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Innovative Non-Releasing Polyphenol Systems as a New Strategy Against Rosé Wine Oxidation

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ABSTRACT

Rosé wines are particularly prone to oxidative degradation due to their low polyphenolic content, which compromises color stability and shelf life. This study introduces an innovative strategy to enhance wine oxidative stability through the immobilization of grape must polyphenols on glutaraldehyde-crosslinked chitosan beads. Polyphenols were simultaneously extracted and immobilized, achieving an immobilization efficiency of up to 74.5% for total polyphenols and over 67% for total anthocyanins. Among all formulations, beads crosslinked with 1% glutaraldehyde (polyphenol-functionalized beads [PFB]-C4) showed the highest bioactive retention and were selected for application in rosé wine subjected to accelerated oxidative stress (60°C). No detectable release of polyphenols was observed in model wine solutions, confirming the stability of the immobilization system. Wines treated with PFB-C4 exhibited significantly higher Fe^{2+} stability and improved color preservation compared to the control. Specifically, a^* and b^* values remained significantly higher ($p < 0.05$), and chroma (C^*) ranged between 28.86 ± 0.43 and 31.79 ± 0.02, indicating greater color intensity (CT) and saturation. Moreover, CI was better retained, whereas tonality (T) remained lower, reflecting reduced browning and pigment degradation. These findings demonstrate that immobilized polyphenols act as both antioxidants and chelating agents, modulating iron redox balance and limiting Fe^{3+} formation. This study provides the first evidence of using immobilized grape polyphenols as a natural, reusable, and regulation-compliant antioxidant strategy to preserve rosé wine color and stability. The immobilization process provides a sustainable and reusable alternative to sulfur dioxide use, although further optimization is required to improve scalability and standardize polyphenol loading efficiency.

1 | Introduction

Wine oxidation is a major concern in modern oenology, as it affects color, aroma, and overall sensory quality, particularly in rosé wines that contain low levels of polyphenolic antioxidants. Traditional antioxidant management relies heavily on sulfur dioxide (SO_2), the use of which is increasingly restricted due to regulatory limits and consumer health concerns. Therefore, the search for sustainable, natural, and reusable alternatives capable of preserving wine stability without altering its sensory profile has become a key research focus. In this context, the present research investigates the immobilization of natural polyphenols on biopolymeric supports to enhance antioxidant protection, minimize oxidative degradation, and potentially reduce SO_2 dependency in winemaking. In recent years, in fact, rosé wine has gained increasing popularity in international markets due to its versatility, freshness, and broad organoleptic appeal. However, its production and preservation present significant challenges, especially in terms of color stability and susceptibility to oxidative degradation. Medina-Plaza et al. (2024) evidenced that, even after only 3 months of storage, rosé wines can undergo a decrease in lightness probably due to the formation of co-pigments and

polymeric pigments. Compared to red wines, rosé wines undergo shorter maceration times, which leads to a reduced extraction of anthocyanins and tannins from grape skins and seeds. Consequently, rosé wine—ranging in color from pale pink to onion-skin hues—is mainly dependent on monomeric anthocyanins, which are highly vulnerable to oxidation (Sacchi et al. 2005). The oxidative stability of wine depends not only on pigment and phenolic integrity but also on the preservation of volatile aroma compounds, which are highly sensitive to metal-catalyzed oxidation (Waterhouse et al. 2017). Polyphenols, through their antioxidant activity and non-covalent interactions with odorants, play a dual role in wine aroma: They can reduce the immediate release of volatile compounds while contributing to their long-term stability and protection from oxidation (Perez-Jiménez et al. 2020).

The antioxidant and chelating capacity of polyphenols and their ability to influence volatile compound preservation were even demonstrated in other plant-based systems like paprika and plant-based milk powders, where phenolic content affected both thermal behavior and volatile composition (Dippong et al. 2023, 2025).

Grape must (GM), especially from red grape varieties, contains a rich array of polyphenolic compounds, including flavonoids, phenolic acids, tannins, and anthocyanins. These substances represent the wine's natural protective shield against oxidative damage. Polyphenols, in particular anthocyanins, play a crucial role in protecting wine against oxidative degradation, with their concentrations varying depending on the grape variety and maceration time. Rosé wines, with low levels of these compounds, are particularly susceptible to oxidation, which may occur during key industrial processes such as racking, filtration, and bottling, as well as throughout storage and distribution. Oxidative reactions may be triggered by several factors, including temperature fluctuations and oxygen exposure.

Oxygen can initiate reactions involving anthocyanins, hydroxycinnamic acids, and flavanols, leading to color loss, browning, and aroma degradation through mechanisms such as quinone formation and radical generation via the Fenton reaction (Nguyen and Waterhouse 2021; Waterhouse et al. 2017). Although certain anthocyanin/tannin ratios in red wines promote the formation of stable pigments, rosé wines generally lack sufficient polyphenolic complexity to counterbalance oxidative processes, resulting in limited shelf life and reduced sensory quality (Zhang et al. 2020).

Rosé category includes a wide range of wines with variable phenolic content. Rosé wines with anthocyanin concentrations below approximately 30 mg/L are particularly prone to oxidation. Their insufficient polyphenolic content makes them vulnerable to oxidative reactions, posing a challenge for researchers and wine industry professionals in developing effective oxidation control strategies (Andújar-Ortiz et al. 2012; Labrador Fernández et al. 2023; Puyo et al. 2023).

Several approaches have been explored to improve the oxidative stability of rosé wines. Soluble polysaccharides extracted from grape pomace have been shown to enhance phenolic stability by promoting acetaldehyde-mediated pigment formation and reducing discoloration (Castro Marín et al. 2019). Oenological tannins combined with bioprotective yeasts have improved color stability by leading to better protection of oxidation-sensitive phenolic compounds in a comparable way to that obtained with the addition of sulfites (Puyo et al. 2023). Additionally, replacement of sulfur dioxide with unripe grape extracts and chitosan has helped preserve the color characteristics of the Sangiovese wine and its sensory attributes (Fia et al. 2023).

Among these strategies, the use of polyphenol-rich extracts from grapes or winery by-products has gained increasing interest due to their antioxidant potential. However, EU legislation prohibits the direct addition of polyphenol extracts, especially anthocyanin-rich ones, as they may alter the wine sensory profile and color (The European Commission 2019).

Therefore, non-releasing systems that exploit the antioxidant properties of polyphenols without modifying wine composition are particularly attractive.

