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The Effects of a *Saccharomyces cerevisiae* Strain Overexpressing the Endopolygalacturonase PGU1 Gene on the Aminoacidic, Volatile, and Phenolic Compositions of *Cabernet Sauvignon* Wines

Mónica Fernández-González [1], Pedro Miguel Izquierdo-Cañas [2,3], Esteban García-Romero [3], Tania Paniagua-Martínez [1] and Sergio Gómez-Alonso [1,*]

[1] Instituto Regional de Investigación Científica Aplicada (IRICA), Universidad de Castilla-La Mancha (UCLM), Avda. Camilo José Cela s/n, 13071 Ciudad Real, Spain; monica.fernandez@uclm.es (M.F.-G.); tania.paniagua@uclm.es (T.P.-M.)
[2] Parque Científico y Tecnológico de Castilla-La Mancha, Paseo de la Innovación, 1, 02006 Albacete, Spain; pmizquierdo@jccm.es
[3] Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF-IVICAM) Crta, Toledo-Albacete s/n, 13700 Tomelloso, Spain; estebang@jccm.es
* Correspondence: sergio.gomez@uclm.es

Abstract: The addition of pectinase enzymes during the maceration stage of grape skins in order to improve the extraction yields and color of red wines is a common practice in many wineries. The objective of this work was to study in depth the changes that occurred in the aminoacidic, volatile, and phenolic compositions of Cabernet Sauvignon wines fermented with a *Saccharomyces cerevisiae* strain genetically modified with the gene encoding for endopolygalacturonase (*PGU1*) in transcriptional fusion with the promoter of the phosphoglycerate kinase (*PGK1*) gene, both from *S. cerevisiae* origin. A higher yield extraction of wine was obtained in wines fermented with the modified strain (PW), increasing by around 6.1% compared to the control wine (CW). Moreover, there was a 40% decrease in the malic acid content in the PW, thus suggesting that this modified yeast could be investigated as a malic acid-reducing agent. There were slight differences in other aroma volatile compounds studied as well as in the phenolic content. However, there was a considerable increase in the amino acid content in the PW.

Keywords: polygalacturonase; phenolic compounds; wine; PGU1

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1. Introduction

The enzymatic processes occurring during winemaking, whether they come from the grapes employed and their endogenous microflora or added in later processes, are essential from primary stages, such as juice extraction, clarification, or filtration, to other more complex ones, such as aroma enhancement or microbiological control [1]. Enzyme production and its subsequent application in wine is regulated by the International Organisation of Vine and Wine (OIV) throughout the European Union; they allow for the production of these enzymes from microorganisms as long as they are GRAS (Generally Recognized as Safe) [2]. Most of the enzymes currently used in the food industry are obtained from microorganisms previously selected and cultivated on an industrial scale [3,4].

Focusing on pectolytic enzymes, these preparations act by breaking down the pectic fraction that appears as the structural polysaccharides in the middle and primary lamellae of the grape cell wall [5,6], facilitating the release of phenolic and aroma compounds [7–9]. In the literature, this technique provides not only an increase in the extracted volume of juice [3] but also increases in the color intensity and phenolic content at the end of fermentation [10–13], but some authors have reported no effect and even a decrease in

the anthocyanin content or in the color of wines [12,14,15]. These discrepancies may be due to differences in the grape polyphenol compositions, in their extraction rates into the wines, and in their subsequent reactions in wine, depending on many parameters, such as vineyard conditions, the maturity of grape berries, and the wine-making techniques [7,16]. These enzymes may also improve the stability, taste, and structure of red wines, because not only may anthocyanins be released from the skins, but also tannins bound to cell walls may be better extracted because of enzymatic action [12,13,16,17]. These pectinase enzyme preparations are normally obtained from *Aspergillus niger*, producing large quantities. However, commercial preparations hardly ever involve single enzymes but a blend of them [18], either because they are released as a consequence of natural side activities of the microorganism or because they are added. This causes, in addition to the main enzymatic process for which they are named, a series of other enzymatic reactions to take place. In this context, the comparison of commercial products becomes very difficult, especially when looking only at the main activity.

Most strains of *Saccharomyces cerevisiae* possess the endopolygalacturonase (PG)-encoding gene named *PGU*1 [19,20] that randomly catalyzes the hydrolysis of α-1,4 glycosidic linkages in polygalacturonic acid; however, under enological conditions, most of them lack pectinase activity. The overexpression of *PGU*1 integrated in one single copy in the genome could be an alternative to the use of fungal enzymes [21]. It has also been reported that a wine strain of *Saccharomyces paradoxus* has strong pectinase activity [19]. Although a few indicators revealed that PG activity was indeed present in wines made by the mutants overexpressing the *PGU*1 gene of either *S. cerevisiae* or *S. paradoxus* (i.e., the enzyme activity in cell-free wine, a higher methanol concentration, and higher free-run wine), no clear impact on wine composition was noticed regarding the phenolic composition [22].

According to the OIV resolution OENO-MICRO 14-546 and the International Oenological CODEX, genetically modified enological yeasts can be used for inoculating grapes, musts, and wines to initiate or ensure the completion of alcoholic fermentation, as well as for producing special wines. However, the same OIV resolution states that authorization from competent authorities is required, and the requirements for genetically modified microorganisms (GMMs) to be authorized vary from country to country. This has limited the use of GMMs in the wine industry [23].

For that reason, the aim of this work was to study the effect of an endopolygalacturonase overexpressing *S. cerevisiae* (CECT11783) strain engineered using the *PGU*1 gene, transcriptionally bonded to the *PGK*1 gene promoter from *S. cerevisiae* [21,24], on the color, general composition, and individual phenolic content of Cabernet Sauvignon wine.

2. Materials and Methods

2.1. Winemaking

Healthy red grapes from *Vitis vinifera* L. var. Cabernet Sauvignon were manually harvested at their optimal ripening stage (25.8 °Brix, 7.3 g/L titratable acidity, pH 3.3). After harvest, grapes were crushed, stemmed, and sulphited (50 mg total SO_2/L). The crushed grapes were then distributed into six homogeneous batches of 6 kg each and placed in 8 L tanks equipped with a submerged cap system in accordance with the winemaking method described by Sampaio et al. (2007) [25]. Three batches were assigned as control wine (CW) and inoculated with 2×10^6 cell/mL of *S. cerevisiae* UCLMS-1 (ScC, *S. cerevisiae* control); the remaining three batches were inoculated with *S. cerevisiae* UCLMS-1M overexpressing the *PGU*1 gene (ScP, *S. cerevisiae* with polygalacturonase activity) [21,24] and labeled as polygalacturonase wine (PW). All of these microvinifications were maintained at a room temperature of 25 ± 1 °C and were controlled daily by measuring the juice temperature and density using a portable density meter (Mettler Toledo, L'Hospitalet de Llobregat, Spain). After completion of fermentation, free-run wine was recovered, and its volume measured. Wines were then racked and sulphited (30 mg total SO_2/L) to prevent malolactic fermentation as it has introduced a new variable in the study which could have masked

the effects of the modified yeast strain. After cold stabilization for one week at 4 °C, wines were bottled and stored in a dark cellar at 15 ± 3 °C until analysis.

2.2. Viability and Analysis of Implantation of Inoculated Strains

Samples were taken periodically to estimate cellular viability; these were plated on YPD agar (Condalab, Madrid, Spain). The resulting colonies were counted after 48 h of incubation at 28 °C. To ascertain the dominance of the inoculated strain, 10 isolates were randomly selected and subjected to delta sequence typing by using the primers δ12–δ21 as previously described [26], and δ-PCR products were analyzed by electrophoresis in 1.5% agarose gel according to the standard procedure for identification at strain level. To confirm that the ScP strain retained pectinase activity, these isolates were grown on plates complemented with polygalacturonic acid (PGA) as a substrate at pH 3.5 [27].

2.3. Chemical Analysis

Wines were analytically characterized to determine the following parameters according to the official analytical methods established by the International Organisation of Vine and Wine [28]: alcohol strength, total acidity (as tartaric acid equivalents), pH, volatile acidity (as acetic acid), free and total SO_2 concentrations, L-malic acid, L-lactic acid, citric acid, tartaric acid, succinic acid, glycerine, color intensity (IC), and tonality. Total polyphenols were determined by measuring absorbance at 280 nm after appropriate sample dilution [29].

CIELAB parameters were obtained following the OIV method [28] using an Agilent 8453 diode array spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) with a homemade program for spectra treatment. The measuring conditions were as follows: transmittance between 770 and 380 nm with 5 nm intervals, 1 mm cuvettes, a D65 illuminant, and a 10° reference pattern observer. Results are expressed with reference to 1 cm optical length.

2.4. Amino Acid Analysis

Amino acid contents were determined by liquid chromatography by following the method proposed in 2007 by Gómez-Alonso et al. [30]. A Varían ProStar HPLC (Varian, Inc., Walnut Creek, CA, USA) was used, equipped with a ProStar 240 pump, a ProStar 410 autosampler, and a ProStar 330 photodiode array detector. Separated compounds were identified based on the aminoenone derivative retention times of the corresponding standard (Sigma-Aldrich, Steinheim, Germany) and quantified by using the internal standard method.

