1.6 to 5.9%.

3.2.2. Flavonols

Flavonols are present in wine as aglycones and glycosylated forms, and among them, the most abundant are quercetin and myricetin [6]. Free aglycone forms are mainly released by hydrolisis during vinification and the storage period from their original grape flavonol-3-glycoside. Flavonols, particularly when they occur in their deglycosylated form, are labile molecules and may be degraded upon exposure to heat, enzymes, as well as common vinification practices influencing significant changes, from a qualitative and a quantitative point of view [35]. In work by Rizzo et al. [36] the high performance liquid chromatography method was developed to be used in the determination of the yeast adsorption profile. Among 23 *S. cerevisiae* strains used in their research, marked yeast selectivity in adsorption phenolic compounds with different chemical structure was detected, identifying the ability of strain Sc 1483 to adsorb the maximum concentration of rutine. In our research, the most abundant flavonol was quercetin-3-*O*-glucoside, whose concentrations significantly differ among wines produced with used yeast strains. Again, strain S16 and strain P showed the lowest adsorption ability, while the strongest ability in Plavac wines from Vis was connected with the GR strain and in Korčula wines, the D21 strain. Quercetin concentrations were markedly influenced by yeast only in wines from Vis, while kaempferol levels were not influenced by yeast strains used. However, when examining the total flavanol concentrations, significant influence was noted, identifying strain S16 as the one with the weakest adsorption capacity and strain D21 with the strongest one.

3.2.3. Phenolic Acids

Phenolic acids present in wines belong to two main groups—hydroxybenzoic acids and hydroxycinnamic acids—and exist in either the free or the conjugated form. Gallic

acid is considered the most abundant benzoic acid while caffeic, coumaric, ferulic and sinapic acids are the most common among hydroxycinnamic acids [37]. The most abundant phenolic acids, regardless of location, were *trans*-caftaric acid and gallic acid, which is in agreement with the findings by Lukic et al. [20] and Žurga et al. [38]. Their concentrations were also significantly influenced by the yeast strains used, with the highest levels detected in Plavac mali wines produced by the S16 strain and the lowest by the D21 strain. The strong capability for galic acid adsorption by yeasts' cell wall was shown by Rizzo et al. [36]. In work by Samoticha et al. [16], phenolic acid concentrations present in Aurora wines also differed in terms of yeasts used. Free phenolic acids (mainly p-coumaric, caffeic and ferulic acids) can be metabolized by microorganisms to form 4-vinyl derivatives, which can be reduced to 4-ethyl derivatives in wine [39]. We can assume that the noted differences in caffeic and ferulic acids between analyzed Plavac mali wines can be partly influenced by their futher transformation to 4-vinyl compounds.

3.2.4. Flavan-3-ols

As one of the principal grape polyphenolic classes in wine, flavan-3-ols are present as monomers ((+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, (−)-epicatechin-3-*O*-gallate) and polymers, usually known as proanthocyanidins or condensed tannins [37]. Proanthocyanidins are composed of chains of flavan-3-ol units, (+)-catechin and (-)-epicatechin, linked together through C4-C6 and C4-C8 interflavanoid bonds [40]. Their composition in wines depends on the cultivar, location, climatological conditions, as well as the winemaking technology and yeast strain used [12]. The selected yeast strains influence the total tannin content in Gaglioppo wines and their contribution in color intensity was published in work by Caridi et al. [9]. From our data, it can be seen that the most abundant compound was procyanidin B1, with the highest concentrations present in Plavac mali wines produced by the D21 strain, regardless of location. The highest procyanidin B1 concentration in Plavac mali wines was also noted in work by Lukic et al. [20]. According to Makris et al. [33], procyanidin B1 and B2 demonstrated a profound influence on cultivar- and geographical origin-based differentiation, which is in accordance with our data, showing significantly higher concentrations of procyanidin B2 but also total flavan-3-ol concentrations in Plavac mali wines from Vis. Between monomers, the most abundant compound was (+)-catechin, followed by (-)-epigallocatechin and (-)-epicatechin with the significantly highest concentrations present in Plavac mali wines from both locations, fermented with the S16 yeast strain. Higher (+)-catechin levels in Plavac mali wines compared to other Croatian wines were also found by Rastija et al. [41] as well as Žurga et al. [38].

