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Effect of pre-fermentative cold soaking and use of different enzymes on the chemical and sensory properties of *Catarratto* wines

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ABSTRACT

The wine industry acknowledges that early harvested grapes or those with uneven ripeness often result in wines with an "unripe fruit" mouthfeel. However, the compounds causing these sensory flaws and the best winemaking techniques to address them remain unclear. This study examines the effects of pre-fermentative cold soaking (PCS) and enzyme addition during fermentation on the chemical composition and sensory properties of *Catarratto* wine, a variety commonly linked to these issues. The hypothesis suggests that grape polysaccharides released during PCS and yeast polysaccharides from fermentation contribute to producing smoother wines. Two winemaking approaches were tested: traditional non-PCS (NPCS) and PCS with 48-hour skin contact at 4°C. Each group included a control and four enzyme treatments: three pectolytic enzymes and a β-glucanase enzyme. Results showed PCS significantly increased grape polysaccharide release, doubling total colloids and enhancing the wine's aromatic complexity. Enzyme treatments increased yeast-derived polysaccharides, with β-glucanase having the greatest impact, raising mannose levels. The addition of enzymes at the beginning of the alcoholic fermentation had no impact on fermentation kinetics but boosted yeast polysaccharide levels. Sensory analysis revealed enzyme-treated wines reduced the "unripe fruit" perception and improved smoothness. This research demonstrates for the first time the potential of PCS and enzymes to enhance the quality of *Catarratto* wines made from early harvested grapes.

1. Introduction

Catarratto, the most cultivated white grape in Sicily and the second in Italy, plays a key role in Italian viticulture (Carimi et al., 2010). Its wines are noted for moderate alcohol, high acidity, and pH levels influenced by vineyard elevation, with hillside grapes yielding higher acidity and malic acid. Aromatically, they feature orange blossom and citrus notes (Leder, 2020). On the palate, they are savory, salty, and have a persistent finish. However, grape heterogeneity ripening at the harvest, lead to presence of underripe, ripe and overripe fruit, simultaneously (Bambina et al., 2024a). The varying proportions of fruit contribute to differences in metabolite composition, which can adversely affect the quality characteristics and the sensory attributes of the wine (Kontoudakis et al., 2011), thereby presenting technical challenges for the winemaker. Nowadays, increasing temperatures due to climate changing condition, are leading to high accumulation of sugars level in the grapes and low total acidity, leading to wines that are too alcoholic and lack freshness. One approach to produce lower-alcohol wines with high acidity level and low pH value involves early grape harvesting (Schelezki et al., 2018). However in white wines from grapes harvested at this stage, or heterogeneous grapes ripening at harvest (where unripe berries predominate), often exhibit unripe sensory notes (Armstrong et al., 2021) often mischaracterized as bitterness. The term "unripe" refers to a perception perceived in the mouth, a sensation of roughness that lingers even after tasting the wine. The high acidity content of the berries does not appear to be directly related to the perception of unripeness. Several wines with naturally high total acidity due to varietal characteristics do not exhibit unripe notes. This perception instead seems to be linked to molecules that have not undergone the appropriate degree of polymerization or depolymerization typically achieved in fully ripe grapes. These compounds may be responsible for the perception of the unripe fruit character and roughness in the mouth, particularly

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noticeable from the mid-palate through the aftertaste. In fully ripe grapes, this phenomenon does not occur or its very low. For instance, Moscato grapes (*V. Vinifera* L.) grown in Aosta Valley or in Sicily (Pantelleria), which are harvested at high ripeness levels, do not exhibit the unripe flavor. In contrast, Moscato from the Asti region, where early harvesting is practiced, often displays this unripe taste. This is the reason why Asti Spumante is traditionally produced with residual sugar. This issue is fundamentally tied to the ripeness level of the grapes. It is also true, that the bitterness of Muscat grape varieties can be due to their high terpenoids content (Jones-Moore et al., 2021). Additionally, the synthesis of volatile compounds in grape berries occurs as they approach full ripeness (Kalua & Boss, 2009). *Catarratto* wines, as wines from grapes grown in hot regions, can sometimes reveals these unripe sensory notes on the palate (Pollon et al., 2024). Even though these characteristics are often observed and well known to winemakers, it is still unknown which compounds they are caused by, and which technique is best suited to solve this problem. During grape ripening, these compounds are transformed and no longer contribute to this sensory unripe perception. One hypothesis suggests that the only compounds undergoing hydrolytic transformations during grape ripening are polysaccharides, which remain intact in unripe grapes. Additionally, polysaccharides can help balance the drying sensation caused by acidity reducing the puckering taste perception. To gain a deeper understanding of this phenomenon and explore potential solutions, various enological techniques have been evaluated, including prefermentative cold soaking (PCS) and the use of different enzymes during the alcoholic fermentation. PCS is widely used in white wine production after crushing and before pressing to enhance aromatic complexity, varietal character, and color stability, while reducing oxidation (Luan et al., 2018). This technique involves keeping the must in contact with grape skins at low temperatures (5–15°C) for a few hours up to days. PCS facilitates the diffusion of polysaccharides and volatile compounds, improving sensory attributes and fermentation efficiency by releasing nitrogen, vitamins, and fatty acids (Gawel et al., 2014). However, it may also increase bitterness and astringency due to the extraction of phenolic compounds like tannins and flavan-3-ols (Sokolowsky et al., 2015). The two main sources of polysaccharides in wine are grapes and microorganisms. Wine colloids are grape-derived type II arabinogalactan-proteins, and rhamnogalacturonans, and yeast-derived mannoproteins. Mannoproteins account for approximately 35 % of the total polysaccharides in wine (Vidal et al., 2003). These polysaccharides are glycoproteins found in the outer layer of the yeast cell wall and are composed of 80 % D-mannose and 10–20 % protein covalently linked with D-glucose and N-acetylglucosamine residues (Rodriguez-Nogales et al., 2012). Mannoproteins, released by *Saccharomyces cerevisiae* during alcoholic fermentation, are excreted primarily during the yeast's exponential growth phase and later through enzymatic activity during wine contact with lees (Charpentier et al., 2004). These compounds have significant commercial value and have been extensively studied for their roles in wine stabilization and sensory improvement (Canalejo et al., 2022). Mannoproteins contribute to tartrate and protein stabilization, enhance sensory properties such as mouthfeel and fullness, add complexity and aromatic persistence, increase viscosity and roundness, and reduce astringency and bitterness (Martínez-Lapuente et al., 2019). Their impact depends on concentration, structural features, size, and type (Brandão et al., 2017). Commercial enzymes are used to modify the structure and levels of wine polysaccharides (Doco et al., 2007). Pectolytic enzymes, often added to must, improve juice yield, aroma, color extraction, and remove colloidal particles and pectin (Aroca et al., 2022). These enzymes also facilitate the release of polysaccharides and other compounds from grape skins (Ducasse et al., 2010). Enzyme preparations often include pectinases, β-glucanases, and glycosidases, which accelerate yeast lysis and release mannoproteins during sur lies élevage, positively influencing wine texture and stability (Rodriguez-Nogales et al., 2012). In this paper, we investigate different enological treatments aimed at reducing or eliminating the unripe character often associated with Catarratto wine, which is obtained from early harvested grapes, thereby enhancing its sensory qualities. To better study the sensory characteristics (unripe attribute) of wines obtained from early harvested grapes, aimed at achieving low pH values and high total acidity, the grapes, typically harvested at 22–23° Brix, were instead picked at 20° Brix. The study evaluates techniques such as PCS and adding pectolytic and β-glucanase enzymes with secondary enzymatic activity during the alcoholic fermentation. Additionally, we assess whether a greater enrichment of grape and yeast polysaccharides in wine by the end of alcoholic fermentation can produce softer wines without "unripe" notes.

2. Materials and methods

2.1. Winemaking process

A total of 550 kg of *Catarratto* grapes (*Vitis vinifera* L.) grown in Menfi (Sicily) were manually harvested in 2022 at 20° Brix. Grapes were collected in optimal sanitary state and processed in the experimental winery of Settesoli (Menfi, Sicily). Traditional white winemaking was employed for NPCS wines, while PCS wines underwent skin contact at 4°C for 48 h before being pressed. Each trial was elaborated in triplicate in 5 L stainless steel tanks.