2.5. Volatile Compound Analysis

Volatile compounds were analyzed by GC–MS using a Thermo Quest model Trace GC gas chromatograph (ThermoQuest, Waltham, MA, USA) and a DSQII mass detector with a single quadrupole analyzer (ThermoQuest, Waltham, MA, USA). The chromatographic column used was a BP21 column (SGE) (50 m × 0.32 mm internal diameter and 0.25 µm phase thick) of Free Fatty Acid Phase (FFAP) (a nitroterephthalic acid (TPA)-modified polyethylene glycol).

For the major volatile compounds, 1 µL of the samples was directly injected with 4-methyl-2-pentanol as an internal standard (final concentration 20 mg/L). The chromatographic conditions were as follows: carrier helium gas (1.7 mL/min, split 1/25), an injector temperature of 220 °C, and an oven temperature of 43 °C for 5 min, increasing by 4 °C/min to 100 °C, increasing by 20 °C/min to 190 °C, and maintaining 190 °C for 45 min.

Minor volatile compounds were extracted by SPE using the method previously described in reference [31]. A volume of 25 mL of wine passed through columns filled with 0.2 g of LiChrolut EN (40–120 µm, Merck, Darmstadt, Germany) using 500 µL of 4-nonanol 0.1 g/L as the internal standard. The column was later washed with 25 mL of Milli-Q water to remove sugars, acids, and other polar substances. The minority fraction of volatile compounds (free flavor) was eluted with 15 mL of pentane/dichloromethane (2:1 v/v).

Extracts were concentrated by distillation with a Vigreux column and subsequently under a stream of N_2 to 150 µL and stored at −20 °C until analysis. A volume of 2 µL of the extract was then injected into the column. Chromatographic conditions were as follows: oven temperature of 43 °C (15 min), increasing by 2 °C/min to 125 °C, by 1 °C/min to 150 °C, and by 4 °C/min to 200 °C (45 min hold). Helium was used as carrier gas (1.4 mL/min, split 1/15, splitless time of 0.5 min.).

Compounds were identified by their mass spectra and chromatographic retention times compared with standard commercial products. Quantification was performed by analyzing m/z fragments characteristic for each compound using the internal standard method. Results for non-available products were expressed in concentration units (µg/L or mg/L), and internal standard equivalents were obtained by normalizing the compound peak area to that of the internal standard and multiplying by the concentration of the internal standard.

2.6. Phenolic Compounds

2.6.1. Sample Preparation for Flavonol and Hydroxycinnamic Acid Derivative Analysis

Anthocyanin-free fractions were prepared to analyze flavonols and hydroxycinnamic acid derivatives by using ECX SPE cartridges (40 µm, 500 mg, 6 mL; Scharlab, Barcelona, Spain) according to the procedure previously described in 2009 by Castillo-Muñoz et al. [32]. The methanolic eluate was dried in a rotary evaporator (35 °C) and re-dissolved in 1.5 mL of 20% methanol in water and directly injected onto the HPLC equipment.

2.6.2. Sample Preparation for Flavan-3-ols Analysis

Flavan-3-ols (monomers, B-type dimers, and polymeric proanthocyanidins) were isolated from wines using SPE on C18 cartridges (Sep-pak Plus C18, Waters Corp., Mildford, MA, USA; cartridges filled with 820 mg of adsorbent) according to the procedure described by Lago-Vanzela et al., 2011 [33], and stored at −18 °C until needed.

2.6.3. HPLC-DAD-ESI-MSn Identification of Phenolic Compounds

Anthocyanins and non-anthocyanin phenolic compounds from wines, namely flavonols and hydroxycinnamic acid derivatives (HCADs), were separately analyzed following the methodology previously described [34]. An analysis was performed on an Agilent 1100 series system equipped with a photodiode array detector (DAD) and a LC/MSD Trap VL electrospray ionization mass spectrometry (ESI-MS/MS) coupled with an Agilent ChemStation workstation for data processing.

For anthocyanin analysis, 10 µL of diluted extracts was injected, whereas 20 µL of anthocyanin-free extract fractions was used for non-anthocyanin phenolic analysis. Injections were made after filtration (0.20 µm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany) on a reversed-phase column, Zorbax Eclipse XDB-C18 (2.1 × 150 mm; 3.5 µm particle; Agilent, Germany), thermostated at 40 °C.

Identification was mainly based on spectroscopic data (UV-Vis and MS/MS) obtained from authentic standards or previously reported in the literature [32,35]. For quantification, DAD chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols), and 320 nm (hydroxycinnamic acid derivatives).

2.6.4. Identification and Quantification of Flavan-3-ols and Stilbenes Using Multiple Reaction Monitoring HPLC-ESI-MS/MS

The analysis was carried out with an HPLC Agilent 1200 series system equipped with DAD (Agilent, Waldbronn, Germany) and coupled to an AB Sciex 3200 QTRAP (Applied Biosystems, Foster City, CA, USA) mass spectroscopy system with a turbo spray source (ESI-MS/MS) controlled through the Analyst MSD software (Applied Biosystems, version 1.5).

Samples were injected before and after acid-catalyzed depolymerization reaction (20 µL) on a reversed-phase Ascentis C18 column (4.6 × 150 mm; 2.7 µm particle; Supelco, Bellefonte, PA, USA) thermostated at 16 °C. The structural information of proantho-

cyanidins was obtained following acid-catalyzed depolymerization induced by pyrogallol, an alternative nucleophile trapping agent that offers similar results when compared to the classic phloroglucinol method, but which also functions under milder experimental conditions [34].

The solvents and gradients used for this analysis and the Multiple Reaction Monitoring (MRM) settings as well as all of the mass transitions (m/z) for identification and quantitation were chosen according to the methodology reported by Lago-Vanzela et al., 2011 [33].

2.7. Sensory Analysis

A descriptive test was performed to identify differences among both wines obtained from the two yeast strains. Wines were analyzed by a panel of expert assessors (between 25 and 50 years of age) who were staff members from the Castilla-La Mancha Institute of Vin and Wine, Spain, with experience in sensory analysis. Previously, assessors were trained in descriptive sensory analysis over several sessions using discriminative tests.

Descriptive sensory analysis was performed by 20 selected panelists following the Sensory Profile method according to ISO Standard 11035:1994 [36]. The descriptors were scored on a structured scale with a range of 0–5 (with 0 being an absence of the descriptor and 5 being the maximum intensity).

2.8. Statistical Analysis

Student's *t*-test was applied to compare results between CW and PW samples to determine whether there were significant differences between them related to chemical characteristics, amino acids, and phenolic compounds as well as sensory characteristics by using SPSS software package version 22.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Fermentation Evolution and Implantation

According to the experimental results, the yeast strain had a significant influence on the fermentation kinetics, with the overexpressed PGU1 gene strain (ScP) being faster than the control (ScC) (Figure 1), which needed three more days (11 vs. 8) to finish the alcoholic fermentation process. This may be explained because nitrogen availability is a fermentation-limiting factor [37], and the free amino acid content in the PW was significantly higher than that of the CW due to polygalacturonase activity, as is shown in Section 3.3.

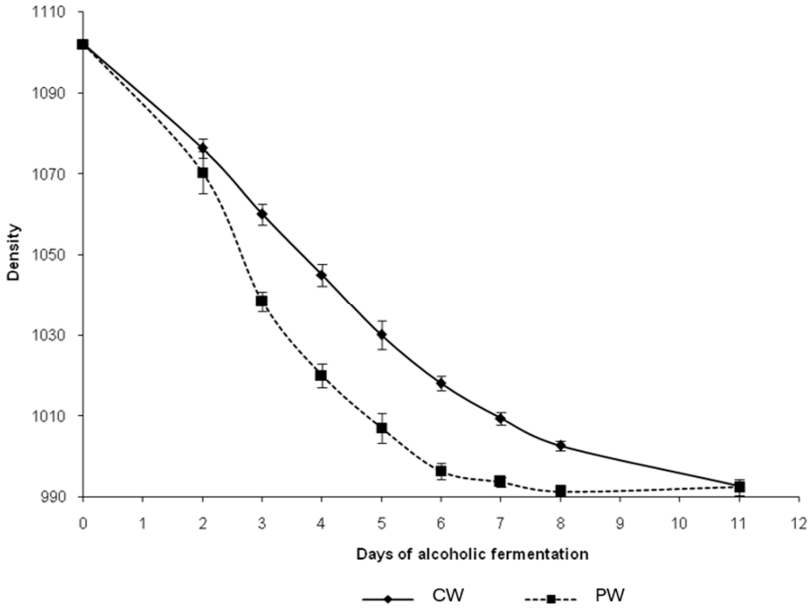

Figure 1. Evolution of alcoholic fermentation, measured as density changes, for both CW and PW samples.

The total yeast population was quite similar in all fermentations irrespective of the yeast strain used (ScC/ScP) and reached a population of around 10^8 cfu/mL after three days. The results of the implantation studies show that in both sets of wines, CW and PW (Figure 2), all of the isolates analyzed presented the same delta profile as that of the starter cultures used, respectively, thus confirming that the yeasts finally implemented in both sets of wines were those initially inoculated. ScC and ScP showed the same genetic profile for the amplified delta regions, as genetic modification does not affect these sequences. Since this delta profile study did not allow for differentiation between ScC and ScP, different colonies obtained from both groups of samples were grown in PGA agar plates at pH 3.5 and incubated at 30 °C/24 h. Only ScP colonies obtained from PW samples were able to produce halos by hydrolysis of polygalacturonic acid on plates.