3.2.5. Stilbenes

Stilbenes are a class of polyphenols that can protect berries from abiotic and biotic stress. Among them, the simplest is *trans*-resveratrol, while *cis*-resveratrol is a less stable isomer. Their 3-O-glucosides are known as *trans*- and *cis*-piceid, respectively [37]. It is well known that the geographical location, cultivar, viticultural as well as oenological practices play a significant role in final resveratrol levels in wines [14,42–44]. A marked resveratrol decrease ranging from 20% in the medium inoculated with *Metschnikowia pulcherrima* yeast up to 32% for the one inoculated with *S. cerevisiae* yeast strain was presented in work by Vacca et al. [45]. Similar results were achieved by Clare et al. [14] showing the significant influence of different yeast strains on resveratrol content in wines. In Plavac mali wines, regardless of location, only *trans*-piceid was detected, with the significantly highest concentrations in ones made by the Fermol Power (P) yeast strain, followed by the S16 strain.

3.2.6. Chromatic Parameters

Table 5 shows the results of Plavac mali wines color evaluation in terms of color intensity (CI), color tonality (T) and proportion of yellow (%Ye), red (%Rd) and blue

(%Bl) pigments. Regarding the A520 value and %Rd, wines produced with the S16 strain exhibited the highest values, followed by strain P and PC. These results are in agreement with the values of total anthocyanins and color intensity values defined in wines produced using these yeast strains. Color tonality values are mainly related to the type of pigments present in the wine, but also to the oxidation degree of the phenolic compounds [9]. Between Plavac mali wines from Korčula, the one produced with the D21 strain exhibited the highest value, while color tonality in Plavac mali wines from Vis was more or less the same, with the lower level present in S16 samples. Similar results were achieved in the work by Blazquez Rojas et al. [46] who investigated the impact of 11 different *Saccharomyces* strains on wine color and found a marked influence on color density and minimal differences in hue values between used yeasts.

Table 5. Chromatic parameters of Plavac mali wines.

Wine	A_{420}	A_{520}	A_{620}	I.C.	T	Chromatic Structure		
						% Yellow Pigments	% Red Pigments	% Blue Pigments
Vis D21	2.21	3.33	0.55	6.09 ^{b}	0.66 ^{a}	36.28 ^{a}	54.67 ^{c}	9.03 ^{b}
Vis P	2.39	3.68	0.55	6.62 ^{a}	0.64 ^{a}	36.10 ^{a}	55.58 ^{b}	8.30 ^{c}
Vis PC	2.45	3.75	0.58	6.78 ^{a}	0.65 ^{a}	36.13 ^{a}	55.30 ^{bc}	8.55 ^{c}
Vis S16	2.43	3.99	0.58	7.00 ^{a}	0.60 ^{b}	34.71 ^{c}	57.00 ^{a}	8.28 ^{c}
Vis GR	2.30	3.50	0.61	6.41 ^{b}	0.65 ^{a}	35.88 ^{b}	54.60 ^{c}	9.51 ^{a}
Korčula D21	1.84	2.57	0.45	4.87 ^{d}	0.71 ^{a}	37.80 ^{a}	52.87 ^{c}	9.33 ^{b}
Korčula P	2.85	4.56	0.84	8.25 ^{b}	0.62 ^{b}	34.54 ^{c}	55.27 ^{a}	10.18 ^{a}
Korčula PC	1.89	2.88	0.51	5.29 ^{d}	0.65 ^{b}	35.72 ^{b}	54.44 ^{b}	9.64 ^{b}
Korčula S16	3.18	5.12	0.89	9.20 ^{a}	0.62 ^{b}	34.56 ^{c}	55.65 ^{a}	9.67 ^{a}
Korčula GR	2.33	3.51	0.60	6.44 ^{c}	0.64 ^{b}	36.18 ^{b}	54.50 ^{b}	9.31 ^{b}

I.C.—color intensity, T—color tonality. Concentrations expressed as mean values (n = 3). Means with different superscript letters, for each location separately, in the same column differ significantly ($p \leq 0.05$).

3.2.7. Multivariate Analyses

Principle components analysis based on 24 phenolic compounds of wine samples obtained using five different yeast strains and grapes from two different locations (Korčula and Vis) explained 85.82% of the total variability among wine samples in the first two canonical factors. A scatter plot was generated (Figure 2), presenting the distribution of wine samples in a two-dimensional space defined by the first two canonical factors, and a vector diagram presenting correlations of polyphenolic compound level with the first two canonical factors. It is evident that the distance between two groups defined by the geographic origin of the samples can be explained by the differences in the content of phenolic compounds located in the II and IV quadrants of the variables coordinate plane. Differences in the phenolic profiles of wines from two locations can be explained by the differences in environmental conditions on two sites where vineyards are in relation to soil characteristics. A similar distribution of wine samples obtained using yeast strains S16 and D21 is evident on the plot, and can be explained by the variables presented in the I quadrant. Wine samples' poitions within the group from the other three yeast strains used (P, PC and GR)are not same, and must be evaluated separately for two groups.