Both PCS and NPCS musts obtained were added with 5 g hL^{-1} of SO^{4+} in SO_2 eq., pectolytic enzymes (3 g hL^{-1} Hzym® Extractive FCE G, HTS enologia) and stored in a cold room (8°C) for 12–18 h to allow settling. On the clear must, five trials were conducted for each group (PCS and NPCS), including three pectolytic enzymes featuring secondary activities (Pectolytic 1; Pectolytic 2; Pectolytic 3), a glucanase enzyme (β-glucanase), and a control without the enzyme (Control) (Table 1). Diammonium phosphate (DAP), containing yeast cell walls and thiamine (0.25 %) (Hnutrix® Dhizote F, HTS Enologia), was added to all the tanks to achieve 200 mg L^{-1} of readily assimilable nitrogen. The must was inoculated with a *pied de cuvée* in full fermentative activity, containing *S. cerevisiae* yeast strain (10 g hL^{-1} K1, Lallemand Wine), alongside the enzymes. The alcoholic fermentation occurred at 16–18°C. Based on the yeast's needs during alcoholic fermentation, open racking of 25 % of the total mass and additions of organic and inorganic nitrogen (5–10 g hL^{-1}) were carried out to promote the completion of the alcoholic fermentation and to avoid the occurrence of sulphury off-flavors. The alcoholic fermentation was finished when the concentration of reducing sugars was less than 2 g L^{-1} and white wines were dry. Then, the wines were racked off the coarse lees derived from grapes and SO_2 was added to achieve a free SO^{4+} in SO_2 eq. of 30 mg L^{-1}. Subsequently, a portion of the wine from each trial was filtered at 1 μm (bright filtration) and bottled.

Table 1
Experimental setup of prefermentative treatments applied to Catarratto wines. The table includes control samples and samples treated with Prefermentative Cold Soaking (PCS) or Non-Prefermentative Cold Soaking (NPCS) in combination with different enzymatic preparations (Pectolytic 1, Pectolytic 2, β-glucanase, and Pectolytic 3). Sample names indicate the treatment and enzyme used.

Catarratto wines	Prefermentative treatment	Enzyme	Sample name
Group 1	Control	-	PCS_Control
	PCS	Pectolytic 1	PCS_Pectolytic 1
	PCS	Pectolytic 2	PCS_Pectolytic 2
	PCS	β-glucanase	PCS_β-glucanase
	PCS	Pectolytic 3	PCS_Pectolytic 3
Group 2	Control	-	NPCS_Control
	NPCS	Pectolytic 1	NPCS_Pectolytic 1
	NPCS	Pectolytic 2	NPCS_Pectolytic 2
	NPCS	β-glucanase	NPCS_β-glucanase
	NPCS	Pectolytic 3	NPCS_Pectolytic 3

2.1.1. Commercial enzyme trials

Pectolytic 1: pectolytic enzyme (*Aspergillus niger)*; Pectolytic 2: pectolytic enzyme featuring β-glucosidase secondary activity (*Aspergillus niger*); β-glucanase: enzyme with pectinase and β-glucanase activity (*Aspergillus niger* and *Trichoderma harzianum*); Pectolytic 3: pectolytic enzyme featuring hemicellulose secondary activity (*Aspergillus niger)*.

2.2. Wine analysis

2.2.1. Chemical-physical parameters

Alcohol content (%v/v), reducing sugars (g L^{-1}), titratable acidity (g L^{-1}), volatile acidity (g L^{-1}), and pH and potassium (%) were determined by means of Fourier-transform infrared (FTIR) technology through a WinescanTM FT 120 Fa. Instrument (FOSS, Hillerød, Denmark) calibrated by applying the EEC 2676 standard procedure. All the analyses were carried out in triplicate.

2.2.2. Total polyphenols and p-DACA (p-Dimethylaminocinnamaldehyde) reactive flavonols

Total polyphenols and *p*-DACA assay were analyzed by means of UV–Vis spectrophotometry (UV-1800 spectrophotometer, Shimazdu Scientific Instruments Inc., Columbia, MD, USA) as being (+)-catechin equivalent. Total polyphenols were determined after the dilution of the wines in hydrochloric ethanol (ethanol:water:hydrochloric acid-37 %, 70:30:1 v:v:v) (Bambina et al., 2024a). To determine the reactivity of flavanols to *p*-dimethylaminocinnamaldehyde (*p*-DACA assay), 1 mL of wine was added to 5 mL of the reagent (prepared by dissolving 100 mg of *p*-DACA in 70 mL of methanol and 25 mL of concentrated HCl, cooling the solution, and bringing the volume to 100 mL with methanol) (Corona, 2010). Total polyphenols and *p*-DACA analysis were performed in triplicate.

2.2.3. Total Colloids and Mannose analysis

To analyze total colloids, the total fraction was isolated from 20 mL of wine, following the method of Usseglio-Tomasset (1976). Polysaccharides were hydrolyzed by adding 2.5 mL of 1 N HCl to the sample, followed by incubation at 100°C for 1 h. Subsequently, the sample was neutralized by the addition of 4 N NaOH until reaching a pH of 7. Mannose was determined by means of UV–Vis spectrophotometry (UV-1800 spectrophotometer, Shimazdu Scientific Instruments Inc., Columbia, MD, USA) using an enzymatic assay kit (Megazyme K-MANGL 04/20, Bray Business Park, Bray, Co. Wicklow, A98 YV29, Ireland), according to the procedure outlined by Gawehn (1988). The procedure involves the phosphorylation of D-glucose, D-fructose, and D-mannose by hexokinase (HK) using ATP, resulting in glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), and mannose-6-phosphate (M-6-P). G-6-P is then oxidized to gluconate-6-phosphate by glucose-6-phosphate dehydrogenase (G6P-DH) with NADP+ , producing NADPH, which is measured at 340 nm. F-6-P is converted to G-6-P by phosphoglucose isomerase (PGI), and M-6-P is converted to F-6-P by phosphomannose isomerase (PMI) and subsequently to G-6-P. The absorbance is analyzed sequentially after each reaction step, allowing the quantification of D-glucose, D-fructose, and D-mannose based on NADPH production. D-Mannose is the major sugar component of the so-called “mannantype” polysaccharides, and in wine, it originates from the hydrolysis of polymeric molecules containing monomeric mannose, such as mannoproteins. Total colloids and mannose content analysis were carried out in triplicate.

2.2.4. Protein Stability

To assess protein stability, 10 mL of wine was filtered using a 0.45 μm membrane filter. The turbidity of the filtered sample was measured both before and after a heat treatment (30 min at 80°C) using a Turbidity meter and a Bentocheck instrument (Wine Time, Hi83749–02). The post-treatment turbidity measurement was taken 40 min after the heat treatment had concluded. The absolute difference in turbidity, expressed in NTU (Nephelometric Turbidity Units), represents the wine’s stability level. The criteria for protein stability were defined as follows: < 2 NTU: Stable

> 2 NTU: Unstable. The analysis was performed in triplicate.

2.2.5. Potassium bitartrate stability

The stability of potassium bitartrate in wines was assessed using a conductivity drop test with the CheckStab Alfa2016Life device (Delta Acque, Florence, Italy). In brief, 100 mL of wine, centrifuged at 3000 rpm for 15 min, was mixed with 2 g of micronized potassium bitartrate for white wines, both at a temperature of −0.5°C. The initial electrical conductivity of the sample at −0.5 °C (denoted as χI) was measured, followed by a second reading after 15 min, post-addition of potassium bitartrate (denoted as χF). The change in conductivity (Δχ) was determined by subtracting χI from χF. Wines were considered stable if the Δχ was less than 30 μS cm^{-1}. The analysis was carried out in triplicate.

2.2.6. Buffer capacity

50 mL of wine was placed in a 150 mL beaker, and the pH was measured to two decimal places (pH_0). If $pH_0 < 3.30$, 5 mL of 0.1 N NaOH was added, and the new pH value was recorded. Conversely, if $pH_0 > 3.30$, 5 mL of 0.1 N HCl or 0.1 N H_2SO_4 was added, and the pH value was recorded. The analysis was performed in triplicate.