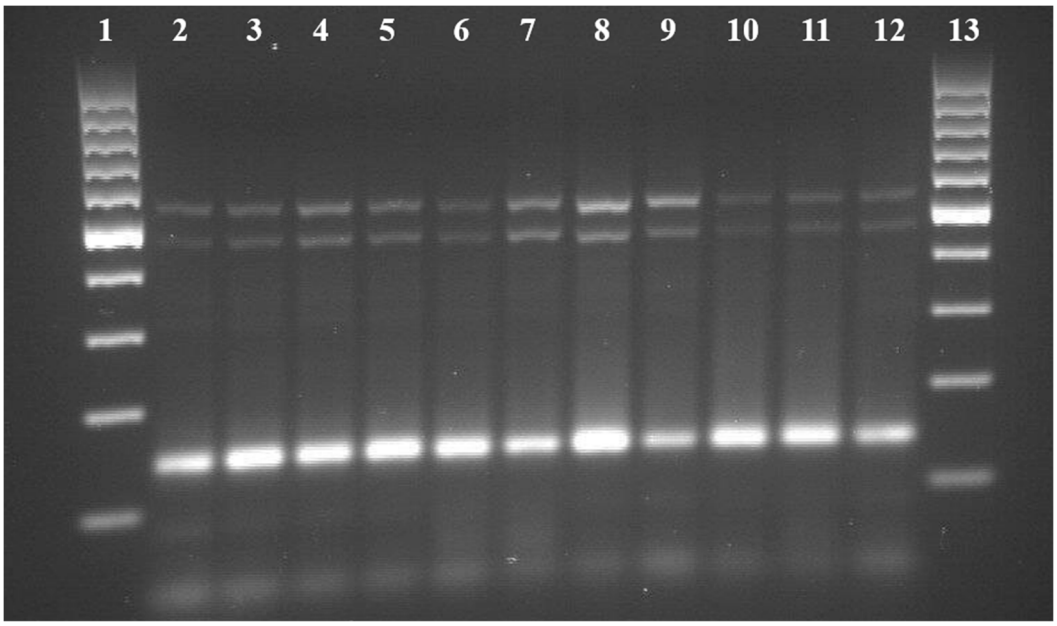

Figure 2. δ-PCR amplification patterns of PW. Implantation was observed by comparing profiles of ten isolated colonies (lanes 2 to 11) of PW to *ScP* strain used for inoculation (lane 12); 100 bp DNA ladder marker (Biotools, Madrid, España) served as size standard for PCR (lanes 1 and 13).

3.2. Physicochemical Analysis

The results of the physicochemical parameters analyzed are summarized in Table 1. These show an increase of 6.1% in the volume of free-run wine obtained for the PW, which was expected due to its polygalacturonase activity, causing a higher degradation of their cellular structure and facilitating juice extraction from grape berries during fermentation [3,22]. This effect was visually observed during vinification by a noticeable degradation of grape berries in the PW just after 3 days of fermentation, while the CW still showed intact berries. However, this increase in free-run wine in the PW had no effect on their alcoholic strength, as they were the same in both set of wines.

On the other side, statistically significant lower malic and succinic acid contents were observed in the PW samples, results that were also reflected in the total acidity; this would also explain the slightly higher value of the pH in the PW samples compared to the control samples. It was excluded that the difference in the malic acid content between both groups was due to malolactic fermentation since the lactic acid content was very low in both groups of wines.

Saccharomyces cerevisiae can degrade malic acid through two pathways during alcoholic fermentation: they can produce ethanol, converting L-malic into pyruvate by malic enzymes, and then transforming it into acetaldehyde by pyruvate decarboxylase, and finally into ethanol by alcohol dehydrogenase, or they can produce succinate, converting L-malic into fumaric acid by fumarase and then transforming it into succinate by fumarate

reductase [38]. In this work, it seems that the pathway followed by ScP is the first one because the alcohol strength is practically the same as that of the CW, even though the final wine volume has increased. On the contrary, the amount of succinate does not increase but decreases compared to the control. More studies would need to be conducted to determine whether ScP could be used as a malic acid-reducing agent since *S. cerevisiae* normally reduces malic acid by about 10–25% [39], and in this case, it was reduced by around 40% compared to the CW.

Table 1. Physicochemical parameters of wine samples inoculated with ScC (CW) and ScP (PW).

	CW	**PW**
Density (g/mL)	0.991 ± 0.002	0.991 ± 0.054
Yield of extraction [1] (%)	66.7 ± 0.6 #	72.8 ± 1.2 #
Alcohol strength (% *v*/*v*)	14.3 ± 0.0	14.4 ± 0.1
Total acidity (g/L)	6.11 ± 0.03 #	5.10 ± 0.10 #
pH	3.45 ± 0.00 #	3.51 ± 0.01 #
Total SO_2 (mg/L)	81.67 ± 2.31	85.67 ± 1.15
Glucose + Fructose (g/L)	2.64 ± 0.52	2.48 ± 0.71
L-malic acid (g/L)	1.87 ± 0.06 #	1.14 ± 0.02 #
Tartaric acid (g/L)	1.11 ± 0.14	1.20 ± 0.03
Citric acid (g/L)	0.22 ± 0.01	0.20 ± 0.01
Succinic acid (g/L)	1.27 ± 0.02 #	1.02 ± 0.03 #
L-lactic acid (g/L)	0.11 ± 0.01 #	0.08 ± 0.02 #
Glycerine (g/L)	10.92 ± 0.18	11.06 ± 0.11
Acetic acid (g/L)	0.23 ± 0.01 #	0.15 ± 0.02 #
Meso-2,3-butanediol (g/L)	0.11 ± 0.01 #	0.19 ± 0.01 #
Levo-2,3-butanediol (g/L)	0.39 ± 0.02 #	0.62 ± 0.04 #
L*	18.02 ± 0.29	18.05 ± 0.30
a*	50.53 ± 1.01	50.58 ± 1.61
b*	29.93 ± 1.33	29.52 ± 2.10
C*	58.73 ± 1.52	58.57 ± 2.45
H	30.63 ± 0.66	30.24 ± 1.00
Colorant Intensity	9.72 ± 0.58	10.12 ± 0.46
Tonality	0.58 ± 0.01	0.58 ± 0.01

# denotes statistically significant differences ($p \leq 0.05$) between different strains used. CW refers to control wine samples inoculated with ScC, while PW refers to samples inoculated with modified ScP strain. [1] Yield of extraction was calculated as liters of free-run wine obtained per kilogram of crushed, stemmed grapes fermented and expressed as percent.

The contents of both 2,3-butanediol isomers were also influenced by the yeast strain applied, with higher values being obtained in the PW samples. This increase has also been observed in some studies with commercial enzymes with pectinase activity [40]. Both levo-2,3-butanediol and meso-2,3-butanediol provide the fruity, sweet, and buttery notes to the wine's aroma, and these higher alcohols are synthesized from acetoin reduction [41], a compound which, as mentioned in a later section, was also significantly higher in the PW samples.

The lower contents of malic and succinic acid in the PW samples could be directly related to the higher content of acetoin in these samples and, consequently, of the 2,3-butanediol isomers since acetoin is a by-product obtained from carbohydrate metabolism that occurs through different routes described in the literature [42].

Finally, as for the color parameters, no differences were found between the two sets of wines, which does not agree with the study concerning the application of commercial enzymes with pectinase activity, which indicates greater color intensity [3]. However, this could be explained by the fact that, with the same quantity of grapes, the volume extracted was higher in the PW samples, thus compensating for the higher release of anthocyanins and total phenols by the higher volume obtained, and also for the higher pH of the PW samples, since it is known that pH has an effect on the coloration of anthocyanins.

3.3. Amino Acid Content

The results concerning the amino acid content determined in the CW and PW are shown in Table 2. The content was 24.7% higher in the PW samples compared to the total amino acid content in the control wines, with proline being the significantly more abundant amino acid, which is in agreement with the results shown in references [43,44], and it is responsible for 99% of this higher total content. This amino acid content increase has recently been observed in some studies after the application of pectinase enzymes during winemaking [45], and it could be explained due to the release of nitrogenous compounds located in the cell walls of grape skins as a consequence of polygalacturonase action. Although proline is one of the most predominant amino acids in grape juice, it is poorly assimilated by wine yeasts under the anaerobic conditions typical of most fermentations; therefore, it is not considered to be an assimilable nitrogen for them. This may explain its higher concentration in the PW due to a greater extraction rate and no consumption by the yeast [46].

Table 2. Free amino acid content found in CW and PW samples, expressed as mg/L.