Recent research studies (Medina-Plaza et al. 2024; Puyo et al. 2023) have highlighted the crucial role of polyphenolic composition in determining the oxidative stability and color preservation of rosé wines. At the same time, immobilization of natural antioxidants on biopolymers such as chitosan has emerged as a promising sustainable strategy to control oxidation processes (Dintcheva et al. 2020; Negm et al. 2020; Nishimoto-Sauceda et al. 2022). However, studies that combine the use of immobilized grape polyphenols with their application as a reusable antioxidant system in wine are still limited, representing the main innovation of the present work.

The present study proposes an innovative approach based on the immobilization of polyphenols onto inert solid supports, which enhances their stability and enables their use as oenological adjuvants by leveraging antioxidant activity without releasing the polyphenols into the wine matrix, thus complying with The European Commission (2019).

Using techniques commonly employed for enzyme immobilization, a protocol was developed to simultaneously isolate and immobilize polyphenols from GM onto glutaraldehyde-crosslinked chitosan beads. Glutaraldehyde is authorized by the US Food and Drug Administration (FDA) as a secondary direct food additive for specific technical purposes, particularly as a crosslinking and stabilizing agent for enzyme preparations used in food processing, but it does not remain in the final food product at levels that pose a health concern (U.S. Food and Drug Administration, Code of Federal Regulations [CFR]). This regulatory framework allows the use of glutaraldehyde as a processing aid, where it plays a functional role during manufacturing but is not intended to have any technological effect in the final food product. This approach has even been widely adopted in the scientific research studies. A recent investigation of Abd Rahman et al. (2025) evaluated the pectic-oligosaccharide production through the use of glutaraldehyde-crosslinked pectinase and xylanase enzymes. Moreover, glutaraldehyde has been widely used to immobilize different enzymes, such as lipases obtained from yeast (Gonawan et al. 2022), glucose oxidase for food packaging applications (Hanušová et al. 2013), and pectinase for fruit juice clarification (Dal Magro et al. 2018; Gür et al. 2018). These studies demonstrate the potential of glutaraldehyde crosslinking

to enhance enzyme stability, reusability, and process efficiency in food-related applications, while adhering to safety and regulatory constraints.

Therefore, the present study aims to develop a system for the GM polyphenols immobilization using chitosan beads crosslinked with glutaraldehyde. Moreover, the protective effect of the immobilized GM polyphenols was evaluated on rosé wine subjected to oxidative stress. Several tests were conducted to optimize the immobilization efficiency (IE) and to develop reactive beads (RB) capable of directly capturing polyphenols from GM, without requiring prior extraction or concentration steps.

Beads with immobilized polyphenols were then added to wine samples, which were exposed to elevated temperature and oxygen to simulate harsh storage conditions.

To the best of our knowledge, this is the first study demonstrating the use of chitosan-based immobilized grape polyphenols as an innovative strategy to control oxidation and color degradation in rosé wine, offering a sustainable alternative to conventional sulfur dioxide treatments.

Specifically, this work aims to (a) irreversibly immobilize polyphenols from GM on the surface of glutaraldehyde-crosslinked chitosan beads; (b) evaluate the immobilization capacity of chitosan beads crosslinked in relation to glutaraldehyde concentrations; (c) verify the absence of polyphenol and glutaraldehyde release into wine-like solutions, ensuring compliance with safety and regulatory requirements; (d) apply immobilized polyphenol beads to rosé wine under oxidative stress to evaluate its effects on oxidation prevention and therefore color stability.

These findings may pave the way for natural, reusable, and potentially sulfite-free stabilization systems in modern oenology.

2 | Materials and Methods

2.1 | GM and Wine Materials

Rosé wine, ready for bottling, was provided by Az. Agr. Floriana Marta Cosentino, a small winery located in Zafferana Etnea, Italy. The wine was produced from *Nerello Mascalese* grapes hand harvested in September 2024. The grapes were destemmed and subjected to a short maceration period (approximately 2 h) to obtain the desired pale color and limited polyphenol extraction. The wine was selected on the basis of its low anthocyanin and total polyphenol content, typical of certain rosé styles, which makes it particularly susceptible to oxidative degradation.

The rosé wine used in this study had the following oenological parameters: pH 2.96, total acidity 7.1 g/L (as tartaric acid), alcohol 12.44% (v/v), total SO_2 39.0 mg/L, and free SO_2 10.0 mg/L. No additional treatments (fining, filtration, or stabilization) were applied beyond standard commercial winemaking practices. Prior to the experiments, the wine was stored in the dark at 4°C and equilibrated to room temperature before use. The same winery provided a GM, used in this experiment for polyphenol immobilization. The must, obtained from *Nerello Mascalese* grapes, was produced through prolonged maceration to ensure optimal extraction of color compounds and polyphenols.

2.2 | Development of Polyphenol-Functionalized Beads (PFBs)

Chitosan RBs were prepared by crosslinking with glutaraldehyde following the protocol described by Gadallah et al. (2023), with minor modifications. These beads were then used to covalently immobilize GM polyphenols, yielding polyphenol-functionalized supports.

In brief, 2% (w/v) chitosan solution was set by dissolving chitosan (GC190-Glentham Life Sciences Ltd.—UK) in 1% (v/v) acetic acid. This solution was extruded dropwise through a syringe needle into a gently stirred 2% (w/v) NaOH bath, yielding spherical chitosan beads. Beads were allowed to harden for 1 h, then rinsed with deionized water until neutral pH was reached. After 1 h, beads were divided into four groups, and each group was crosslinked with different concentrations (C1, C2, C3, and C4) of glutaraldehyde in phosphate buffer (pH 7 0.1 M): C1 at 0.1% (v/v), C2 at 0.25% (v/v), C3 at 0.5% (v/v), and C4 at 1.0% (v/v) and incubated for 24 h at room temperature. During this step, Schiff base linkages formed between chitosan's free amino groups and glutaraldehyde's aldehyde moieties, and RBs were made. RBs were then washed repeatedly with deionized water until no residual glutaraldehyde was detected (Kaiser test) and stored at 4°C until use. In order to determine the optimal weight of beads to be added per mL of wine, preliminary trials were carried out (data not shown). Different bead-to-wine ratios were tested to identify the condition under which the beads reached saturation while leaving only a minimal amount of total polyphenols unabsorbed in the wine. Therefore, 2 g of RB, prepared as described above, were introduced into 50 mL of GM and gently agitated at room temperature for 6–8 h. Under these conditions, must-derived polyphenols were immobilized onto the bead surface through residual aldehyde groups and non-covalent interactions. The RB functionalized with must polyphenols were labeled as PFBs followed by C1, C2, C3, or C4 in relation to the glutaraldehyde concentration used.

Each group of PFB (PBF-C1, PBF-C2, PBF-C3, and PBF-C4) was then washed four times with sterilized distilled water and stored under refrigerated conditions until its use in the following experiment, whereas the unbounded must fraction (UMF), obtained after the removal of each PFB group, was recovered by filtration and analyzed in a subsequent report. The preparation steps of PFB are summarized in Figure 1.