2.3. Volatile organic composition

Volatile organic compounds (VOCs) were analyzed by means of Gas Chromatography (Agilent 6890 Series GC system, Milan, Italy) coupled with Mass Spectrometry (Agilent 5973 NetWork Mass Selective Detector, Milan, Italy) following the method described by Bambina et al., (2024b). In brief, 25 mL of wine was diluted to 75 mL using deionized water and supplemented with 1-heptanol standard 98 % purchased from Thermofisher (Milan, Italy) (0.25 mL of a 40 mg L^{-1} hydroalcoholic solution). The diluted sample was then processed through a 1 g C18 cartridge (Isolute, SPE Columns, Uppsala, Sweden, part no. 221–0100-C) that had been preconditioned with 3 mL of methanol followed by 4 mL of deionized water. After washing the cartridge with 30 mL of deionized water, the volatile compounds were extracted using 12 mL of dichloromethane, dehydrated, and concentrated to a final volume of 0.5 mL. Gas chromatography was performed using a DB-WAX column (Agilent Technologies, 30 m, 0.250 mm i.d., film thickness 0.25 μm, part no. 122–7032). The oven temperature program was as follows: 40 °C for 2 min, ramping from 40 to 60 °C at 3 °C min^{-1}, holding at 60 °C for 2 min, then increasing to 190 °C at 2 °C min^{-1}, holding for 10 min, followed by an increase to 230 °C at 5 °C min^{-1}, and a final hold at 230 °C for 15 min. The injection mode was splitless, with the injector set at 250 °C and the transfer line at 230 °C. Helium served as the carrier gas, with a column flow rate of 1 mL/min. Volatile organic compounds (VOCs) were identified by comparing their mass spectra and retention indices of pure commercial standards (Table S1). When no standards were available, the NIST/EPA/NIH Mass Spectral Library (Version 2.0d, 2015 build) was used. The linear retention indices (LRI) used for compound identification are provided in Table S2. VOCs were semi-quantified relative to the peak area of 1-heptanol as the internal standard, with results expressed in μg L^{-1} .

2.4. Sensory analysis

2.4.1. Duo-trio test

The sensory evaluation of wines by duo-trio test (UNI ISO 10399 [45]) and the paired comparison test (UNI ISO 5495 [46] was carried out. The ISO guidelines (UNI ISO 8589 [47]) were followed with a panel of 18 or 14 tasters. The panel was composed of technicians and students of the Degree Course in Viticulture and Oenology of the University of

Palermo, who have experience with the evaluation of wines. Test significance was evaluated according to Roessler et al. (1978).

2.4.2. Sorting test

The wines were also evaluated using a sorting test, following the method described by Corona et al. (2021), in which panelists ranked each attribute from the weakest to the strongest sensation. The panel consisted of 17–19 trained evaluators, who considered two variables: "Unripe fruit" and "Overall preference."

2.5. Statistical analysis

A factorial analysis of variance (ANOVA) was applied to assess each treatment's individual impact. The analysis was carried out using Rstudio (RStudio Team, Boston, MA, USA) version [4.0.3]. The main factors were prefermentative cold soaking and enzyme addition treatments. Differences with p values of less than 5 % ($p < 0.05$) were considered statistically significant. In case of significant differences, a *post hoc* HSD Tukey's test was conducted. Analysis was in triplicate for each fermentation replicate. The results were expressed as mean values with corresponding standard error. To point out the differences of the wines with and without the prefermentative cold soaking treatment, the Orthogonal Partial Least Squares-Discriminant Analysis (O-PLS-DA) was conducted. Additionally, an O-PLS-DA was carried out to differentiate between the Control and each enzyme addition, both analyses performed using the MetaboAnalyst web-based tool suite (Pang et al., 2021). The permutation test in O-PLS-DA assesses whether the model's classification is statistically significant or due to random chance. It randomly shuffles class labels to generate a distribution of model performance (R^2Y, Q^2) under the null hypothesis. If the original model significantly outperforms the permuted models, it confirms its validity. The PCA of sensory attributes and chemical composition of wines was performed using XLSTAT software (Addinsoft, Paris, France), version [2024.4.0].

3. Results

3.1. Alcoholic fermentation and wines physical-chemical composition

The sugar fermentation kinetics of *S. cerevisiae* in PCS and NPCS *Catarratto* wines supplemented with various enzymes followed a consistent pattern (Fig. 1). Alcohol content increased more rapidly after nitrogen additions and partial open racking of the must. In PCS wines, enzyme-supplemented trials exhibited slightly enhanced fermentation kinetics compared to the control and the pectolytic enzyme 2 trial, with final alcohol content ranging from 11.65 % to 11.79 % vol (Table 2). In NPCS wines, fermentation was more favorable with pectolytic enzyme 2 up to day 8, while other trials showed similar final alcohol content (12.05 –12.5 % vol), except for pectolytic enzyme 3 (11.19 % vol). All trials completed fermentation in 13–14 days. As the ethanol production was similar in all the trials, the addition of pectolytic and β-glucanase enzymes did not influence sugar fermentation kinetics or the performance of *S. cerevisiae*, as enzymatic activity targeted non-viable yeast cells, causing their lysis during fermentation (Garcia-Moruno et al., 2001). Table 2 highlights that enzyme treatments did not significantly affect the wines' physical-chemical properties or phenolic composition, with no notable differences in alcohol content, residual sugars, pH, acidity, or polyphenols. Consistent with our findings, a previous study examining the effect of five commercial enzyme preparations on white wine composition reported that enzymes did not significantly impact the basic physical-chemical properties (Scutarașu et al., 2022). However, the PCS treatment had a notable influence on specific parameters. This process significantly decreased total acidity, with a corresponding increase in pH, as well as an impact on *p*-DACA reactive flavanols and total

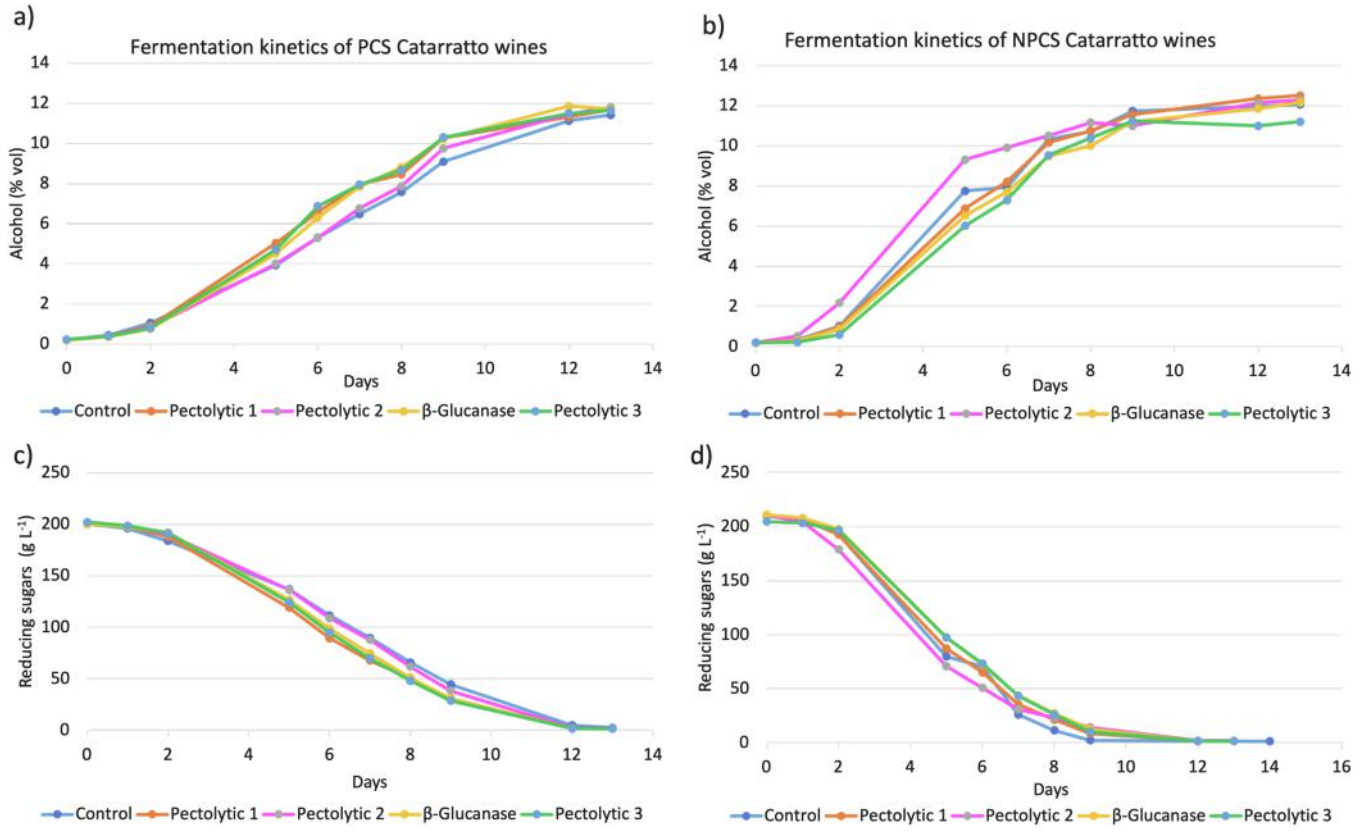

Fig. 1. Fermentation kinetics of prefermentative cold soaked (PCS) (a,c) and non-prefermentative cold soaked (NPCS) (b, d) Catarratto wines with the addition of different pectolytic and β-glucanase enzymes.