	CW	PW	Percentage Variation (%)
Glutamic Acid + Glutamine	7.34 ± 0.66 #	12.33 ± 0.09 #	168
Asparagine + Hydroxyproline	40.01 ± 1.73 #	30.18 ± 0.43 #	75.4
Serine	2.42 ± 0.02 #	3.37 ± 0.45 #	139
Histidine	2.36 ± 0.33 #	3.41 ± 0.06 #	144
Glycine	1.80 ± 0.04 #	5.26 ± 0.63 #	292
Threonine	0.43 ± 0.03 #	0.93 ± 0.01 #	216
β-Alanine + Arginine	3.96 ± 0.33 #	6.15 ± 0.17 #	155
L-Alanine	1.35 ± 0.09 #	5.30 ± 0.14 #	392
γ-Aminobutyric Acid	0.61 ± 0.06 #	1.14 ± 0.02 #	187
Proline	3802 ± 71 #	4749 ± 28 #	125
Tyrosine	2.73 ± 0.40 #	4.00 ± 0.17 #	146
Amonia	0.26 ± 0.04 #	0.35 ± 0.03 #	135
Valine	0.33 ± 0.05 #	0.77 ± 0.02 #	233
Cysteine	0.83 ± 0.12 #	1.05 ± 0.01 #	126
Methionine	0.54 ± 0.11	0.45 ± 0.02	-
Tryptophan	1.37 ± 0.26	1.08 ± 0.11	-
Isoleucine	0.30 ± 0.03 #	0.44 ± 0.07 #	147
Leucine	0.35 ± 0.08 #	0.57 ± 0.03 #	163
Phenylalanine	0.14 ± 0.02 #	0.35 ± 0.04 #	250
Ornithine	0.60 ± 0.06 #	1.34 ± 0.03 #	223
Lysine	0.38 ± 0.07	0.37 ± 0.11	-
TOTAL	3870 ± 76 #	4827 ± 31 #	125

# denotes statistically significant differences ($p \leq 0.05$) between different strains used. CW refers to control wine samples inoculated with ScC, while PW refers to samples inoculated with modified ScP strain.

As for the rest of the amino acids, all of them increased except for methionine, tryptophan, and lysine, for which no significant differences were found between both groups of samples, as well as for asparagine and hydroxyproline, which decreased significantly in the PW.

Percentagewise, the amino acids that increased the most with the use of the modified yeast were glycine, threonine, L-alanine, valine, phenylalanine, and ornithine, with all of them having variations greater than 200% compared to those in the control wines. The different proportions in each of the amino acid changes can be explained by their different contents in the grape tissues, which determine the amounts in which they are released, and the different utilization rates by the yeast for each one.

3.4. Volatile Compound Analysis

It is during the alcoholic fermentation stage that a large amount of volatile compounds are produced, which will influence the final wine aroma; the availability of sugars and

nitrogen compounds is a decisive factor in the development of these compounds, especially those belonging to higher alcohols and esters [47]. Therefore, due to the results described in the previous section, it is to be expected that the PW samples, with a higher amino acid content, will have a more complex aromatic profile [48].

Table 3 shows the concentrations of different volatile compounds determined in both the CW and PW groups of wines by GC/MS. As expected, the methanol production increased in the PW, confirming the results shown in previous studies by applying different pectinase enzymes [20,22,49]. This higher methanol content in the PW samples may be explained as a release due to the demethylesterification of the cell wall polymethylpolygalacturonans of the grape. Nevertheless, the methanol content in all samples was still much lower than the highest concentration permitted by the International Organisation for Vine and Wine, i.e., 400 mg/L in red wines (Resolution OENO 19/2004).

Table 3. Volatile compounds identified in control and PW samples.

	CW	PW		CW	PW
Esters			Carbonylic compounds		
Ethyl acetate [1]	33.5 ± 2.2	29.3 ± 0.9	Acetaldehyde [1]	12.5 ± 0.5	19.3 ± 0.9
Isoamyl acetate [1]	0.75 ± 0.10	1.02 ± 0.11	Acetoin [1]	2.03 ± 0.24	4.98 ± 0.22
2-Phenylethyl acetate [2]	0.13 ± 0.02	0.31 ± 0.04	2-methyl-tetrahydro-thiofen-3-ona [2]	15.9 ± 3.4	4.3 ± 1.6
Ethyl dodecanoate [2]	15.7 ± 2.1	9.8 ± 1.7	3-hydroxy-4-phenyl-2-butanone [2]	17.6 ± 5.0	44.4 ± 13.1
Ethyl hexadecanoate [2]	25.9 ± 4.2	18.2 ± 0.7	Lactones		
Ethyl lactate [1]	4.49 ± 0.25	3.12 ± 0.24	δ-Decalactone [2]	10.9 ± 0.4	4.71 ± 0.87
Ethyl 3-hydroxy-decanoate [2]	11.01 ± 3.06	3.34 ± 1.14	γ-Decalactone [2]	1.51 ± 0.27	0.84 ± 0.30
Ethyl 2-hydroxy-phenylpropanoate [1]	19.5 ± 1.9	49.4 ± 4.8	4(1-OH-ethyl)-γ-butyrolactone [2]	7.43 ± 1.87	2.92 ± 0.28
Ethyl and methyl succinate [1]	7.16 ± 0.80	5.45 ± 0.61	Norisoprenoids		
Diethyl malate [2]	72.9 ± 9.0	35.6 ± 4.4	Damascenone [2]	6.75 ± 0.74	3.50 ± 0.28
Alcohols			3-Oxo-α-ionol [2]	42.8 ± 5.3	15.9 ± 2.4
Methanol [1]	65.9 ± 6.9	133.2 ± 4.8	Methoxyphenols		
Propanol [1]	21.4 ± 1.9	17.6 ± 0.8	Vanillin [2]	3.47 ± 0.66	1.47 ± 0.51
1-Butanol [1]	2.01 ± 0.12	3.31 ± 0.08	Acetovainillone [2]	54.9 ± 7.6	22.0 ± 2.2
Isobutanol [1]	50.0 ± 1.3	42.7 ± 2.0	Propiovainillone [2]	3.21 ± 0.95	1.17 ± 0.15
1-Octanol [2]	21.8 ± 1.6	26.4 ± 2.0	Methyl vanillate [2]	19.8 ± 3.5	9.9 ± 1.9
3-Methyl-thio-propanol [1]	0.25 ± 0.07	0.05 ± 0.01	t-Isoeugenol [2]	0.50 ± 0.13	0.20 ± 0.05
Acids			Volatile phenols		
Octanoic acid [1]	5.56 ± 1.76	2.31 ± 0.59	Guayacol [2]	30.6 ± 3.4	22.1 ± 2.6
Decanoic acid [1]	1.54 ± 0.16	0.58 ± 0.17	4-Ethyl-phenol [2]	1.66 ± 0.35	0.79 ± 0.21
9-Decenoic acid [2]	9.28 ± 2.66	3.36 ± 0.71	4-Propyl-guaiacol [2]	0.69 ± 0.09	0.25 ± 0.04
Geranic acid [2]	71.0 ± 16.1	29.1 ± 6.6	Bencenic alcohols		
Furanic compounds			2-Phenylethanol [1]	60.5 ± 8.6	104.5 ± 14.8
Hydroxymethylfurfural [2]	19.2 ± 3.4	9.5 ± 1.2			

[1, 2] denote concentrations expressed in mg/L (1) or ug/L (2). Only compounds with statistically significant differences ($p \leq 0.05$) are shown. CW refers to control wine samples inoculated with ScC, while PW refers to samples inoculated with modified ScP strain.

Mayor volatile compounds, such as acetoin, 1-butanol, isobutanol, and isoamyl acetate, also increased in the PW. The higher acetoin content observed in the PW samples studied is in agreement with the increase observed in both 2,3-butanediol isomers, since acetoin is its precursor, and the 1-butanol and isobutanol alcohols and isoamyl acetate ester increases can be explained by the higher content of amino acids, which are metabolic precursors of this group of volatile compounds [45].

Regarding minor volatile compounds, no differences were found for most of them. However, statistically significant differences were observed in 10 out of all esters analyzed. Given that the PW had a lower acetic acid concentration, this was subsequently reflected in a lower ethyl acetate content (4.2 mg/L units). However, the ScP strain produced 0.27 mg/L of isoamyl and 0.18 mg/L of 2-phenylethyl acetates more than the yeast control. The increases in some ethyl ester compounds in the PW samples can easily be explained because of their higher content of amino acids, which, as mentioned above, are metabolic precursors of this kind of compounds [45]. Also, the higher 2-phenylethyl acetate concentration can be explained by a higher 2-phenylethanol content in the PW; this increase was also reported

in studies with pectinase enzymes [50]. Based on other studies, increases in isoamyl and 2-phenylethyl acetate could create more fruity aromas [51] as well as floral/rose hints [52] in PW. The significantly lower contents of ethyl and methyl succinate, ethyl lactate, and diethyl malate in the PW samples are attributed to the lesser observed contents of their precursors, succinic, lactic, and malic acid.

The concentrations of the acids analyzed varied depending on the type of yeast strain employed. The octanoic, decanoic, 9-decenoic, and geranic acid contents were higher in the CW samples. These acids impart herbaceous and fruity, fatty or rancid notes to wine [53,54], even in low concentrations, because of their low perception threshold.

Higher concentrations of acetaldehyde and 3-OH-2-butanone were observed in the PW, with acetaldehyde being one of the most important sensory carbonyl compounds formed during alcoholic fermentation as it is associated with herbaceous and oxidative notes in wines [55], and 3-OH-2-butanone, at low concentrations, makes a positive contribution to the wine aroma, supplying buttery notes and adding complexity [56].