2.3 | Evaluation of a PFB System

All groups of PBFs (PBF-C1, PBF-C2, PBF-C3, and PBF-C4) (Figure 1) were evaluated for their ability to immobilize polyphenols. This was assessed indirectly by measuring the concentration of bioactive compounds in the GM before and after contact with the RBs (Figure 1). The analyses of the must samples after contact with the beads, representing the UMF (UMF-C1, UMF-C2, UMF-C3, and UMF-C4) (Figure 1), allowed the quantification of the decrease in polyphenol content and antioxidant activity in the

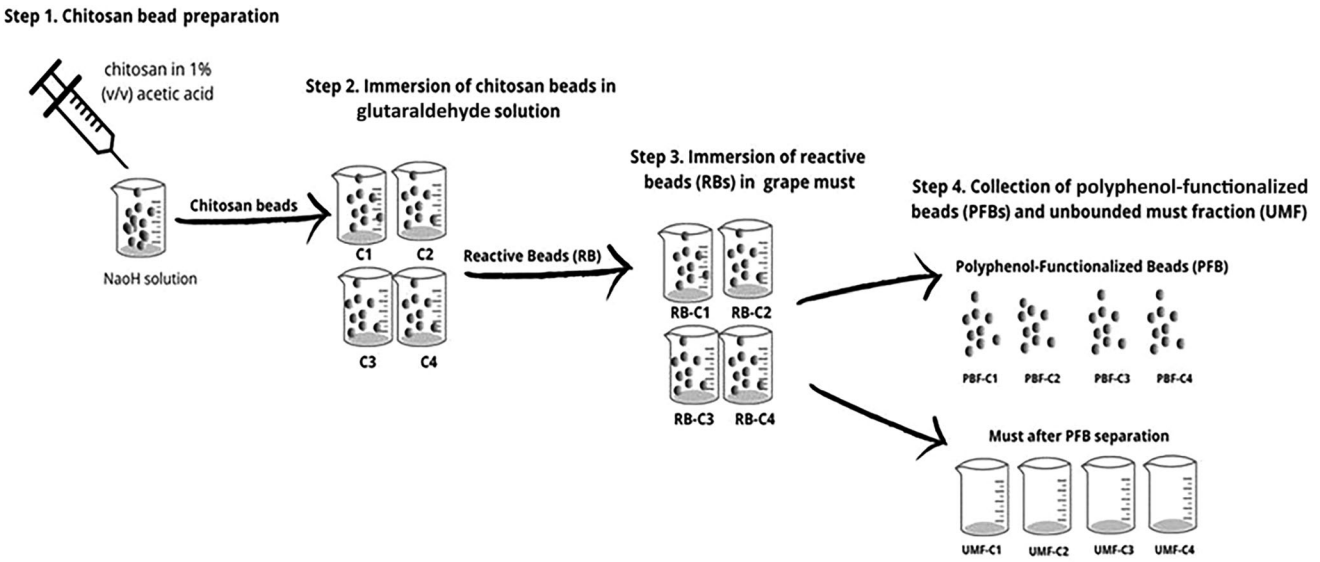

FIGURE 1 | Steps in the preparation of polyphenol-functionalized beads (PFB).

liquid phase, serving as an indirect measure of the beads' capacity to retain bioactive compounds from the must.

2.3.1 | Total Polyphenol Evaluation, Anthocyanin Content, and Antioxidant Activity

Total polyphenols were quantified using the Folin–Ciocalteu method, as described by Singleton et al. (1999). The reaction mixture consisted of 250 μL of each UMF, 1.25 mL of Folin–Ciocalteu reagent, and, after a 3-min incubation, 2.5 mL of 20% sodium carbonate (Na_2CO_3), resulting in a total volume of 25 mL. The mixture was then incubated for 60 min in the dark to allow the reaction to complete. The absorbance of the solution was measured at 725 nm using a Perkin Elmer Lambda 25 ultraviolet–visible (UV–Vis) spectrometer (Perkin Elmer Inc., Waltham, MA, USA). Results were expressed as mg of gallic acid equivalents (GAE) per liter of sample (mg GAE/L). A standard curve was constructed using standard concentrations of gallic acid ranging from 0 to 80 mg/mL.

Anthocyanin contents were determined on wine samples under study following the method described by Ribéreau-Gayon (2003). This method allows to make an estimation of the different forms of anthocyanins. In order to determine the free, combined, and total anthocyanin content, 1 mL of each wine sample was mixed with 1 mL of ethanol acidified with 0.1% HCl and 20 mL of 2% HCl; this solution of 10 mL was, respectively, placed in two test tubes labeled test tube A and test tube B. In test tube A, 4 mL of water was added, whereas 4 mL of a 15% sodium bisulfite ($NaHSO_3$) solution was added to test tube B. The absorbance was then read after 15 min at 520 nm using a Perkin Elmer lambda 25 UV–Vis spectrometer (Perkin Elmer Inc., Waltham, WA, USA). The concentrations (C) of free, combined, and total anthocyanins were expressed as mg of cyanidin-3-glucoside equivalents (CGE) per liter of sample (mg CGE/L) and determined using the following equations:

$$C_{\text{free anthocyanins}} = (\text{Abs}_A - \text{Abs}_B) \times 875$$

$$C_{\text{total anthocyanins}} = \text{Abs}_A \times 875$$

$$C_{\text{combined anthocyanins}} = C_{\text{total anthocyanins}} - C_{\text{free anthocyanins}}$$

where Abs_A represents the absorbance measured in test tube A, and Abs_B represents the absorbance measured in test tube B.

The IE representing the capacity of each PFB group (Figure 1) to bind the bioactive compounds (polyphenols, total, combined, or free anthocyanins) was calculated according to the following formula:

$$\text{IE}(\%) = \left(\frac{C_i - C_f}{C_i}\right) \times 100$$

where C_i is the initial concentration of each compound (total polyphenols, total combined, or free anthocyanins), and C_f is the concentration after immobilization.

Moreover, the amount of bioactive compounds immobilized per gram of beads (mg compound/g beads) was calculated using the following formula:

$$\text{Bioactive compound immobilization capacity (mg/g)} = (C_i - C_f) \times V/m$$

where C_i and C_f are the initial and final concentrations of the bioactive compound in solution (mg/L), V is the volume of the solution (L), and m is the mass of the beads (g).

Antioxidant capacity of UMF-C1, UMF-C2, UMF-C3, and UMF-C4 samples under study (Figure 1) was evaluated using the ferric reducing antioxidant power (FRAP) method and compared with the previous GM RB inoculation.