Table 2
Wine physical-chemical composition and polyphenols.

Factor	Alcohol (% vol)	Residual sugars (g L^{-1})	pH	Total acidity (g L^{-1})	Volatil acidity (g L^{-1})	K (%)	*p*-DACA (mg L^{-1})	Total polyphenols (mg L^{-1})
Prefermentative (n = 5)								
PCS	11.7 ± 0.1	1.59 ± 0.04	3.6 ± 0.1	4.3 ± 0.5	0.27 ± 0.01	1.2 ± 0.3	4.6 ± 0.1	60 ± 1
NPCS	12.0 ± 0.3	1.6 ± 0.2	3.31 ± 0.03	6.2 ± 0.1	0.22 ± 0.02	0.95 ± 0.04	3.1 ± 0.3	15 ± 2
Sign.	ns	ns	**	**	*	ns	***	***
Enzyme (n = 2)								
Control	11.7 ± 0.3	1.6 ± 0.1	3.5 ± 0.3	5 ± 1	0.25 ± 0.01	1.0 ± 0.1	4 ± 1	40 ± 24
Pectolytic 1	12.0 ± 0.5	1.7 ± 0.1	3.5 ± 0.2	5 ± 1	0.3 ± 0.0	1.04 ± 0.04	4 ± 1	36 ± 21
Pectolytic 2	12.0 ± 0.3	1.7 ± 0.1	3.4 ± 0.1	6 ± 1	0.2 ± 0.0	0.96 ± 0.01	4 ± 1	35 ± 26
β-Glucanase	12.0 ± 0.1	1.60 ± 0.01	3.5 ± 0.3	5 ± 2	0.3 ± 0.0	1.0 ± 0.1	4 ± 1	40 ± 20
Pectolytic 3	11.6 ± 0.2	1.5 ± 0.1	3.5 ± 0.3	5 ± 2	0.2 ± 0.1	1 ± 1	4 ± 1	38 ± 22
Sign.	ns	ns	ns	ns	ns	ns	ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant,
* = 90 % of significance,
** = 99 % of significance,
*** = 99 % of significance. PCS prefermentative cold soaked wines, NPCS = non-prefermentative cold soaked wines, with the addition of different pectolytic and β-glucanase enzymes.

polyphenols. These results are in agreement with Alti-Palacios et al. (2023).

3.2. Potassium bitartrate data, protein stability and buffer capacity

NPCS treatment significantly increased protein stability (ΔNTU < 2) and decreased buffer capacity compared to PCS wines, while the conductivity drop was not affected (Table 3). Enzyme treatments had no significant impact on conductivity drop, protein stability and buffer capacity. These findings suggest that the increase of yeast-polysaccharide during must fermentation is insufficient for effective protein and tartaric stabilization. Similarly, the extraction of grape polysaccharides did not demonstrate a significant effect on tartrate stabilization. As expected, the conductivity drop showed no significant difference ($\Delta\chi > 30$ μS cm^{-1}), as the wines were not subjected to tartaric stabilization. However, the release of mannoproteins MP40 and MP32 from yeast lysis exhibited, in various instances, a beneficial impact on the conductivity drop and protein stability in white wines (Pollon et al., 2024). Likewise, RG-II, released from grapes, have also been found to contribute to tartrate stabilization (Doco et al., 2007).

3.3. Total colloids and Mannose content

Polysaccharides are the main macromolecules in wine with colloidal properties, derived either from pectic polysaccharides found in grape cell walls or mannoproteins originating from yeast cell walls. They play a pivotal role in the chemical composition and sensory profile of wine, affecting characteristics such as bitterness, astringency, and viscosity, key elements in high-quality wines (Vidal et al., 2004). Table 4 presents the results of the colloid content and mannose concentration at the end of the alcoholic fermentation for PCS and NPCS trials, with the addition of pectolytic and β-glucanase enzymes. There are two-fold higher total colloid content in PCS wines compared to NPCS wines. The release of polysaccharides in PCS trials indicates that enhanced extraction of pectins had occurred from the grape solids. This result is in agreement with Gil et al. (2015) and Kassara et al. (2019), whose works show that increased maceration length resulted in increased grape polysaccharide content of the final wines, while Kassara et al. (2019) discovered that polysaccharide-associated rhamnose (RG-II) was elevated by pectinase treatment. Pectolytic-treated wines show significantly higher levels of colloids compared to the control, with Pectolytic 2 enzyme exhibiting the highest concentration. The amount of polysaccharides released in wine from grape berry cell walls depends on different parameters that include the grape variety, terroir, maturity stage, vintage, the wine-making techniques, and they can be modified to a great extent by enzyme treatments (Cejudo-Bastante et al., 2018). The concentration of

Table 3
Conductivity drop, protein stability and buffer capacity at the end of alcoholic fermentation.

Factor	Δχ Conductivity drop (μS cm^{-1})	Δ NTU	Buffer capacity (meq L^{-1})
Prefermentative (n = 5)			
PCS	149 ± 8	2.6 ± 0.5	48 ± 1
NPCS	138 ± 16	0.9 ± 0.3	32 ± 3
Sign.	ns	**	***
Enzyme (n = 2)			
Control	141 ± 24	1.7 ± 0.5	40 ± 6
Pectolytic 1	147 ± 14	1 ± 1	40 ± 5
Pectolytic 2	124 ± 25	1.2 ± 0.4	41 ± 6
β-Glucanase	139 ± 12	2 ± 1	38 ± 6
Pectolytic 3	167 ± 26	2 ± 1	41 ± 7
Sign.	ns	ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, * = 90 % of significance, ** = 99 % of significance, *** = 99 % of significance. PCS prefermentative cold soaked wines, NPCS = non-prefermentative cold soaked wines, with the addition of different pectolytic and β-glucanase enzymes.

Table 4
Mannose content and total colloids at the end of the alcoholic fermentation in prefermentative cold soaked (PCS) and non-prefermentative cold soaked (NPCS) Catarratto wines, with the addition of different pectolytic and β-glucanase enzymes.

Factor	Colloids (g L^{-1})	Mannose (mg L^{-1})
Prefermentative (n = 5)		
PCS	0.5 ± 0.1	105 ± 9
NPCS	0.25 ± 0.03	55 ± 3
Sign.	**	.
Enzyme (n = 2)		
Control	0.2 ± 0.1 d	52 ± 6
Pectolytic 1	0.4 ± 0.1 c	81 ± 15
Pectolytic 2	0.5 ± 0.1. a	83 ± 11
β-Glucanase	0.4 ± 0.1 bc	106 ± 25
Pectolytic 3	0.5 ± 0.1 ab	79 ± 17
Sign.	**	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, * = 90 % of significance, ** = 99 % of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. PCS = prefermentative cold soaked wines, NPCS = non-prefermentative cold soaked wines, with the addition of different pectolytic and β-glucanase enzymes

mannose increased in all enzyme-treated samples compared to the control, although the differences are not statistically significant. Mannoproteins are located in the outermost layer of the yeast cell wall and can constitute up to 50 % of the dry mass of *S. cerevisiae*. Their release is influenced by the yeast strain, as well as winemaking and aging conditions, and they exhibit a broad molecular mass distribution with multiple populations, characterized by a high mannose residue content compared to other sugars (Gawel et al., 2014). A steady increase in mannose concentration of the must during fermentation and ageing processes occurs, to become one of the most prevalent polysaccharides in wine (Guadalupe et al., 2007). Mannose is present in higher concentrations in the sample treated with the β-glucanase enzyme due to its direct action on yeast cell walls. This suggests that the β-glucanase enzyme acts directly on yeast cell walls, increasing the release of yeast-derived molecules into the medium by the end of the alcoholic fermentation. Meanwhile, pectolytic enzymes act on *S. cerevisiae* cell walls, likely due to a glycosidase activity present in the commercial enzyme formulation, as a side activity (Garcia-Moruno et al., 2001). Glucans from *S. cerevisiae* are mainly β-D-1,3-linked glucose units with β-D-1,6-linked lateral glucose chains and some branched β-D-1,6-glucans with some β-D-1,3-links are also present (Pollon et al., 2024). It can be confirmed that pectolytic enzymes are able to hydrolyze glucan chains, thereby indirectly releasing yeast mannans into the must. This finding was previously reported by Garcia-Moruno et al. (2001) in their study on Barbera wine. The wine treated with pectolytic enzyme was richer in grape polysaccharides and mannose content with respect to the control, indicating that pectolytic enzyme works on both grape and yeast cell walls. The greatest release of yeast-polysaccharides occurs during the exponential growth. Gil et al. (2015) reported that yeast polysaccharides (mannoproteins) were mainly released during the first week of alcoholic fermentation. Moreover, the increase in mannose content in wine submitted to the PCS technique is due to the higher presence of grape solids, which provide greater nutrition for yeasts in the must, leading to increased production and release of mannoproteins into wine (Gawel et al., 2014).