Finally, only a few differences were found in the contents of lactones, norisoprenoids, methoxyphenols, and volatile phenols, with all of these differences being due to a significantly lower content in the wines treated with the modified yeast. These four families of compounds exert a significant effect on the sensory quality of wine, as lactones contribute a fruity aroma [57], norisoprenoids contribute fruity, floral, or spicy notes [53], and methoxyphenol compounds contribute highly appreciated spicy and smoky aromas [58]. As for the group of volatile phenols, ethyl phenols are particularly important because they undermine the final quality of wine. Significant differences were observed between the two wines, with lower contents of 4-ethylphenol and 4-propilguaiacol being obtained in the PW samples. These compounds have an unpleasant animal odor described as leather and even as horse sweat and are serious defects in wine when they exceed the perception thresholds [59], so this reduction in the samples treated with the modified yeast is therefore of great interest, especially considering the results observed in one study, which shows how after the application of commercial pectinase enzymes such as vinylphenols, volatile phenol precursors increase due to the residual cinnamate esterase activity present in these enzyme preparations [40], increasing the contents of cinnamic acids in the medium, which, in turn, are precursors of vinylphenols.

According to some results found in the literature, enzymatic application during winemaking resulted in wines with a higher volatile content, which is responsible for giving wines fruitier notes [40], an increased monoterpene content [60], as well as norisoprenoid and benzene compounds, allowing for wines with more honey, lime, and smoky attributes to be obtained [61]. Most of these volatile compounds are found in their glycosylated form in grape berries and would thus be transferred to the wine without contributing to its aroma. For these compounds to contribute to the sensory perception of wine, they must be released by the action of glycosidase, which may be present in enzymatic preparations either intentionally or as residual activities [2]. However, this increase was not observed after the application of the modified yeast ScP, thus suggesting that it does not exhibit glycosidase activity.

3.5. Phenolic Compounds

The total polyphenol content was determined in both groups of samples prior to the analysis using independent groups of phenolic compositions, obtaining a total polyphenol index of 47.67 in the wines treated with the control yeast (ScC) and a value of 50.33 for the wines treated with the modified one (ScP). These results indicate that there was no significant increase in the total polyphenol concentration, contrary to several studies that used an enzymatic application of pectinase activity [62,63], but there was a higher extraction value of phenolic compounds since their concentration in the PW did not diminish in spite of the significative increase in the wine extraction yield.

There were only slight differences in the phenolic compositions of the wines. A total of 19 anthocyanins were identified (Table 4), 4 of which were higher in the PW, namely

delphinidin and cyanidin 3-glucosides, vitisin B, and the caffeoylated derivative of malvidin 3-glucoside; however, this increase was not reflected in the total anthocyanin content. On the other hand, the acetylated derivative of malvidin 3-glucoside was significantly lower in the wines treated with the modified yeast. Previous studies using pectolytic enzymes reflect a general increase for most of the anthocyanins studied [2,13], but there have also been studies in which this content did not vary [64]. In any case, the samples treated with the modified yeast did not show such significant results in terms of anthocyanin enrichment.

Table 4. Anthocyanin contents of CW and PW samples expressed as mg/L.

	CW	PW
Delphinidin 3-glucoside	13.57 ± 1.31 #	16.42 ± 0.96 #
Cyanidin 3-glucoside	0.51 ± 0.05 #	0.82 ± 0.05 #
Petunidin 3-glucoside	18.10 ± 2.00	18.46 ± 0.90
Peonidin 3-glucoside	5.55 ± 0.36	6.39 ± 0.42
Malvidin 3-glucoside	209.68 ± 13.10	192.04 ± 12.63
Delphinidin 3-acetylglucoside	6.29 ± 0.33	6.12 ± 0.21
Vitisin A	6.01 ± 0.31	5.91 ± 1.04
Vitisin B	10.02 ± 0.05 #	11.29 ± 0.34 #
Acetyl vitisin A	4.00 ± 0.16	3.88 ± 0.38
Petunidin 3-acetylglucoside	8.13 ± 0.47	8.26 ± 0.06
t-Delphinidin 3-coumaroylglucoside	1.11 ± 0.16	0.91 ± 0.09
Peonidin 3-acetylglucoside	3.04 ± 0.26	3.02 ± 0.07
Malvidin 3-acetylglucoside	82.97 ± 4.26 #	75.19 ± 3.42 #
t-Cyanidin 3-coumaroylglucoside	0.24 ± 0.03	0.26 ± 0.01
Malvidin 3-caffeoylglucoside	0.25 ± 0.07 #	0.46 ± 0.08 #
t-Petunidin 3-coumaroylglucoside	1.33 ± 0.14	1.46 ± 0.06
c-Malvidin 3-coumaroylglucoside	0.72 ± 0.04	0.80 ± 0.03
t-Peonidin 3-coumaroylglucoside	1.09 ± 0.08	1.04 ± 0.07
t-Malvidin 3-coumaroylglucoside	23.62 ± 1.92	20.89 ± 1.25
Total content	396.24 ± 23.86	373.61 ± 20.89

# denotes statistically significant differences ($p \leq 0.05$) between different strains used. CW refers to control wine samples inoculated with ScC, while PW refers to samples inoculated with modified ScP strain.

Regarding the flavonol content (Table 5), a total of 23 compounds were identified; 9 of them showed significant differences between the two groups of samples, with 6 of them being significantly lower in the PW samples (myricetin 3-glucoside, laricitrin 3-glucoside, and free myricetin, quercetin, laricitrin, and isorhamnetin), and 3 presented higher concentrations in those samples, namely the 3-galactoside derivatives of myricetin, quercetin, and syringetin. However, these small differences did not result in a significant change in the total flavonol content.

Table 5. Flavonol contents in CW and PW samples expressed as mg/L.

	CW	PW
Myricetin 3-glucuronide	2.56 ± 0.02 #	2.27 ± 0.11 #
Myricetin 3-galactoside	0.59 ± 0.01 #	0.71 ± 0.09 #
Myricetin 3-glucoside	16.61 ± 0.01	15.50 ± 0.28
Quercetin 3-galactoside	0.68 ± 0.01 #	0.74 ± 0.03 #
Quercetin 3-glucuronide	10.23 ± 0.26	9.92 ± 0.17
Quercetin 3-glucoside	4.72 ± 0.05	4.79 ± 0.30
Q-3-Rut	0.45 ± 0.08	0.45 ± 0.11
Laricitrin 3-galactoside	0.08 ± 0.02	0.16 ± 0.04
Myricetin	6.64 ± 0.08 #	4.84 ± 0.15 #
Laricitrin 3-glucoside	4.54 ± 0.04 #	4.26 ± 0.08 #
Kaempferol 3-galactoside	0.37 ± 0.11	0.42 ± 0.03
Kaempferol 3-glucoside	0.08 ± 0.04	0.09 ± 0.01
Kaempferol 3-glucuronide	0.52 ± 0.08	0.61 ± 0.05

Table 5. *Cont.*

	CW	PW
Isorhamnetin 3-galactoside	0.17 ± 0.04	0.12 ± 0.01
Isorhamnetin 3-glucoside	0.34 ± 0.01	0.31 ± 0.01
Isorhamnetin 3-glucuronide	0.14 ± 0.01	0.09 ± 0.01
Syringetin 3-galactoside	0.94 ± 0.01 #	0.99 ± 0.03 #
Syringetin 3-glucoside	4.07 ± 0.06	3.77 ± 0.15
Quercetin	7.21 ± 0.02 #	5.54 ± 0.23 #
Laricitrin	0.36 ± 0.01 #	0.28 ± 0.01 #
Kaempferol	0.90 ± 0.03	0.79 ± 0.17
Isorhamnetin	0.30 ± 0.02 #	0.24 ± 0.02 #
Syringetin	0.28 ± 0.01	0.23 ± 0.05
Total flavonols	62.70 ± 0.07	57.07 ± 0.33
Myricetin fraction	26.40 ± 0.05	23.32 ± 0.15
Quercetin fraction	23.26 ± 0.21	21.43 ± 0.04
Laricitrin fraction	4.97 ± 0.01	4.69 ± 0.06
Kaempferol fraction	1.86 ± 0.20	1.91 ± 0.14
Isorhamnetin fraction	0.94 ± 0.05	0.75 ± 0.01
Syringetin fraction	5.28 ± 0.06	4.98 ± 0.23
Trisubstituted	36.64 ± 0.01	32.98 ± 0.42
Disubstituted	24.20 ± 0.26	22.18 ± 0.04
Monosubstituted	1.86 ± 0.20	1.91 ± 0.14

# denotes statistically significant differences ($p \leq 0.05$) between different strains used. CW refers to control wine samples inoculated with ScC, while PW refers to samples inoculated with modified ScP strain.

For flavan-3-ols (Table 6), the results are similar to those of the other two groups of flavonoids previously commented on, with only slight differences in flavan-3-ol (−)-epicatechin, which was significantly higher in the PW samples, an increase that has also been observed in previous studies [9], but no changes were observed in the total flavan-3-ols content or in their mean degree of polymerisation (mDP) nor in the percentages of galloyllation and prodelphinidins.

Table 6. Flavan-3-ols contents in CW and PW simples expressed as mg/L.