The FRAP assay, described by Benzie et al. (1996), was used to indirectly evaluate the antioxidant activity of the PFBs (PF-B). The assay measures the increase in absorbance at 593 nm due to the formation of the ferrous–tripyridyl-*S*-triazine complex (TPTZ–Fe(II)) in the presence of reducing agents.

In brief, a volume of 2.9 mL of freshly prepared FRAP reagent, composed of 10 mL of 300 mM acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ in 40 mM HCl, and 1 mL of 20 mM $FeCl_3$ (10:1:1, v/v/v), was added to each must sample. After incubation at 37°C for 30 min, absorbance was measured at 593 nm using a Perkin Elmer Lambda 25 UV–Vis spectrophotometer (Perkin Elmer Inc., Waltham, MA, USA), using the acetate buffer as a blank. All the analyses were conducted at least in triplicate, and results were expressed as mg of Trolox equivalents (TE)/L of sample.

The potential antioxidant activity of the different PFBs (PFB-C1, PFB-C2, PFB-C3, and PFB-C4) was indirectly calculated using the above-described assay by measuring the decrease in antioxidant activity of each UMF (similarly as previously reported) and calculated with the following formula:

$$\text{Antioxidant activity potential (mg/g)} = (C_i - C_f) \times V/m$$

where C_i and C_f are the initial and final concentrations of the bioactive compound in solution (mg/L), V is the volume of the solution (L), and m is the mass of the beads (g).

2.3.2 | Release of Polyphenols and Glutaraldehyde on Model Wine

Among the three groups of chitosan beads crosslinked with different concentrations of glutaraldehyde, the C4 group treated with 1% glutaraldehyde exhibited the highest capacity to adsorb bioactive compounds, as also reported in the results section (see Section 3.1). Therefore, this group was selected for the further experiments made on wine matrix. However, before application, the ability of the beads to retain both polyphenols and glutaraldehyde under prolonged stress conditions was evaluated.

A model wine solution was prepared to mimic the main characteristics of the rosé wine used in the study. The solution consisted of 12.44% (v/v) ethanol in water and 0.033 M tartaric acid, adjusted to pH 3.0 using 1 M NaOH.

Approximately 2 g of PFBs (PFB-C4) were immersed in 50 mL of the model wine solution, which was then subjected to oxidative stress following the protocol described by Deshaies et al. (2020) and incubated at 60°C overnight.

After incubation, the model wine was filtered to remove the PFB-C4, and the possible release of polyphenols into the solution was assessed using the Folin–Ciocalteu assay, as previously described in Section 2.3.1.

Glutaraldehyde release from PFB into the model wine was evaluated using a colorimetric method adapted from Jolibois et al. (2002). Aliquots of the model wine were let to react with a phenol–perchloric acid reagent to form a yellow chromophore, and absorbance was measured at 479 nm using a UV–Vis spectrophotometer.

Quantification was performed using an external calibration curve prepared with standard glutaraldehyde solutions ranging from 0.01 to 2.5 mg/L.

In addition, the presence of residual substance in the solution was assessed by recording the UV–Vis absorption spectra in the 340–840 nm range using a Perkin Elmer lambda 25 UV–Vis spectrometer (Perkin Elmer Inc., Waltham, MA, USA). This analysis enabled the detection of characteristic absorption peaks attributable to polyphenolic compounds, allowing evaluation of any potential release from the PFB-C4 into the surrounding medium.

2.4 | Accelerated Aging Tests Made on Rosé Wine Treated With PFBs (PFB-C4

To simulate oxidative stress under controlled and accelerated conditions, 50 mL of rosé wine (see Section 2.1) was transferred to a 500 mL flask and inoculated with PFBs that had previously been shown to have the highest polyphenol immobilization capacity (PFB-C4). The wine sample containing the PFB-C4 was labeled W-PFB-C4. Accelerated aging tests were then conducted according to the protocols described by Coppola et al. (2021) and Deshaies et al. (2020), which are commonly used to replicate, within a shortened time frame, the oxidative processes typically occurring during wine aging.

The wine was saturated with air by vigorously shaking the flask for 10 s. This aeration step was repeated three times. A control sample, labeled W-CONTROL, consisted of wine treated under identical conditions but without the addition of PFB-C4. All oxygen-saturated samples were subsequently incubated at 60°C to induce oxidative stress and analyzed after 15, 30, 60, and 120 min of incubation. To assess the oxidative evolution of wine during the accelerated oxidation treatment, several markers were monitored, including acetaldehyde formation, iron content, and color variation.

2.4.1 | Determination of Fe(II), Fe(III), Fe (total), and Acetaldehyde

The concentrations of Fe(II) and total iron in wine samples were determined using a colorimetric method adapted from Nguyen and Waterhouse (2019), employing ferrozine as the complexing agent. Ferrozine reacts specifically with Fe(II) to form a stable purple complex, the absorbance of which was measured at 562 nm.

For Fe(II) determination, 1 mL of wine was mixed with 10 µL of ferrozine solution (3.5%), vortexed, and then 1.5 mL of EDTA solution (0.005%) was added. Absorbance was recorded after 1 min of reaction. Total iron was measured using the same protocol, with the addition of 1.5 mL of ascorbic acid solution (0.1%) instead of EDTA to reduce Fe(III) to Fe(II) prior to complexation and recording the absorbance after 30 min of incubation. Iron

concentrations were calculated using a calibration curve prepared with $FeSO_4$ standard solutions and expressed as mg/L.

Acetaldehyde concentration was determined using the Megazyme Acetaldehyde Assay Kit (Megazyme International, Ireland), following the manufacturer's instructions. Briefly, wine samples were prepared and incubated with the specific reagents provided in the kit, which enzymatically convert acetaldehyde to a detectable product. The absorbance was measured spectrophotometrically at 340 nm. Acetaldehyde concentration was calculated by comparison with a calibration curve prepared from standards supplied in the kit, and results were expressed as mg/L.

2.4.2 | Color Evaluation

UV–Vis absorption spectra of the wine samples were recorded using a PerkinElmer Lambda 25 UV–Vis spectrophotometer (Perkin Elmer Inc., Waltham, MA, USA) equipped with a 1 mm quartz cuvette. Spectra were acquired over the wavelength range of 340–830 nm, providing detailed insight into the wine's color and absorbance characteristics. Multiple linear regression model proposed by Prez-Magario and Gonzalez-SanJose (2002) was used for the calculation of the CIELab parameters L^*, a^*, and b^*. Chroma of each sample was calculated as follows:

$$C = \sqrt{(a^*2 + b^*2)}$$

In the CIELAB system, L^* represents lightness on a scale from 0 (black) to 100 (white); a^* indicates position on the green–red axis, with negative values corresponding to green and positive values to red; b^* reflects position on the blue–yellow axis, where negative values indicate blue and positive values yellow. Chroma (C) quantifies color saturation (intensity).