3.4. Volatile organic compounds (VOCs)

The investigation into volatile organic compounds (VOCs) led to the identification and quantification of 46 distinct compounds in *Catarratto* wines. These compounds were categorized into seven chemical families: acids, benzenoids, acetate esters, ethyl esters, C_{13}-norisoprenoids, alcohols, and monoterpenes shown in Tables S3 – S8 of the Supplementary Information. Table S3 provides the data on the influence of the cold soak treatments and use of enzymes during alcoholic fermentation on the fatty acid composition of the wines. Acids are generated by yeast during the breakdown of fatty acids (Molina et al., 2007). This group of chemicals in wine is related to unpleasant odors, when they exceed their odor thresholds (Molina et al., 2007). However, when they are present in low concentration, these compounds add complexity to the wine's bouquet. Observing the prefermentative factor, it is possible to note that cold soak enhances the concentration of fatty acids in wines, particularly hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, and hexadecanoic acid. In contrast, the use of enzymes during fermentation did not result in significant variations in the content of these compounds across the different experimental trials. Esters, which include ethyl esters and acetates, represent a group of chemicals in wine that play a crucial role in enhancing the complexity of its aroma. The formation of esters is influenced by several factors, including yeast strain, must aeration, fermentation technology, and fruit maturity. Key nutrients, including nitrogen compounds and must solids, play a particularly important role (Alti-Palacios et al., 2023). Table S4 provides the data for the ethyl esters evaluated for the factor cold soak treatment and enzyme. It was possible to observe that cold soak treatment has a positive influence on the concentration of these compounds, especially ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate. This finding is in agreement with Alti-Palacios et al. (2023). The factor enzyme substantially exhibits very slight differences at the level of ethyl octanoate and ethyl hexadecanoate (10 % significance). Alcohols are secondary products of yeast alcoholic fermentation, and based on their concentration, this chemical family can have either a positive or negative influence on wine aroma (Cai et al., 2014). Table S5 reports the data of alcohols evaluated on the two factors studied. The enzyme factor did not show any significant effect on the concentration of these latter compounds, while cold soak treatment led to an increase in the concentrations of isoamyl alcohol, 3-ethoxypropanol and methionol. Acetate esters consist of an acid group (acetate) and an alcohol group, which can either be ethanol or a more complex alcohol derived from the metabolism of amino acids (Zhang et al., 2015). Table S6 presents the effect of the factor cold soak and enzyme on the acetate esters of the related wines. The enzyme factor did not show any significant effect on the concentration of these compounds, except for tryptophol content, while cold soak treatment led to an increase in the concentrations of hexyl acetate, phenylethyl acetate, and isoamyl acetate (10 % significance). Benzyl alcohol, 2-phenylethanol, acetovanillone, and 4-vinylguiacol were identified in the related *Catarratto* wines (Table S7). The factor enzyme did not show any significant effect on the concentration of this class of compounds. The cold soak treatment resulted in significantly higher concentrations of benzyl alcohol. Norisoprenoids in wine originate primarily from the oxidative degradation of carotenoids, such as beta-carotene and lutein, present in grapes. During grape maturation and vinification processes, carotenoids undergo chemical cleavage, leading to the formation of aromatic compounds (Mendes-Pinto, 2009). Monoterpenes are regarded as key odorants in aromatic grape varieties of *Vitis vinifera* L., to which they impart their characteristic floral aromas. Monoterpenes, such as linalool, geraniol, nerol, citronellol, and α-terpineol, generally carrying floral and/or citrus odours, are the most abundant forms of terpenes (Panighel & Flamini, 2014). Table S8 represents the effect of the factor cold soak and enzyme on the concentration of C_{13}-norisoprenoids and monoterpenes. The factor enzyme did not show any significant difference between these two chemical families. The cold soak treatment, on the other hand, increased the concentrations of C_{13}-norisoprenoids, specifically blumenol C and vomifoliol. In contrast, the treatment resulted in a decrease in the concentration of monoterpenes, although this change was not statistically significant. However, in model systems, polysaccharides typically reduce aroma release, either by altering the viscosity of the medium or through direct molecular interactions with aromatic compounds (Jones-Moore et al., 2021). Several studies have investigated the influence of polysaccharides on wine aroma compounds, with most concluding that the reduction in aroma volatility is influenced by the hydrophobicity of both the aroma compounds and the polysaccharides (Rinaldi et al., 2019). This fact implies a longer aromatic perception because the volatile compounds retained by polysaccharides will be slowly released (Del Barrio-Galán et al., 2011). The impact of some pectic polysaccharides on some volatile compounds in a wine model solution was studied (Jones-Moore et al., 2021). It was observed that uronic-rich polysaccharides could have two opposite effects: a suppressing effect, decreasing the amount of aroma in the headspace, or a "salting out" effect, causing an increase in the headspace concentration of a volatile compound because of the increase in the ionic strength of the solution. Pérez-Magariño et al. (2022) found that grape and yeast-derived polysaccharides increased the concentration of most of the chemical families of positive aroma compounds in white wine, such as ethyl esters of straight-chain fatty acids, alcohol acetates and terpenes associated with the fresh, fruity, and floral notes. These results suggest that polysaccharides can influence the hydrolysis/esterification balance between ethyl esters and alcohols acetate during aging or storage, favoring the maintenance of the volatile compounds associated with fruity and floral notes over time (Pérez-Magariño et al., 2022).