	CW	PW
(+)-Catechin	50.83 ± 3.48	48.10 ± 2.30
(-)-Epicatechin	18.41 ± 0.66 #	19.94 ± 1.03 #
(+)-Catechin-3-gallate	18.95 ± 0.90	14.54 ± 0.10
(-)-Epigallocatechin	4.93 ± 0.38	3.73 ± 0.21
(-)-Epicatechin-3-gallate	0.59 ± 0.08	0.60 ± 0.07
(-)-Epigallocatechin-3-gallate	0.24 ± 0.04	0.18 ± 0.01
Procyanidin B1	31.97 ± 1.70	26.01 ± 1.60
Procyanidin B2	11.07 ± 0.63	11.50 ± 0.57
PB RT 31,9	2.23 ± 0.11	1.88 ± 0.32
PB RT 38,9	0.38 ± 0.01	0.40 ± 0.08
Terminal units	162.42 ± 4.31	125.86 ± 1.46
Extension units	319.42 ± 22.79	289.57 ± 1.80
Total flavan-3-ols	575.76 ± 15.50	502.50 ± 2.02
mDP (n monomeric units)	2.96 ± 0.18	3.30 ± 0.01
% Galloylation	3.59 ± 0.01	3.80 ± 0.12
% Prodelphinidins	37.01 ± 0.79	35.81 ± 0.76

# denotes statistically significant differences ($p \leq 0.05$) between different strains used. CW refers to control wine samples inoculated with ScC, while PW refers to samples inoculated with modified ScP strain.

Among the non-flavonoid phenolic compounds analyzed, hydroxycinnamic acid derivative (Table 7) and stilbene (Table 8) differences were only found for the hydroxycinnamic acid derivative cis-fertaric acid, which was lower in the samples treated with the modified yeast.

Table 7. Hydroxycinnamic acid derivative contents in CW and PW samples expressed as mg/L.

	CW	PW
t-Caftaric acid	62.26 ± 2.52	54.03 ± 1.55
t-Coutaric acid	23.82 ± 0.56	20.11 ± 0.59
c-Coutaric acid	4.30 ± 0.19	3.64 ± 0.18
t-Fertaric acid	10.93 ± 0.05	10.19 ± 0.47
c-Fertaric acid	5.57 ± 0.04 #	4.60 ± 0.25 #

# denotes statistically significant differences ($p \leq 0.05$) between different strains used. CW refers to control wine samples inoculated with ScC, while PW refers to samples inoculated with modified ScP strain.

Table 8. Stilbene contents in CW and PW simples expressed as mg/L.

	CW	PW
t-Resveratrol-glucoside	0.67 ± 0.03	0.63 ± 0.10
c-Resveratrol-glucoside	0.67 ± 0.01	0.60 ± 0.04
c-Resveratrol	0.55 ± 0.11	0.37 ± 0.02

No statistically significant differences ($p \leq 0.05$) between the different strains used were found. CW refers to the control wine samples inoculated with ScC, while PW refers to the samples inoculated with the modified ScP strain.

The results of all groups of phenolic compounds analyzed showed that, despite what was expected due to the higher degradation of grape berry skins by enzymatic action in the samples treated with the modified yeast ScP, which has shown a great impact on the amino acid concentrations of these wines, overall, there were no differences between the two groups of samples studied for phenolic compounds. These results agree with the conclusions of some studies found in the literature with enzymatic preparations [2,9,14]. Conversely, Eschstruth et al. [22] obtained different results by working with two different *S. cerevisiae* strains overexpressing the PGU1 gene, given that the total flavan-3-ol and B2 dimer contents were significantly lower in the PW, whilst these two groups of phenolic compounds did not vary in our study. On the other hand, quercetin showed the opposite tendency of our study, increasing significantly in the PW. A possible explanation for this effect is the interactions between the grape cell wall material released by pectinase at the same time as grape skin phenolic compounds, as demonstrated in the model solutions by Beaver et al., 2020 [65]. Another factor to consider is the higher yield of wine obtained when fermenting with ScP yeast, which could partially compensate a higher extraction of phenolic compounds from the skins due to the dilution effect.

3.6. Sensory Analysis

A descriptive sensory analysis was performed according to ISO Standard 11035 [36]. Figure 3 indicates the results of the descriptive sensory assay performed on both sets of wine. Despite slight variations, the statistical results indicate that the samples did not differ significantly for any of the attributes studied, except for the violet hue, which was higher in the CW. Although some studies have described an increase in the color intensity of wines treated with enzyme preparations [2], in our study, both the physicochemical and sensory results indicate that this effect was not significant with the use of the modified yeast ScP. There were also no significant changes in the perception of the cherry red color nor in its corresponding instrumental measurement, $a^{\#}$ component of the CIELab system. Furthermore, the greater violaceous color observed by the tasters was not supported by the instrumental color determination since no significant differences were observed in terms of the CIELab color parameters.

Regarding smell and taste perceptions, some studies have described the influence of the application of pectolytic enzymes on these wine characteristics [66–68], with all of them concluding that their application resulted in a wine with greater aromatic and mouth complexities as a consequence of the greater presence of phenolic and volatile compounds. However, although some significant changes were detected in the volatile composition, these differences were not detected by the tasters in our study.

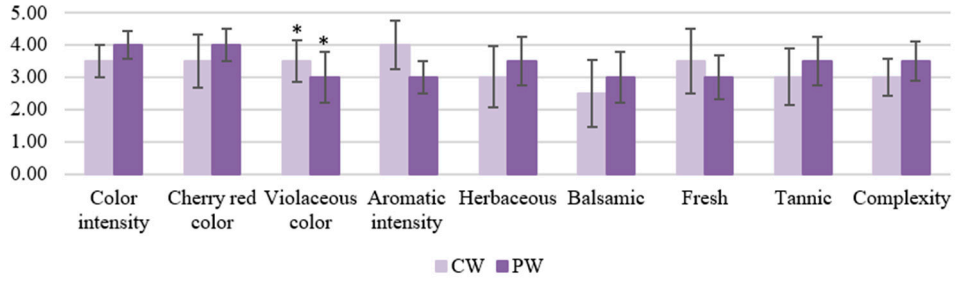

Figure 3. Descriptive sensory analysis results of samples treated with control (CW) and modified yeast (PW). * denotes statistically significant differences ($p \leq 0.05$) between different strains used.

4. Conclusions

The fermentation process of the Cabernet Sauvignon samples was accelerated after the inoculation of the yeast modified with ScP mainly due to the higher availability of nitrogen, resulting in fermentation ending 3 days earlier compared to the control sample with the ScC strain. The correct implantation of this modified ScP strain was confirmed by δ-PCR.

The application of the ScP strain caused a higher free-run juice volume compared to the unmodified strain due to the higher polygalacturonase activity of these yeasts, an effect also observed after the application of pectinase enzymes; however, to our surprise, no colorimetric changes were observed in the wines obtained with ScP [3]. The results concerning the physical–chemical analysis also reflect a slightly higher acidity in the control samples derived from a higher malic and succinic acid content; on the other hand, the 2,3-butanediol isomers were significantly increased in the PW samples [40]. As expected, there was a significant increase in methanol production due to the demethylesterification of the cell wall polymethylpolygalacturonans of the grape [20,22,49], but it was still within the maximum limits established by the OIV.

Regarding the amino acid composition, the PW samples presented a 24.7% higher content than the control samples, with proline being the most abundant amino acid in both groups of samples, as reflected in the literature [43,44]. This, together with other factors, affected the volatile composition, and some significant changes were observed in some major volatile compounds due to the increase in their amino acid precursors [45].

Finally, the sensory analysis showed that the samples treated with the ScP strain produced wines only differing in the lower violet hue of the PW when a descriptive sensory analysis was applied. However, this sensory color change was not supported by the CIELab measurement.

In conclusion, we could say that the use of the ScP strain in the production of Cabernet Sauvignon wine produces a significant increase in the extraction yield of the wine without affecting its sensory characteristics. However, some issues that arise in this work should be studied further, for example, the quantification of the polygalacturonase enzyme produced by the ScP strain, a detailed study on the effect of this yeast on the malic acid content and pH of wine, and the extraction rate of phenolic compounds, which should also include an analysis of the phenolic compounds that remain in the solids of the grape (skin and seed) after the fermentation and isolation of the wine.

5. Patents

This *S. cerevisiae* (CECT11783) strain that was studied, engineered using the PGU1 gene and transcriptionally bonded to the PGK1 gene promoter from *S. cerevisiae* [21,24], was patented by Fernández-González, M.; Briones Pérez, A.I.; and Úbeda Iranzo, J. in 2010 [24].

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writing—review and editing, T.P.-M., M.F.-G. and S.G.-A.; visualization, T.P.-M. and S.G.-A.; supervision, M.F.-G. and S.G.-A.; project administration, E.G.-R.; funding acquisition, M.F.-G., P.M.I.-C., S.G.-A. and E.G.-R. All authors have read and agreed to the published version of the manuscript.