Color intensity (CI) and tint (T) of each sample under study were evaluated by measuring the absorbance at 420, 520, and 620 nm.

These values were used to calculate the CI and tint (T), following the equations reported by (Gayon et al. 2006):

$$CI = Abs_{420} + Abs_{520} + Abs_{620}$$

$$T = Abs_{420}/Abs_{520}$$

All measurements were performed at least in triplicate to ensure accuracy and reproducibility.

2.5 | Statistical Analysis

Data obtained from each experiment, conducted at least in triplicate, were statistically analyzed using Minitab software (version 20.0, Minitab Inc., State College, PA, USA). A one-way analysis of variance (ANOVA) was performed on the mean values to evaluate the effect of different treatments. When significant differences were found ($p \leq 0.05$), Fisher's least significant difference (LSD) test was applied for pairwise comparisons among treatments. Results are presented as mean ± standard deviation (SD). In all tables, different lowercase letters within the same column indicate statistically significant differences ($p \leq 0.05$) among treatments, whereas the same letter denotes no significant difference.

3 | Results and Discussion

3.1 | Efficiency of Bioactive Compound Immobilization and Potential Release of Polyphenols and Glutaraldehyde on Model Wine

Table 1 presents the concentration of total polyphenols, anthocyanin content (free, combined, and total), and antioxidant activity evaluated in GM and in UMF (UMF-C1, UMF-C2, UMF-C3, and UMF-C4) (Figure 1), as well as the corresponding IE (IE%).

Compared to the original GM, all UMF samples showed a significant reduction in polyphenol and anthocyanin content, confirming the effective adsorption of these compounds onto bead surface. The total polyphenol content decreased from 690.00 ± 0.35 mg/L in the GM to 176.17 ± 0.26 mg/L in UMF-C4, with significant differences between all conditions ($p < 0.05$) and a maximum IE of 74.46%. A similar trend was observed for free anthocyanins, which dropped from 54.78 ± 0.46 mg/L in GM to 22.17 ± 0.41 mg/L in UMF-C4, with immobilization efficiencies of 59.53%. Again, all conditions differed significantly ($p < 0.05$), confirming that different immobilization setups affected anthocyanin retention differently (Table 1).

Interestingly, combined anthocyanins showed the highest IE, reaching 78.48% in UMF-C4 and suggesting a strong interaction between this anthocyanin form and the immobilization matrix.

Regarding total anthocyanins, the concentration in the unbounded fraction decreased by up to 67.30% in UMF-C4. The statistical grouping confirms that all tested conditions resulted in significantly different ($p < 0.05$) anthocyanin retention in the unbounded must.

The untreated GM exhibited the highest antioxidant activity (1078.2 ± 13.7 mg TE/L), consistent with its rich content in total phenolics (690.00 ± 0.35 mg GAE/L) and total anthocyanins (92.84 ± 0.23 mg CGE/L). In contrast, the antioxidant activity in the UMF samples decreased proportionally with increasing IE%. In particular, UMF-C4, which showed the highest IE% for total polyphenols (74.46%) and total anthocyanins (67.30%), retained only 196.5 ± 29.6 mg TE/L of antioxidant activity.

On the basis of each UMF analysis, the content of immobilized compounds was expressed as mg of compound per gram of beads (PFB) (Figure 2). As displayed in Figure 2, the immobilized polyphenols ranged from 8.13 ± 0.00 to 12.85 ± 0.01 mg GAE/g of beads, whereas the anthocyanins varied between 0.23 ± 0.00 and 0.75 ± 0.00 mg CGE/g. Notably, samples of PFB-C4 exhibited the significantly highest ($p < 0.05$) bioactive compound content, and in line with this, the antioxidant activity of the beads, also indirectly inferred from the unbound fractions, was estimated to

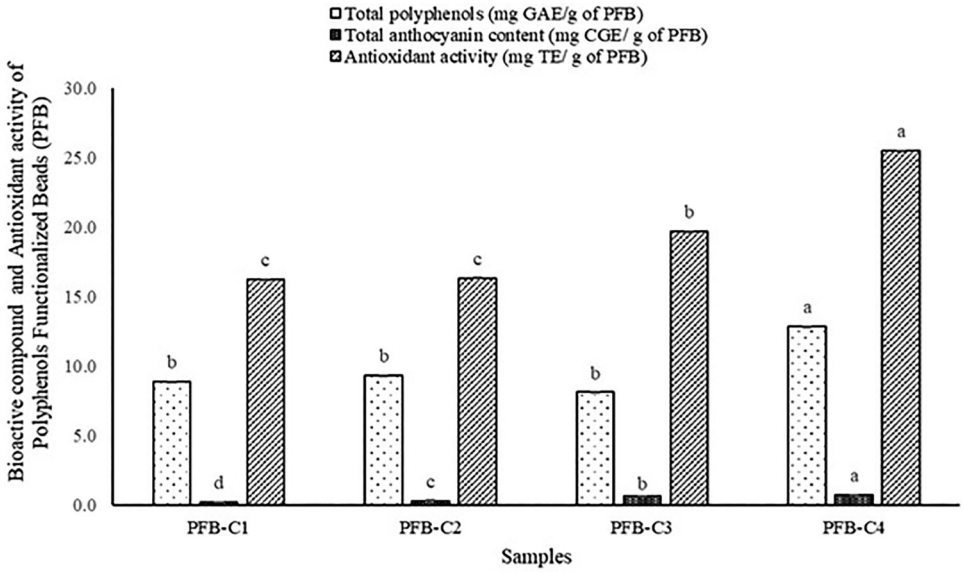

FIGURE 2 | Bioactive compounds and antioxidant activity of polyphenol-functionalized bead (PFB). Mean value and standard deviation bars are shown. Within each parameter (total polyphenols, total anthocyanins, and antioxidant activity), means marked by different letters differ significantly ($p < 0.05$) according to Fisher's least significant difference test.

range from 16.26 ± 0.00 to 25.49 ± 0.00 mg TE/g, with PFB-C4 again showing the highest ($p < 0.05$) value.

Moreover, before application in wine, the stability of PFB-C4 beads was tested in a model wine solution under prolonged oxidative stress to assess the potential release of immobilized polyphenols and residual glutaraldehyde. As confirmed by spectrophotometric assays and UV–Vis analysis, no detectable release of either polyphenols or glutaraldehyde was observed in the treated samples compared to the model wine control. The absence of characteristic absorption peaks associated with polyphenolic compounds and glutaraldehyde in the supernatant confirmed the effective retention of both components within the bead matrix, even under accelerated degradation conditions. Therefore, PFB-C4 beads can be considered stable and safe for wine application, without concerns of compound leaching into the surrounding medium.