3.5. Sensory analysis

The sensory analysis of *Catarratto* wines was carried out by comparison of the experimental wines, performing a duo-trio test and a preference test. In Table 5, the results show the outcomes of the duo-trio sensory test regarding the "unripe fruit" character. In both NPCS and PCS wines, the control wines exhibited a higher perception of unripe fruit compared to enzyme-treated wines. Specifically, in NPCS wines, the addition of the enzyme Pectolytic 1 and Pectolytic 2 showed significant differences, with 14 panelists perceiving the control having a stronger unripe fruit perception versus 4 ($p < 0.04$). In PCS wines, the addition of Pectolytic 1, Pectolytic 2, and β-glucanase enzymes also showed significant differences, with *p*-values of $p < 0.03$ and $p < 0.02$, respectively. This finding supports the hypothesis that the PCS technique, along with the addition of pectolytic and β-glucanase enzymes during must fermentation, which facilitates the release of grape and yeast-derived polysaccharides, positively impacts wine mouthfeel, contributing to reducing the perception of unripe fruit. When the judges were asked to express a preference (Table 6), NPCS wines treated with Pectolytic 1 and Pectolytic 2 enzymes were preferred over the control, with 14 answers out of 18 ($p < 0.04$). Similarly, in PCS wines, those treated with Pectolytic 1 and Pectolytic 2 showed to be preferred over the control ($p < 0.02$), while other enzyme treatments did not reach significance. Judges described these enzyme-treated wines as less astringent, smoother, and better balanced (Table 6). These results align with some studies found in the literature. Pérez-Magariño et al. (2022) in their work found that polysaccharides reduced the perception of acidity and bitterness in white wines, giving rise to sweeter wines, while Del Barrio-Galán et al. (2011) observed a reduction in bitterness due to the addition of commercial yeast derivatives primarily composed of mannoproteins. Quijada-Morin et al. (2014) and Gawel et al. (2014), in their studies, reported that polysaccharide concentrations enhanced mouthfeel properties such as fullness and viscosity, reducing the perceived astringency in red wines. Quijada-Morin et al. (2014) found that among all the polysaccharide families, RG-II and mannoproteins showed the strongest effect to reduce the astringency. Mannoproteins released by yeasts may interact with polyphenol aggregates as steric stabilizers (Poncet-Legrand et al., 2007). This feature is related to the ability of mannoproteins to interact with polyphenols found in wine, forming hydrogen-bonded complexes with them. In this way, bitter polyphenols would not be able to interact with taste receptors, developing the astringency perception (Poncet-Legrand et al., 2007). Polysaccharides are also capable of inhibiting salivary protein-polymerized polyphenols interactions by forming ternary complexes with proteins and polyphenols through hydrophobic interactions (Gawel et al., 2016). In white wines, the reduction in unripe fruit perception may also be due to the masking effect of the sapid peptide (Hsp12p), a heat-shock protein from mannoprotein released during the autolysis of *S. cerevisiae*, which exhibits a sweet taste and thereby contributes to the reduction of wine astringency (Rinaldi et al., 2019). The RG-II polysaccharides have also been described to reduce the overall wine astringency and indirectly influence sweetness (Quijada-Morin et al., 2014). RG-II is the grape polysaccharide that most effectively reduces the interaction between salivary proteins and wine polyphenols. The ability to smooth the perception of astringency is likely related to the branched structure and the presence of unusual sugars (which can contribute to a high-sugar characteristic), as well as the linkages between them (Quijada-Morin et al., 2014). In red wines, the interaction of polysaccharides with other compounds increases with the greater presence of the glucose moiety in the compounds they bind with, as it promotes a high number of binding sites for interactions with polysaccharides, such as gallotannins (Brandão et al., 2024). Thus, the interaction of all the structures containing glucose residues will be highly reduced. However, in white wines, specially made from grapes not fully ripened, the total content of phenolic compounds is lower or should be absent and their role in the astringency is less clear (Simoes Costa et al., 2015). Polysaccharides can also interact with other polysaccharide molecules. The interactions of polysaccharides with other polysaccharide molecules were already described, although it was reported that they had lower affinity than the interactions of polysaccharides with other ligands (Won et al., 2018). White wines made from grapes that have not reached full ripeness are low in polyphenolic content and polysaccharide-polysaccharide binding could occur. Polysaccharides, such as RG-II and mannoproteins, may interact with other grape polysaccharide molecules that have not undergone the hydrolysis transformation yet. One hypothesis suggests that non-hydrolyzed polysaccharides (from unripe grape berry cell walls) are responsible for the unripe character. If this hypothesis is true, the binding between polysaccharides themselves could mitigate the perception of unripe flavor by reducing the mouth puckering sensation. Furthermore, the high acidity and the low alcohol content of the resulting wine could be involved in the astringency perception (Brandão et al., 2024), contributing to the unripe character. As these astringent attributes are localized on the mouth surfaces, the presence of polysaccharides with viscous properties may compensate for the decrease in mouth lubrication (Brandão et al., 2024). However, the astringency should not be confused with the drying sensation caused by organic acids in white wines (Gawel et al., 2016). RG-II is the most abundant grape polysaccharide in juice, and its presence is largely due to its ease of enzymatic solubilization from the cell wall and its resistance to fragmentation by pectinases used in must production (Vidal, 2001). This group of polysaccharides will be more abundant in red wines, where fermentation occurs in the presence of the grape skins, compared to white wines, which are produced by fermenting the juice of crushed and pressed grapes (Gawel et al., 2016). Therefore, we expect the impact of RG-II on the perception of astringency in traditionally vinified white wines (without skin contact) to be lower than in white wines subjected to a pre-fermentative cold soaking treatment, and even lower than in red wines. Red wines are expected to contain 100–150 mg L^{-1} of RG-II, while white wines should contain 30–50 mg L^{-1}. Thus, in the vinification of white grape varieties that do not undergo maceration for enological purposes, the focus should be on extracting polysaccharides from yeast cell walls during alcoholic fermentation to mitigate the perception of unripe flavors in the mouth. Additionally, Vidal et al. (2004) observed that in a wine model solution, RG-II had no effect on bitterness in the absence of mannoproteins, but enhanced it in its presence. However, Quijada-Morin et al. (2014) found that an increase in mannose and rhamnose in the oligosaccharide

Table 5
Results of duo-trio test for the "unripe fruit" perception: performed on different pectolytic and β-glucanase enzyme-treated Catarratto wines, both pre-fermentative cold-soaked (bottom) and non-prefermentative cold soaked (top).

	Pairs of Wines	Number of Judges	Number of Correct answers	Number of Correct answers required for a $p < \alpha$	Significance
Catarratto NPCS	Control vs. Pectolytic 1	18	14 vs. 4	14 for a $p < 0.04$	$p < 0.04$
	Control vs. Pectolytic 2	18	14 vs. 4	14 for a $p < 0.04$	$p < 0.04$
	Control vs. β-Glucanase	18	12 vs. 6	14 for a $p < 0.04$	ns.
	Control vs. Pectolytic 3	14	8 vs. 6	12 for a $p < 0.02$	ns.
Catarratto PCS	Control vs. Pectolytic 1	14	11 vs. 3	11 for a $p < 0.03$	$p < 0.03$
	Control vs. Pectolytic 2	14	11 vs. 3	11 for a $p < 0.03$	$p < 0.03$
	Control vs. β-Glucanase	14	12 vs. 2	12 for a $p < 0.02$	$p < 0.02$
	Control vs. Pectolytic 3	14	10 vs. 4	12 for a $p < 0.02$	ns.

Note: *p*: *p*-value; α: significance level; ns: not significant.

Table 6
Results of preference test: performed comparing different pectolytic and β-glucanase enzyme-treated Catarratto wines, both prefermentative cold soaked (bottom) and non-prefermentative cold soaked (top) to control.

	Pairs of Wines	Number of Judges	Number of Correct answers	Number of Correct answers required for a $p < \alpha$	Significance	Less astringent	Smoother	More balanced
Catarratto NPCS	Control vs. Pectolytic 1	18	4 vs.14	14 for a $p < 0.04$	$p < 0.04$	35 %	35 %	30 %
	Control vs. Pectolytic 2	18	4 vs.14	14 for a $p < 0.04$	$p < 0.04$	27 %	44 %	30 %
	Control vs. β-Glucanase	18	6 vs.12	14 for a $p < 0.04$	ns.	-	-	-
	Control vs. Pectolytic 3	14	7 vs.7	12 for a $p < 0.02$	ns.	-	-	-
Catarratto PCS	Control vs. Pectolytic 1	14	2 vs.12	12 for a $p < 0.02$	$p < 0.02$	40 %	40 %	20 %
	Control vs. Pectolytic 2	14	2 vs.12	12 for a $p < 0.02$	$p < 0.02$	34 %	42 %	24 %
	Control vs. β-Glucanase	14	3 vs.11	12 for a $p < 0.02$	ns.	-	-	-
	Control vs. Pectolytic 3	14	8 vs.6	12 for a $p < 0.02$	ns.	-	-	-

Note: p: p-value; α: significance level; ns: not significant.

fraction, resulting from the degradation of mannoproteins and rhamnogalacturonans, was positively correlated with astringency. This finding supports the idea that the complete polysaccharide molecule is essential for effective interaction with other astringent ligands, highlighting the need for careful evaluation of enzymatic preparations in enology to prevent undesirable polysaccharide degradation. Although polysaccharides seem to play important roles in the wine's astringency, some characteristics and composition of the initial wine also affect the sensory properties of the wine. Rinaldi et al. (2012) found that in red wine, salivary protein precipitation, a key factor in astringency perception, is influenced by ethanol, tartaric acid, fructose, and mannoproteins. Tartaric acid significantly increased salivary protein precipitation and thus the perceived astringency, while ethanol, fructose and mannoproteins reduced them. Moreover, as the concentration of each influencing factor increases, its effect on salivary protein precipitation becomes more pronounced (Rinaldi et al., 2012).