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References

1. Bayindirli, A. Chapter 9: Use of Enzymes for Wine Production. In *Enzymes in Fruit and Vegetable Processing: Chemistry and Engineering Applications*; CRC Press: Boca Raton, FL, USA, 2010; ISBN 978-1-4200-9434-3.
2. Claus, H.; Mojsov, K. Enzymes for Wine Fermentation: Current and Perspective Applications. *Fermentation* **2018**, *4*, 52. [CrossRef]
3. Espejo, F. Role of commercial enzymes in wine production: A critical review of recent research. *J. Food Sci. Technol.* **2021**, *58*, 9–21. [CrossRef]
4. Shrestha, S.; Rahman, M.S.; Qin, W. New insights in pectinase production development and industrial applications. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 9069–9087. [CrossRef] [PubMed]
5. Martínez-Lapuente, L.; Guadalupe, Z.; Ayestarán, B. Chapter 6: Properties of Wine Polysaccharides. In *Pectins: Extraction, Purification, Characterization and Applications*; Masuelli, M., Ed.; IntechOpen: London, UK, 2020; Volume 6, pp. 125–146.
6. Pinelo, M.; Arnous, A.; Meyer, A.S. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci. Technol.* **2006**, *17*, 579–590. [CrossRef]
7. Kassara, S.; Li, S.; Smith, P.; Blando, F.; Bindon, K. Pectolytic enzyme reduces the concentration of colloidal particles in wine due to changes in polysaccharide structure and aggregation properties. *Int. J. Biol. Macromol.* **2019**, *140*, 546–555. [CrossRef]
8. Guadalupe, Z.; Palacios, A.; Ayestarán, B. Maceration enzymes and mannoproteins: A possible strategy to increase colloidal stability and color extraction in red wines. *J. Agric. Food Chem.* **2007**, *55*, 4854–4862. [CrossRef]
9. Revilla, I.; González-SanJosé, M.L. Compositional changes during the storage of red wines treated with pectolytic enzymes: Low molecular-weight phenols and flavan-3-ol derivative levels. *Food Chem.* **2003**, *80*, 205–214. [CrossRef]
10. Smith, P.A.; McRae, J.M.; Bindon, K.A. Impact of winemaking practices on the concentration and composition of tannins in red wine. *Aust. J. Grape Wine Res.* **2015**, *21*, 601–614. [CrossRef]
11. Bautista-Ortín, A.B.; Jiménez-Pascual, E.; Busse-Valverde, N.; López-Roca, J.M.; Ros-García, J.M.; Gómez-Plaza, E. Effect of wine maceration enzymes on the extraction of grape seed proanthocyanidins. *Food Bioprocess Technol.* **2013**, *6*, 2207–2212. [CrossRef]
12. Bautista-Ortín, A.; Martínez-Cutillas, A.; Ros-García, J.; López-Roca, J.; Gómez-Plaza, E. Improving colour extraction and stability in red wines: The use of maceration enzymes and enological tannins. *Int. J. Food Sci. Technol.* **2005**, *40*, 867–878. [CrossRef]
13. Kelebek, H.; Canbas, A.; Cabaroglu, T.; Selli, S. Improvement of anthocyanin content in the cv. Öküzgözü wines by using pectolytic enzymes. *Food Chem.* **2007**, *105*, 334–339. [CrossRef]
14. Nogales-Bueno, J.; Baca-Bocanegra, B.; Heredia, F.J.; Hernández-Hierro, J.M. Phenolic compounds extraction in enzymatic macerations of grape skins identified as low-level extractable total anthocyanin content. *J. Food Sci.* **2020**, *85*, 324–331. [CrossRef] [PubMed]
15. Revilla, I.; González-Sanjosé, M.L. Evolution During the Storage of Red Wines Treated with Pectolytic Enzymes: New Anthocyanin Pigment Formation. *J. Wine Res.* **2001**, *12*, 183–197. [CrossRef]
16. Ducasse, M.-A.; Canal-Llauberes, R.-M.; de Lumley, M.; Williams, P.; Souquet, J.-M.; Fulcrand, H.; Doco, T.; Cheynier, V. Effect of macerating enzyme treatment on the polyphenol and polysaccharide composition of red wines. *Food Chem.* **2010**, *118*, 369–376. [CrossRef]
17. Romero-Cascales, I.; Fernández-Fernández, J.I.; Ros-García, J.M.; López-Roca, J.M.; Gómez-Plaza, E. Characterisation of the main enzymatic activities present in six commercial macerating enzymes and their effects on extracting colour during winemaking of Monastrell grapes. *Int. J. Food Sci. Technol.* **2008**, *43*, 1295–1305. [CrossRef]
18. Hüfner, E.; Haßelbeck, G. Application of microbial enzymes during winemaking. In *Biology of Microorganisms on Grapes, in Must and in Wine*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 635–658.
19. Borovkova, A.N.; Shalamitskiy, M.Y.; Naumova, E.S. Pectinolytic Yeast Saccharomyces paradoxus as a New Gene Pool for Winemaking. *Microbiology* **2023**, *92*, 256–268. [CrossRef]
20. Fernández-González, M.; Ubeda, J.; Vasudevan, T.; Otero, R.C.; Briones, A. Evaluation of polygalacturonase activity in Saccharomyces cerevisiae wine strains. *FEMS Microbiol. Lett.* **2004**, *237*, 261–266. [CrossRef] [PubMed]

21. Fernandez-Gonzalez, M.; Ubeda, J.; Cordero-Otero, R.; Gururajan, V.T.; Briones, A. Engineering of an oenological Saccharomyces cerevisiae strain with pectinolytic activity and its effect on wine. *Int. J. Food Microbiol.* **2005**, *102*, 173–183. [CrossRef]
22. Eschstruth, A.; Divol, B. Comparative characterization of endo-polygalacturonase (Pgu1) from Saccharomyces cerevisiae and Saccharomyces paradoxus under winemaking conditions. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 623–634. [CrossRef]
23. Ferreira, D.J.M.; Noble, J. Yeast strain optimization for enological applications. In *Advances in Grape and Wine Biotechnology*; IntechOpen: London, UK, 2019; pp. 1–17.
24. Gonzalez, M.F.; Perez, A.I.B.; Iranzo, J.U. Cepa de Levadura *Saccharomyces cerevisiae* CECT 11783, Modificada Genéticamente con Actividad Pectinolítica: Su Método de Obtención y Aplicación. Spain Patent No. 2,328,323, 3 September 2010.
25. Sampaio, T.L.; Kennedy, J.A.; Vasconcelos, M.C. Use of microscale fermentations in grape and wine research. *Am. J. Enol. Vitic.* **2007**, *58*, 534–539. [CrossRef]
26. Legras, J.-L.; Karst, F. Optimisation of interdelta analysis for Saccharomyces cerevisiae strain characterisation. *FEMS Microbiol. Lett.* **2003**, *221*, 249–255. [CrossRef]
27. Charoenchai, C.; Fleet, G.H.; Henschke, P.A.; Todd, B. Screening of non-Saccharomyces wine yeasts for the presence of extracellular hydrolytic enzymes. *Aust. J. Grape Wine Res.* **1997**, *3*, 2–8. [CrossRef]
28. OIV. *Compendium of International Methods of Wine and Must Analysis*; International Organisation of Vine and Wine: Paris, France, 2016; Volume 1.
29. Somers, T.C.; Evans, M.E. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "Chemical age". *J. Sci. Food Agric.* **1977**, *28*, 279–287. [CrossRef]
30. Gómez-Alonso, S.; Hermosín-Gutiérrez, I.; García-Romero, E. Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. *J. Agric. Food Chem.* **2007**, *55*, 608–613. [CrossRef]
31. Pérez-Martín, F.; Izquierdo-Cañas, P.M.; Seseña, S.; García-Romero, E.; Palop, M.L. Aromatic compounds released from natural precursors by selected Oenococcus oeni strains during malolactic fermentation. *Eur. Food Res. Technol.* **2015**, *240*, 609–618. [CrossRef]
32. Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Gómez, M.V.; Velders, A.H.; Hermosín-Gutiérrez, I. Flavonol 3-O-glycosides series of Vitis vinifera cv. Petit Verdot red wine grapes. *J. Agric. Food Chem.* **2009**, *57*, 209–219. [CrossRef]
33. Lago-Vanzela, E.S.; Da-Silva, R.; Gomes, E.; García-Romero, E.; Hermosín-Gutiérrez, I. Phenolic Composition of the Edible Parts (Flesh and Skin) of Bordô Grape (Vitis labrusca) Using HPLC–DAD–ESI-MS/MS. *J. Agric. Food Chem.* **2011**, *59*, 13136–13146. [CrossRef]
34. Pérez-Navarro, J.; Izquierdo-Cañas, P.M.; Mena-Morales, A.; Martínez-Gascueña, J.; Chacón-Vozmediano, J.L.; García-Romero, E.; Gómez-Alonso, S.; Hermosín-Gutiérrez, I. First chemical and sensory characterization of Moribel and Tinto Fragoso wines using HPLC-DAD-ESI-MS/MS, GC-MS, and Napping® techniques: Comparison with Tempranillo. *J. Sci. Food Agric.* **2019**, *99*, 2108–2123. [CrossRef]
35. Castillo-Munoz, N.; Fernandez-Gonzalez, M.; Gomez-Alonso, S.; Garcia-Romero, E.; Hermosin-Gutierrez, I. Red-color related phenolic composition of Garnacha Tintorera (*Vitis vinifera* L.) grapes and red wines. *J. Agric. Food Chem.* **2009**, *57*, 7883–7891. [CrossRef]
36. *ISO 11035:1994*; Sensory Analysis—Identification and Selection of Descriptors for Establishing a Sensory Profile by a Multidimensional Approach. International Organization for Standardization: Geneva, Switzerland, 2007.
37. Gobert, A.; Tourdot-Maréchal, R.; Sparrow, C.; Morge, C.; Alexandre, H. Influence of nitrogen status in wine alcoholic fermentation. *Food Microbiol.* **2019**, *83*, 71–85. [CrossRef]
38. Su, J.; Wang, T.; Wang, Y.; Li, Y.Y.; Li, H. The use of lactic acid-producing, malic acid-producing, or malic acid-degrading yeast strains for acidity adjustment in the wine industry. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2395–2413. [CrossRef] [PubMed]
39. Redzepovic, S.; Orlic, S.; Majdak, A.; Kozina, B.; Volschenk, H.; Viljoen-Bloom, M. Differential malic acid degradation by selected strains of *Saccharomyces* during alcoholic fermentation. *Int. J. Food Microbiol.* **2003**, *83*, 49–61. [CrossRef] [PubMed]
40. Scutarașu, E.C.; Luchian, C.E.; Vlase, L.; Nagy, K.; Colibaba, L.C.; Trinca, L.C.; Cotea, V.V. Influence Evaluation of Enzyme Treatments on Aroma Profile of White Wines. *Agronomy* **2022**, *12*, 2897. [CrossRef]
41. Cai, J.; Zhu, B.Q.; Wang, Y.H.; Lu, L.; Lan, Y.B.; Reeves, M.J.; Duan, C.Q. Influence of pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. *Food Chem.* **2014**, *154*, 217–229. [CrossRef] [PubMed]
42. Romano, P.; Suzzi, G. Origin and production of acetoin during wine yeast fermentation. *Appl. Environ. Microbiol.* **1996**, *62*, 309–315. [CrossRef] [PubMed]
43. Herbert, P.; Santos, L.; Alves, A. Simultaneous Quantification of Primary, Secondary Amino Acids, and Biogenic Amines in Musts and Wines Using OPA/3-MPA/FMOC-CI Fluorescent Derivatives. *J. Food Sci.* **2001**, *66*, 1319–1325. [CrossRef]
44. Long, D.; Wilkinson, K.L.; Poole, K.; Taylor, D.K.; Warren, T.; Astorga, A.M.; Jiranek, V. Rapid Method for Proline Determination in Grape Juice and Wine. *J. Agric. Food Chem.* **2012**, *60*, 4259–4264. [CrossRef] [PubMed]
45. Scutarașu, E.C.; Luchian, C.E.; Cioroiu, I.B.; Trincă, L.C.; Cotea, V.V. Increasing Amino Acids Content of White Wines with Enzymes Treatments. *Agronomy* **2022**, *12*, 1406. [CrossRef]
46. Long, D.; Wilkinson, K.L.; Taylor, D.K.; Jiranek, V. Novel Wine Yeast for Improved Utilisation of Proline during Fermentation. *Fermentation* **2018**, *4*, 10. [CrossRef]