As also reported by Gadallah et al. (2023), glutaraldehyde concentration plays a key role both in the function of the polymeric formulation of beads and target compounds. Therefore, the present results suggest that the concentration of the crosslinking agent significantly affects the binding efficiency of different polyphenolic subclasses, highlighting the importance of optimizing bead formulation according to the specific chemical nature of the target compounds.

3.2 | Evaluation of Oxidative Evolution of Wine Subjected to Accelerated Oxidation Process

3.2.1 | Assessment of Fe(II), Fe(III), Fe (total), and Acetaldehyde

In order to assess the potential protective effect of immobilized polyphenols against wine oxidation, rosé wine samples were subjected to an accelerated oxidative stress protocol. Wines were incubated at 60°C for up to 4 h to simulate oxidative conditions that may occur during storage and handling. Figure 3a–c displays the iron speciation, over 240 min of incubation at 60°C, of W-PFB-C4 and untreated wine sample (W-CONTROL). At all-time points, W-PFB-C4 exhibited significantly ($p < 0.05$) higher concentrations of Fe(II) (Figure 3a) in comparison to W-CONTROL. Notably, Fe(II) increased progressively over time in the treated samples, reaching 0.373 ± 0.003 mg/L after 240 min, whereas in the control sample, Fe(II) decreased significantly, dropping to 0.103 ± 0.003 mg/L.

Indeed, the calculated Fe(III) content (Figure 3b) in the treated wine was consistently lower than in the control, reaching a minimum of 0.049 mg/L at 240 min, compared to 0.604 mg/L in the control. The sharp decline of Fe(III) in treated samples suggests that polyphenol-based spheres preferentially interact with or reduce Fe(III), thereby shifting the redox balance toward the ferrous form. Moreover, Fe (total) (Figure 3c) decreased in treated wines compared to the control, with values dropping from 0.506 ± 0.008 to 0.423 ± 0.003 mg/L over the 240 min incubation, whereas the control remained above 0.7 mg/L throughout. The reduction in total iron content indicates a likely chelating or adsorptive action of the spheres, which may have retained iron species, particularly Fe(III), on their surface.

Table 2 reports the data concerning the acetaldehyde concentration, determined as a primary indicator of ethanol oxidation.

After 15 min of oxidative stress at 60°C, the W-CONTROL sample showed a marked increase in acetaldehyde concentration, reaching approximately 23.2 ± 3.7 mg/L. In contrast, the wine containing immobilized polyphenol spheres exhibited significantly lower acetaldehyde levels (12.1 ± 0.53 mg/L), suggesting a protective effect of the immobilized antioxidants (Table 2). At subsequent time points (30–240 min), acetaldehyde concentrations decreased in both samples, with more stable values observed

TABLE 1 | Evaluation of polyphenols' unbounded must fraction (UMF[a]) and immobilization efficiency (IE%[b]).

Samples	Parameters								
	Total polyphenols		Free anthocyanins		Combined anthocyanins		Total anthocyanins		Antioxidant activity
	mg GAE/L	IE%	mg CGE/L	IE%	mg CGE/L	IE%	mg CGE/L	IE%	mg TE/L
GM	690.00 ± 0.35[a]	—	54.78 ± 0.46[a]	—	38.06 ± 0.61[a]	—	92.84 ± 0.23[a]	—	1078.2 ± 13.7[a]
UMF-C1	334.94 ± 0.30[c]	51.46	33.78 ± 0.63[b]	38.34	28.76 ± 0.13[b]	24.41	62.53 ± 0.53[b]	32.64	418.5 ± 7.8[b]
UMF-C2	315.67 ± 0.13[d]	54.25	27.80 ± 0.75[c]	49.25	25.38 ± 0.92[c]	33.33	53.17 ± 0.27[c]	42.73	421.2 ± 17.1[b]
UMF-C3	365.01 ± 0.17[b]	47.10	23.92 ± 0.41[d]	56.33	12.28 ± 0.41[d]	67.72	36.2 ± 0.25[d]	61.01	326.4 ± 2.8[c]
UMF-C4	176.17 ± 0.26[e]	74.46	22.17 ± 0.41[e]	59.53	8.20 ± 0.13[e]	78.48	30.36 ± 0.35[e]	67.30	196.5 ± 29.6[c]

Note: Data are presented as mean ± standard deviation of the mean. In each column, within the same parameter (total polyphenols, free anthocyanins, combined anthocyanins, total anthocyanins, and antioxidant activity), values followed by different letters are significantly different according to Fisher's least significant difference test ($p < 0.05$).

[a]UMF: Unbounded must fraction is the residual must fraction after immobilization.

[b]IE%: Immobilization efficiency, calculated as the percentage reduction in each compound with respect to the original grape must (GM).

FIGURE 3 | (a) Fe(II), (b) Fe(III), and (c) Fe (total) of control wine sample (W-CONTROL) and wine containing polyphenol-functionalized beads (W-PFB-C4). Mean value and standard deviation bars are shown. Means marked by different letters differ significantly ($p < 0.05$) according to Fisher's least significant difference test.

TABLE 2 | Acetaldehyde concentration of wine samples during oxidative stress at 60°C.

Incubation time (min)	Samples	
	W-CONTROL	W-PFB-C4
15	23.2 ± 3.7°	12.1 ± 0.53[b]
30	13.0 ± 2.1°	12.3 ± 3.57[a]
60	13.0 ± 1.8°	11.2±1.60[a]
120	10.9 ± 1.1°	12.3 ± 1.78[a]
240	9.5 ± 1.3°	11.2 ± 1.25[a]

Note: Data are presented as mean ± standard deviation of the mean. In each row, values followed by different letters are significantly different according to Fisher's least significant difference test ($p < 0.05$).

Abbreviation: PFB, polyphenol-functionalized bead.

in the treated wine, likely due to the scavenging and binding capacity of the immobilized polyphenols.

Acetaldehyde formation in wine is strongly influenced by oxygen exposure and microbial activity (Dai et al. 2022) and is widely recognized as a marker of oxidative degradation, color instability, and sensory deterioration (Arias-Pérez et al. 2021). It is mainly produced through ethanol oxidation catalyzed by transition metals such as Fe^{3+} and Cu^{2+}, or through enzymatic and non-enzymatic pathways involving polyphenols (Kasai and Kawai 2021; Xue et al. 2021). These metals catalyze redox reactions (e.g., via Fenton-type pathways), accelerating ethanol oxidation to acetaldehyde and promoting its subsequent reactions with phenolic substrates, especially in the presence of iron, which favors acetaldehyde formation and its combination with wine phenolics (Cacho et al. 1995; Danilewicz 2007; Nguyen and Waterhouse 2022). In the present study, acetaldehyde accumulation was significantly lower in the wine treated with immobilized polyphenols (W-PFB-C4) compared to the untreated control, suggesting that the immobilized antioxidants effectively limited ethanol oxidation and subsequent aldehyde formation.