3.6. Multivariate statistical analysis

3.6.1. O-PLS-DA based on the overall wine composition

To further understand the difference between control and PCS technique and the impact of each enzyme added during the alcoholic fermentation on the chemical properties of *Catarratto* wines a supervised Orthogonal Partial Least Squares Discriminant Analysis (O-PLS-DA) was carried out. A preselective O-PLS-DA was performed to select the variables with a VIP score greater than 1. Fig. 2(a) presents the O-PLS-DA Score Plot for PCS and NPCS wines. The score plot shows that the two groups, PCS and NPCS wines, are distinctly clustered. The 15 most important variable importance in projections (VIPs) are shown in Panel (b) of Fig. 2. The colored boxes on the right represent the direction of association of each variable with the two experimental groups. Specifically, each color indicates whether a variable is more closely associated with one group than the other. It can be observed that the features mostly contributing to discrimination were *p*-DACA, total colloids, buffer capacity, diendiol I, linalool, hexyl acetate, and mannose. PCS treatment increased key volatile compounds such as esters (hexyl acetate, phenylethyl acetate) and alcohols ((*E*)-hexenol, (*Z*)-3-hexenol), which contribute to aroma complexity. Previous studies identified hexyl acetate and phenylethyl acetate as some of the aroma compound markers that discriminated PCS from the NPCS wines. Their corresponding odor sensory descriptors are green and fruity for hexyl acetate and rose and floral for phenylethyl acetate (Alti-Palacios et al. 2023). In

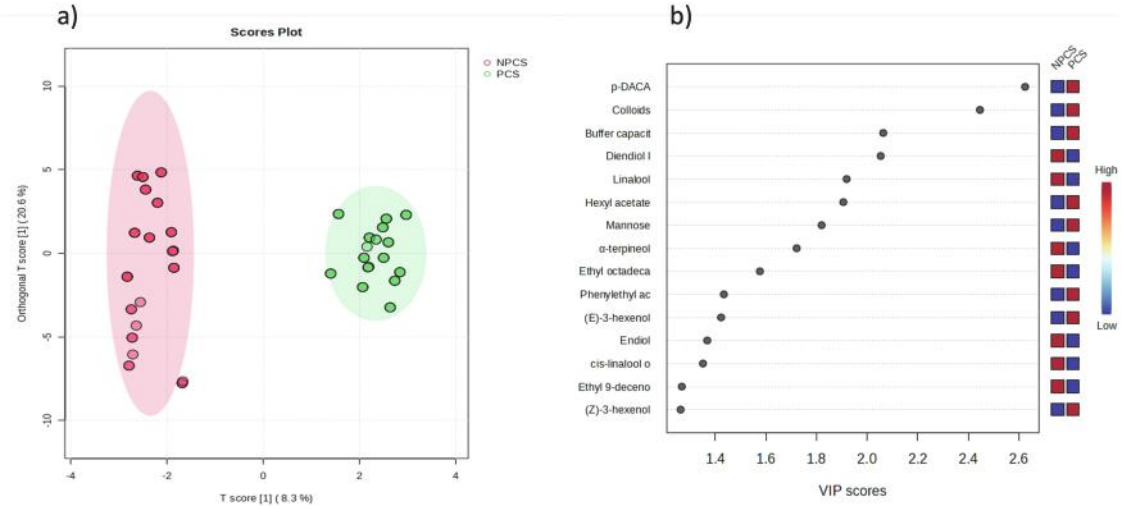

Fig. 2. O-PLS-DA Score Plot (a) showing the separation between Catarratto wines with prefermentative cold soak (PCS) and non-prefermentative cold soak (NPCS) as based on the volatile organic composition and the chemical-physical parameters. Important variables in projections (VIPs) are reported in (b). The colored boxes on the right of the figure indicate the relative concentrations of the corresponding metabolites.

addition, previous studies showed that maceration of the crushed and destemmed grapes before fermentation led to a higher abundance of low molecular weight phenolic compounds (Cai et al., 2014). The pre-fermentative maceration also increases the content of C6 alcohols extracted in the must, as earlier reported (Cejudo-Bastante et al., 2011). Meanwhile, terpenes compounds such as diendiol I, linalool, α-terpineol, endiol were found in higher concentration in wines that were not submitted to the PCS technique. In general, Alti-Palacios et al. (2023) observed that the concentration of the aroma compounds was higher in the wines submitted to the PCS technique, except for terpene compounds, which showed different trends between the control and PCS trials. PCS also enhanced structural components such as colloids and mannose, which play a crucial role in mouthfeel properties of a white wine, such as reducing astringency and bitterness (Gawel et al., 2016). Additionally, buffer capacity enhances the perception of acidity on the palate and improves the wine's taste persistence (Obreque-Slier et al., 2016). The permutation test results confirmed the statistical significance of the O-PLS-DA model, with empirical p-values of R^2Y: $p = 0.001$ (Supplementary Information, Figure S1 (a,b). In Fig. 3, the O-PLS-DA scores plot in each panel (A, C, E, G) shows clear clustering of the control wines (red, left side) and enzyme-treated wines (green, right side). This separation indicates a distinct impact of enzymatic treatments (Pectolytic 1, Pectolytic 2, Pectolytic 3, and β-glucanase) on the volatile organic composition and the chemical-physical parameters of the wines compared to the untreated controls. VIPs (panels B, D, F, H) of Fig. 3 identify the most influential variables driving the separation. The increase in volatile compounds varies among the enzymatic treatments, reflecting the specificities of each enzyme. However, it can be observed that the addition of Pectolytic 1, Pectolytic 2, and β-glucanase to the fermenting must led to an increase in terpenoid molecules. In most cases, terpenes are more abundant in their glycosylated form rather than in their free (unglycosylated) form. Their increase in wine can be explained by the β-glucosidase activity as a side function of certain enzymes (Rodríguez-Nogales et al., 2024). It has been stated that the increase of some aroma compounds could reduce astringency and bitterness of wines (Ferrer-Gallego et al., 2014). Volatile compounds may play a role in modulating astringency, likely due to a cognitive association between smell, taste, and mouthfeel, as flavor perception involves olfaction, gustation, and chemesthesis (Ferrer-Gallego et al., 2014). Colloids appear prominently in the VIP scores across the enzyme treatments, highlighting their impact by enzyme activity. Mannose is specifically highlighted in the β-glucanase treatment (Panel F). These results align with the findings of Pellerin & Tessarolo (2001), where the addition of commercial enzyme preparations to the fermenting must optimized and accelerated the autolysis of yeast cell walls, releasing yeast-derived polysaccharides into the medium.

3.6.2. *Principal component analysis (PCA) of sensory attributes and chemical composition*

To further explore the impact of the PCS technique and the studied enzymes, as well as the relationship between the chemical composition and the examined sensory attributes, an unsupervised PCA was performed. PCA was conducted for the two groups (NPCS and PCS) using wine chemical composition data, the sum of the chemical classes of volatile organic compounds, and the two key sensory attributes, including the unripe fruit attribute and overall satisfaction, as variables (Fig. 4(a) and (b), respectively). Data related to the sorting test can be found in Figure S3 of the Supplementary Information. In Fig. 4(a), for NPCS wines, the PCA biplot visualizes the first two principal components, PC1, which explains 49.98 % of the variance, and PC2, which accounts for 25.87 % of the variance, together explaining 75.85 % of the total variance. For PC1, the variables with strong positive correlations (Pearson's coefficient > 0.7) include benzenoids, acetate esters, ethyl esters, C_{13}-norisoprenoids, alcohols, mannose, esters of organic acids, and colloids. Acids exhibit a weaker positive correlation. The unripe fruit attribute has a weak negative correlation with PC1. For PC2, the variables with strong positive correlations (Pearson's coefficient > 0.7) are buffer capacity and total polyphenols. On the negative side, *p*-DACA shows a strong negative correlation, while lactones and acids are negatively correlated, though less strongly. The overall satisfaction score is also correlated with PC2. NPCS_Control and NPCS_Pectolytic 1 are separated on the negative side of PC1; NPCS_Pectolytic 2 is separated on the positive side of both PC1 and PC2; NPCS_Pectolytic 3 is separated on the negative side of both PC1 and PC2, while NPCS_β-glucanase is located on the positive side of PC1 and the negative side of PC2. In Fig. 4 (b), for PCS wines, the PCA biplot illustrates that PC1 explains 50.26 % of the variance, PC2 accounts for 27.73 % of the variance, together explaining 78 % of the total variance. For PC1, variables with strong positive correlations (Pearson's coefficient > 0.7) include acids, esters of organic acids and benzenoids, which contribute to the separation of samples in the positive PC1 region. On the negative side, variables with strong negative correlations (Pearson's coefficient < -0.7) include *p*-DACA, total polyphenols, acetate esters, ethyl esters, alcohols, and lactones, driving the separation of samples in the negative PC1 region. For PC2, variables with strong positive correlations include mannose, overall satisfaction, and colloids, which contribute to the separation of samples in the positive PC2 region. On the negative side, the variable unripe fruit shows a strong negative correlation, driving the separation of samples in the negative PC2 region. PCS_Control is located on the negative side of both PC1 and PC2. PCS_Pectolytic 3 is located on the negative side of PC1. PCS_Pectolytic 1 aligns in the positive side of PC1. PCS_β-glucanase and PCS_Pectolytic 2 are located on the positive side of PC2 and the negative side of PC1. It can be observed that in both groups (NPCS and PCS), the enzymes Pectolytic 2 and Pectolytic 3 often display similar behavior to the β-glucanase enzyme. They show increased concentrations of colloids and mannose, which result in higher scores for overall satisfaction. Additionally, these enzymes are consistently positioned on the opposite side of the unripe fruit attribute, exhibiting an opposite behavior compared to the control. An increase of grape- and yeast-derived polysaccharides in white wines at the end of the alcoholic fermentation helps to improve the mouthfeel attributes, reducing the unripe fruit character, saving time and storage space by avoiding the need for an extended maturation period of the wine on the lees. There is a difference in behavior among the enzymes, but there is also a distinction between the enzymes and the control. These latter analyses suggested that there is a marked impact of the addition of commercial enzymatic preparation featuring secondary activity along with fermenting yeasts on wine chemical and sensory profiles (Chong et al., 2019) from early harvested grapes.