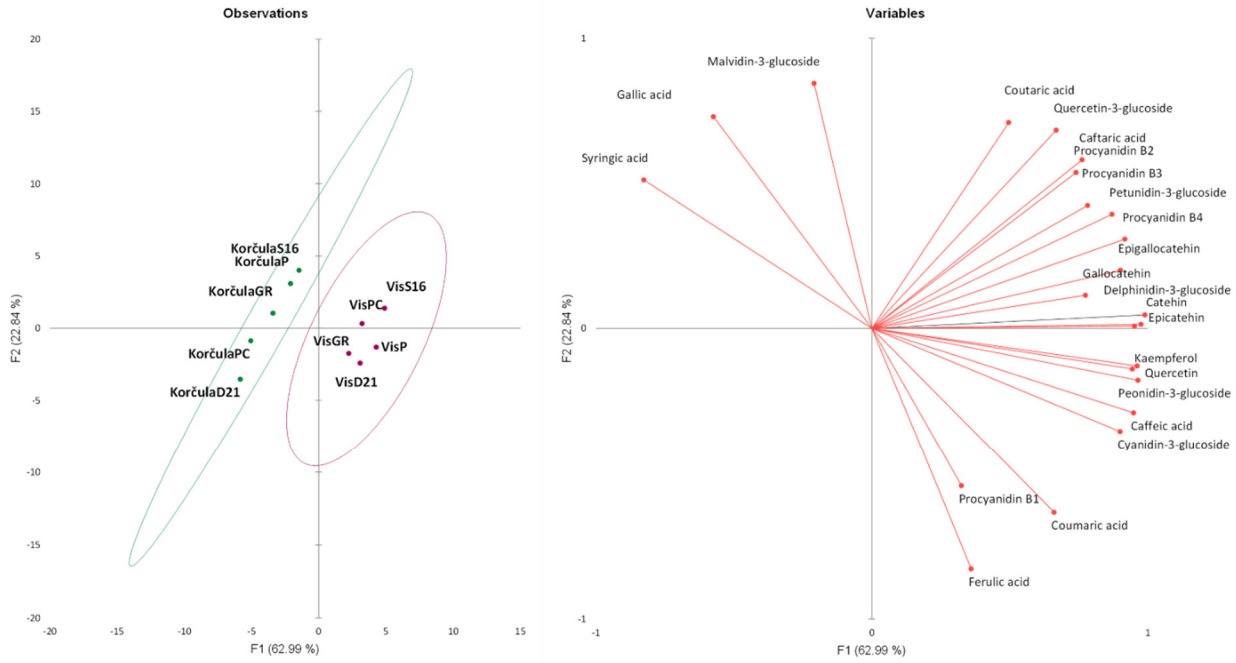

Figure 2. Principle component analysis (PCA): distribution of the cv. Plavac mali wine samples (observations) from two different locations (Islands Vis and Korčula) in two-dimensional coordinate system defined by the first two principal components (PC1 and PC2) according to the polyphenolic profiles (variables). Confidence ellipses are presenting 95% confidence intervals of two group of variables.

4. Conclusions

This work regarding Croatian Plavac mali wines made by five commercially available *Saccharomyces* yeast strains has pointed out the important role that yeast can have in defining the chemical composition as well as visual properties of red wines. The results presented here showed that target yeast selection can help wine producers to achieve an easily desired wine style with greater ageing capacity and color intensity. The presented results pointed out a marked difference in individual polyphenol compound adsorption, namely delphinidin and petunidin-3-*O*-glucoside, as well as gallic acid and some flavan-3-ols between tested yeasts, with Fermol Super 16 being the one with the lowest and Lalvin D21 with the strongest adsorption ability. Wine color intensity was also influenced by the tested strains, which indicated out the possibility of modulating the style of a Plavac mali by the use of commercial yeasts. Finally, we have confirmed the impact of the geographical and pedological heterogeneity of vine growing locations on the polyphenol composition of Plavac mali wines produced by different yeasts.

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