These results highlight the dual role of the immobilized polyphenols: as antioxidants limiting iron redox cycling and as potential chelating agents removing iron from the wine matrix. These findings are consistent with previous studies reporting the ability of polyphenols to reduce Fe(III) to Fe(II) and to form stable Fe–polyphenol complexes that lower free iron levels in solution (Sánchez-Vioque et al. 2013). Additionally, the observed antioxidant protection may be attributed to the presence of immobilized anthocyanins, which can act as preferential redox substrates. By undergoing oxidation themselves, they protect native anthocyanins and other wine phenolics from oxidative degradation, thus contributing to the stabilization of color and sensory properties (He et al. 2012). Even Baris et al. (2024) evidenced that in different rosé wine models, the amount of anthocyanins is crucial to preserve the higher concentration of Fe(II) over time, suggesting as the antioxidant role is both correlated with concentration and type of anthocyanin.

The observed stabilization of Fe^{2+} and the lower Fe^{3+} formation in W-PFB-C4 reflect a protective redox buffering effect. Polyphenols, particularly catechins and anthocyanins, are known to form stable Fe^{2+} complexes, thereby limiting Fenton-type reactions responsible for oxidative chain propagation (Arias-Pérez et al. 2021; Bueno et al. 2018). Moreover, recent findings have demonstrated that the antioxidant effectiveness of individual anthocyanins varies markedly during wine oxidation. In particular, delphinidin-3-glucoside acts as the most efficient "sacrificial" antioxidant, rapidly consuming oxygen and preventing ethanol oxidation, whereas malvidin-3-glucoside shows lower protective efficiency and promotes Strecker aldehyde formation (Escudero et al. 2025).

3.2.2 | Color Evaluation

Table 3 reports the evolution of CIELab parameters (L^*, a^*, b^*, and C) in rosé wine, monitored over 240 min (at 60°C) of accelerated oxidation.

At time zero, the wine before oxidation exhibited an L^* value of 79.34 ± 0.01, a^* of 33.00 ± 0.01, b^* of 17.42 ± 0.01, and chroma (C^*) of 37.32 ± 0.02, reflecting a vivid red–pink hue with a bluish component. After 15 min of oxidative stress, the control sample (W-CONTROL) showed a significant increase in lightness (L^*), reaching 84.94 ± 0.09 ($p < 0.05$), indicating a rapid loss of CI. In contrast, W-PFB-C4 exhibited a lower and more moderate increase in lightness (81.83 ± 0.01), suggesting a better retention of the original wine appearance. At this same time point, W-CONTROL also showed a marked decrease in a^* (18.51 ± 0.18) and b^* (8.67 ± 0.16), indicating a strong reduction in red and yellow components, respectively. These reductions were significantly higher ($p < 0.05$) than those observed in W-PFB-C4, which maintained higher values (a^* equal to 25.22 ± 0.01; b^* equal to 12.61 ± 0.01), reflecting greater color stability. By 60 min, W-CONTROL reached an L^* value of 84.50 ± 0.08, significantly high ($p > 0.05$) in comparison to W-PFB-C4, which, in contrast, showed no further significant change compared to the previous time points, remaining around 81.31 ± 0.00. Likewise, W-CONTROL's a^* and b^* values (respectively equal to 18.86 ± 0.18 and 8.72 ± 0.11) still retain significantly ($p < 0.05$) lower values in comparison to W-PFB-C4, which registered the a^* value of 23.56 ± 0.01 and b^* value of 11.36 ± 0.01, highlighting the continued protective effect of the polyphenol-based material.

After 240 min, L^* decreased to 82.76 ± 0.11, whereas a^* increased to 21.02 ± 0.26 and b^* became less negative (−9.88 ± 0.15), consistent with pigment degradation and oxidation-induced discoloration. In contrast, the sample treated with immobilized polyphenols (W-PFB-C4) maintained a more stable L^* and a^* profile. Most notably, a^* values remained significantly ($p < 0.05$) higher than in W-CONTROL throughout all time points (Table 3), suggesting better preservation of red tonalities (Baris et al. 2024). Chroma values (C^*) were markedly higher in the immobilized polyphenol sample (ranging from 28.86 ± 0.43 to 31.79 ± 0.02) in comparison to W-CONTROL (ranging from 20.43 ± 0.00 to 22.47 ± 0.27), further confirming improved CI and therefore the protective activity exerted by PFB on wine under oxidative stress, in accordance with the effect that tannins can exert on wine color already observed by different authors (Chen et al. 2016; Liu et al. 2013; Puyo et al. 2023) in rosé, red wines, and model wine solutions.

Additional parameters related to wine color, such as CI and tonality (T), were also evaluated. The rosé wine, prior to oxidative stress, exhibited a CI of 0.84 and a tint (T) of 1.42, values typical of wines with good pigment content and freshness. Following exposure to oxidative conditions, the behavior of the control wine (W-CONTROL) and the treated wine (W-PFB-C4) was markedly different over time.

From Figure 4a,b, it is possible to observed that after 15 m of forced oxidation, the CI dropped in both samples, and in particular, W-CONTROL decreased to 0.57, whereas W-PFB-C4 retained a significantly ($p < 0.05$) higher value of 0.71, closer to the initial level. At the same time, the tint (T) (Figure 4b) increased in the control wine (W-CONTROL) to 1.66, indicating a shift toward browning. Even after 60 and 120 min, the CI value in W-PFB-C4 still remained above that of the sample W-CONTROL, indicating a better color freshness. Moreover, in comparison to W-CONTROL, sample W-PFB-C4 maintained at all-time points (15,

TABLE 3 | Color parameters of wine samples during storage during oxidative stress at 60°C.