47. Barrajón-Simancas, N.; Giese, E.; Arévalo-Villena, M.; Úbeda, J.; Briones, A. Amino acid uptake by wild and commercial yeasts in single fermentations and co-fermentations. *Food Chem.* **2011**, *127*, 441–446. [CrossRef]
48. Mirás-Avalos, J.M.; Bouzas-Cid, Y.; Trigo-Córdoba, E.; Orriols, I.; Falqué, E. Amino Acid Profiles to Differentiate White Wines from Three Autochtonous Galician Varieties. *Foods* **2020**, *9*, 114. [CrossRef] [PubMed]
49. Han, Y.; Du, J. Relationship of the methanol production, pectin and pectinase activity during apple wine fermentation and aging. *Food Res. Int.* **2022**, *159*, 111645. [CrossRef]
50. Rollero, S.; Zietsman, A.J.J.; Buffetto, F.; Schückel, J.; Ortiz-Julien, A.; Divol, B. Kluyveromyces marxianus Secretes a Pectinase in Shiraz Grape Must That Impacts Technological Properties and Aroma Profile of Wine. *J. Agric. Food Chem.* **2018**, *66*, 11739–11747. [CrossRef] [PubMed]
51. Rojas, V.; Gil, J.V.; Piñaga, F.; Manzanares, P. Acetate ester formation in wine by mixed cultures in laboratory fermentations. *Int. J. Food Microbiol.* **2003**, *86*, 181–188. [CrossRef] [PubMed]
52. Izquierdo Cañas, P.M.; García Romero, E.; Gómez Alonso, S.; Palop Herreros, M.L.L. Changes in the aromatic composition of Tempranillo wines during spontaneous malolactic fermentation. *J. Food Compos. Anal.* **2008**, *21*, 724–730. [CrossRef]
53. Mansfield, A.K.; Schirle-Keller, J.-P.; Reineccius, G.A. Identification of Odor-Impact Compounds in Red Table Wines Produced from Frontenac Grapes. *Am. J. Enol. Vitic.* **2011**, *62*, 169–176. [CrossRef]
54. Cañas, P.M.I.; Pérez-Martín, F.; Romero, E.G.; Prieto, S.S.; Herreros, M. de los L.P. Influence of inoculation time of an autochthonous selected malolactic bacterium on volatile and sensory profile of Tempranillo and Merlot wines. *Int. J. Food Microbiol.* **2012**, *156*, 245–254. [CrossRef] [PubMed]
55. Liu, S.-Q.; Pilone, G.J. An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. *Int. J. Food Sci. Technol.* **2000**, *35*, 49–61. [CrossRef]
56. Swiegers, J.H.; Bartowsky, E.J.; Henschke, P.A.; Pretorius, I.S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139–173. [CrossRef]
57. Miller, G.C.; Pilkington, L.I.; Barker, D.; Deed, R.C. Saturated Linear Aliphatic γ- and δ-Lactones in Wine: A Review. *J. Agric. Food Chem.* **2022**, *70*, 15325–15346. [CrossRef]
58. Kelly, D.; Zerihun, A. The Effect of Phenol Composition on the Sensory Profile of Smoke Affected Wines. *Molecules* **2015**, *20*, 9536–9549. [CrossRef] [PubMed]
59. Milheiro, J.; Filipe-Ribeiro, L.; Vilela, A.; Cosme, F.; Nunes, F.M. 4-Ethylphenol, 4-ethylguaiacol and 4-ethylcatechol in red wines: Microbial formation, prevention, remediation and overview of analytical approaches. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 1367–1391. [CrossRef] [PubMed]
60. Rusjan, D.; Strlič, M.S.; KoÅ, T.; Prosen, H. The response of monoterpenes to different enzyme preparations in Gewürztraminer (*Vitis vinifera* L.) wines. *South Afr. J. Enol. Vitic.* **2009**, *30*, 56–64. [CrossRef]
61. Cabaroglu, T.; Selli, S.; Canbas, A.; Lepoutre, J.-P.; Günata, Z. Wine flavor enhancement through the use of exogenous fungal glycosidases. *Enzym. Microb. Technol.* **2003**, *33*, 581–587. [CrossRef]
62. Alpeza, I.; Kovačević, K.; Vanzo, A. Total phenols, stilbene and antioxidative activity in Babić and Plavac mali wines; Eficiency of pectolytic enzymes. *Glas. Zašt. Bilja* **2019**, *42*, 50. [CrossRef]
63. Revilla, I.; González-San José, M. Addition of pectolytic enzymes: An enological practice which improves the chromaticity and stability of red wines. *Int. J. Food Sci. Technol.* **2003**, *38*, 29–36. [CrossRef]
64. Borazan, A.A.; Bozan, B. The influence of pectolytic enzyme addition and prefermentative mash heating during the winemaking process on the phenolic composition of Okuzgozu red wine. *Food Chem.* **2013**, *138*, 389–395. [CrossRef]
65. Beaver, J.W.; Miller, K.V.; Medina-Plaza, C.; Dokoozlian, N.; Ponangi, R.; Blair, T.; Block, D.; Oberholster, A. The Effects of Temperature and Ethanol on Proanthocyanidin Adsorption to Grape Cell Wall Material in the Presence of Anthocyanins. *Molecules* **2020**, *25*, 4139. [CrossRef] [PubMed]
66. Bakker, J.; Bellworthy, S.J.; Reader, H.P.; Watkins, S.J. Effect of Enzymes During Vinification on Color and Sensory Properties of Port Wines. *Am. J. Enol. Vitic.* **1999**, *50*, 271–276. [CrossRef]
67. Alpeza, I.; Kovačević Ganić, K.; Vanzo, A.; Herjavec, S. Improved Chromatic and Sensory Characteristics of Plavac Mali Wines-Efficiency of Maceration Enzymes: Czech Journal of Food Sciences. *Czech J. Food Sci.* **2017**, *35*, 236–245. [CrossRef]
68. Armada, L.; Fernández, E.; Falqué, E. Influence of several enzymatic treatments on aromatic composition of white wines. *LWT-Food Sci. Technol.* **2010**, *43*, 1517–1525. [CrossRef]