4. Conclusion

The results of this study provide compelling evidence that PCS treatment and enzyme addition during alcoholic fermentation significantly improve the chemical and sensory properties of *Catarratto* wines. PCS enhanced the release of grape polysaccharides, doubling the total colloidal fraction, while enzymes, particularly β-glucanase, increased mannose concentration. Sensory analysis confirmed reduced "unripe fruit" perception and overall preference of the resulting wines. These findings highlight, for the first time, the potential of these techniques to optimize the quality of wines made from *Catarratto* grapes obtained from early harvested grapes. These results suggest that these techniques may be applicable to all *Vitis vinifera* L. grape varieties that are harvested early for technological purposes or display berry ripening heterogeneity at harvest, conditions that often result in wines with "unripe" sensory notes on the palate. This technique will be further investigated in other grape varieties, including red grapes, to assess its potential effects on phenolic compounds (such as anthocyanins and polyphenols) extracted during fermentative maceration, as well as its impact on unripe sensory perceptions.

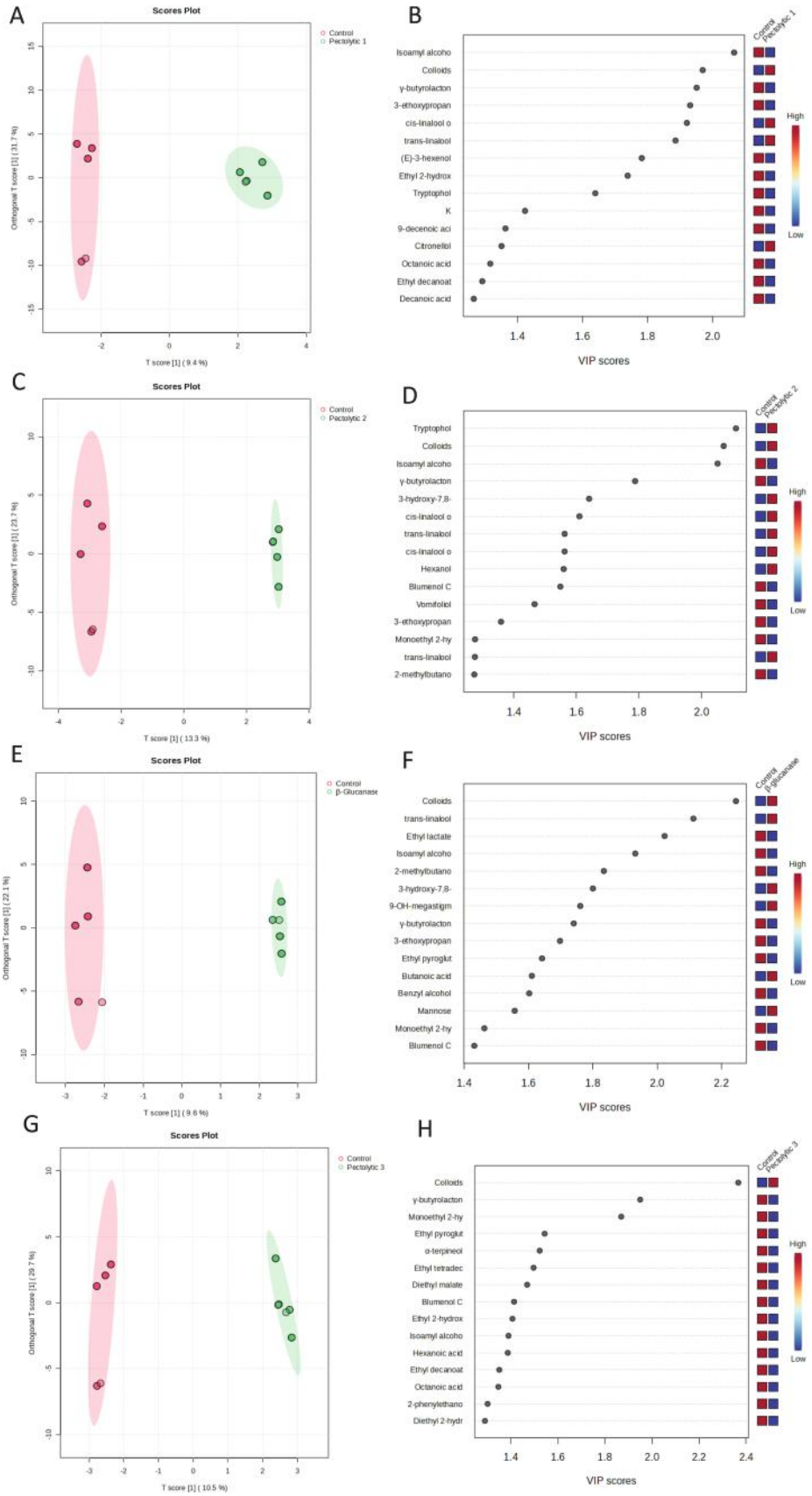

Fig. 3. O-PLS-DA scores plots (panels A, C, E, G) illustrating clear clustering between control wines (red, left) and enzyme-treated wines (green, right), demonstrating the distinct impact of enzymatic treatments (Pectolytic 1, Pectolytic 2, β-glucanase and Pectolytic 3) on the volatile organic composition and chemical-physical parameters. Panels B, D, F, and H show the corresponding Variable Importance in Projection (VIP) scores, highlighting the key variables driving these differences.

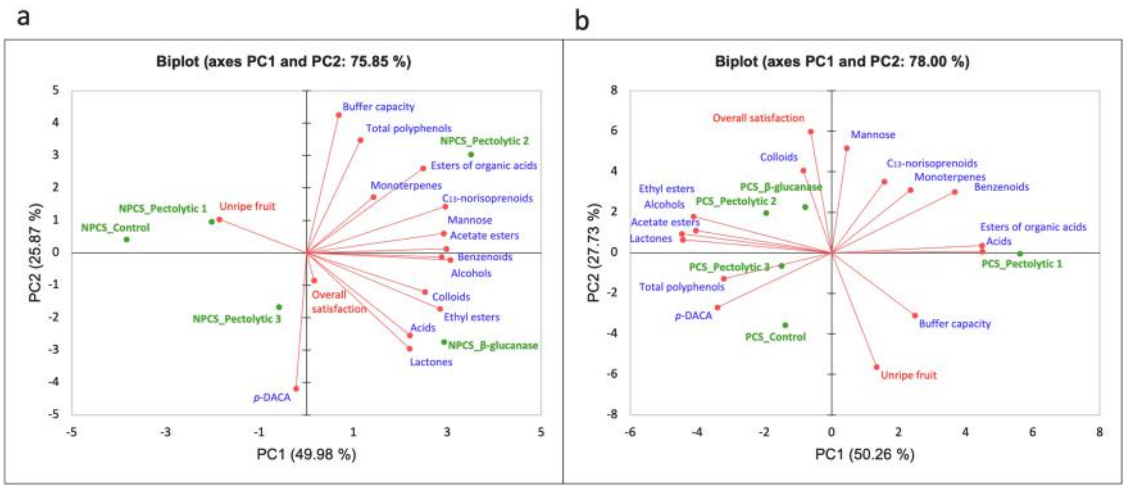

Fig. 4. PCA biplots for NPCS wines (a) and PCS wines (b) treated with the addition of different pectolytic and β-glucanase enzymes (green text), based on the two sensory attributes scores (unripe fruit and overall satisfaction) (red text) and compositional parameters (blue text) as variables.

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CRediT authorship contribution statement

Corona Onofrio: Writing – review & editing, Methodology, Data curation, Conceptualization. **Cinquanta Luciano:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Caraci Valentina:** Formal analysis. **Schnitter Manuel:** Investigation, Formal analysis. **Pollon Matteo:** Investigation, Formal analysis. **Vitaggio Clara:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.107560.

Data availability

Data will be made available on request.

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