Color parameters	Samples	Incubation time (min)				
		15	30	60	120	240
L^*	W-CONTROL	84.94 ± 0.09^a	84.58 ± 0.09^a	84.50 ± 0.08^a	83.51 ± 0.10^a	82.76 ± 0.11^b
	W-PFB-C4	81.83 ± 0.01a	80.55 ± 0.01^b	81.31 ± 0.00^b	83.13 ± 0.01^a	83.97 ± 0.01^a
a^*	W-CONTROL	18.51 ± 0.18^b	19.09 ± 0.19^b	18.86 ± 0.18^b	20.34 ± 0.23^b	21.02 ± 0.26^b
	W-PFB-C4	25.58 ± 0.02^a	28.32 ± 0.03^a	27.04 ± 0.01^a	24.42 ± 0.02^a	24.31 ± 0.01^a
b^*	W-CONTROL	8.67 ± 0.16^b	8.89 ± 0.12^b	8.72 ± 0.11^b	9.55 ± 0.14^b	9.88 ± 0.15^b
	W-PFB-C4	13.34 ± 0.93^a	14.44 ± 0.02^a	13.71 ± 0.01^a	12.24 ± 0.01^a	12.32 ± 0.01^a
C^*	W-CONTROL	20.44 ± 0.19^b	21.06 ± 0.22^b	20.78 ± 0.21^b	22.47 ± 0.27^b	20.43 ± 0.00^b
	W-PFB-C4	28.86 ± 0.43^a	31.79 ± 0.04^a	30.32 ± 0.02^a	27.31 ± 0.02^a	27.26 ± 0.02^a

Note: Data presented as mean ± standard deviation of the mean. In each column, within the same parameter (L^*, a^*, b^*, and C) and the same time point (15, 30, 60, 120, and 240 min), values followed by different letters are significantly different according to Fisher's least significant difference test ($p < 0.05$).
Abbreviation: PFB, polyphenol-functionalized bead.

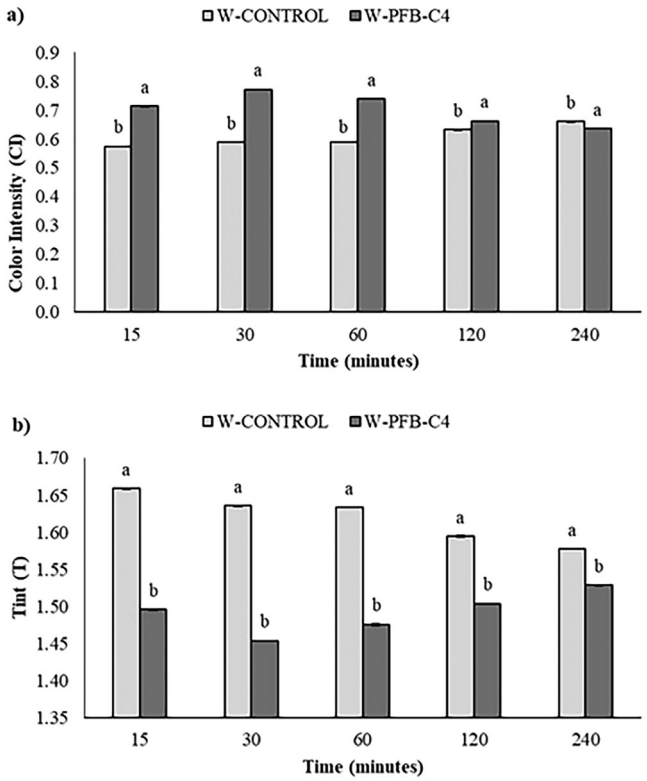

FIGURE 4 | (a) Color intensity (CI) and (b) tint (T) of control wine sample (W-CONTROL) and wine-containing polyphenol-functionalized beads (W-PFB-C4). Mean value and standard deviation bars are shown. Means marked by different letters differ significantly ($p < 0.05$) according to Fisher's least significant difference test.

30, 60, 120, and 240 min) a significantly ($p < 0.05$) lower T value (Figure 4b), suggesting better pigment preservation over time. Results obtained suggest that although the total pigment content (CI) may converge at later stages, the quality and oxidation state of pigments (reflected by T) remain better preserved in the treated wine.

Overall, the wine treated with immobilized polyphenols (W-PFB-C4) underwent less pronounced color changes compared to the untreated control, maintaining values that more closely resemble the pre-oxidation state. These results suggest that the immobilization of polyphenols in glutaraldehyde-crosslinked chitosan beads contributes to the preservation of color attributes in rosé wine under oxidative conditions. Such stabilization may result from the chelation of transition metals (particularly Fe^{3+}), which catalyze oxidative pigment degradation (Oliveira et al. 2011; Pyrzynska 2007). Moreover, the maintenance of C^* values suggests that the immobilized system preserved pigment vibrancy, consistent with reduced browning reactions and quinone formation (Garrido and Borges 2013). As reported by Puyo et al. (2023), the use of oenological tannins can stabilize the color of bioprotected rosé wines in a manner comparable to sulfur dioxide (SO_2) addition; however, such additives may also influence the sensory profile of the final product. In contrast, the proposed immobilization strategy offers a dual advantage: It enhances color stability and redox balance while avoiding the direct addition of active compounds to the wine matrix, thus preserving its sensory integrity and ensuring regulatory compliance. The anthocyanin profile of *Nerello Mascalese* is dominated by malvidin-3-glucoside, followed by minor amounts of delphinidin-, petunidin-, and peonidin-3-glucosides. Due to the low total anthocyanin content and the prevalence of non-acylated forms, rosé wines derived from this grape variety are particularly prone to color loss and oxidative degradation. The application of immobilized polyphenol systems in this study likely contributed to preserving the most oxidation-sensitive anthocyanins (Alabbosh et al. 2025), thus limiting pigment degradation.

4 | Conclusions

This study demonstrated that immobilized polyphenols, covalently bound to glutaraldehyde-crosslinked chitosan beads, effectively protected rosé wine from oxidative degradation, preserving its color and redox stability without any release of compounds into the matrix. The treatment enhanced Fe(II) stability, limited pigment oxidation, and maintained chromatic freshness under accelerated oxidative stress.

The proposed immobilization method presents several advantages, including the use of natural grape–derived compounds, the reusability of the antioxidant matrix, and full compliance with EU oenological regulations, as no polyphenol release occurs. However, as this is a preliminary study, some limitations must be acknowledged. The quantification of immobilized polyphenols was performed indirectly, and the specific identity of the adsorbed and retained polyphenolic compounds was not determined. Additionally, the accelerated oxidation model may not fully represent natural wine aging conditions but provides a valuable preliminary model to assess oxidative stability under controlled stress. Future studies will therefore focus on identifying which polyphenols are selectively adsorbed from the must and which are effectively protected during wine aging. Furthermore, efforts will be directed toward optimizing process scalability and applying this immobilization strategy under standard and commercial winemaking conditions, including the evaluation of volatile compounds and sensory attributes, to better assess its long-term impact on wine quality.

Author Contributions

Lucia Parafati: conception and design of study, acquisition of data, interpretation of data, drafting the manuscript. **Ilaria Proetto**: conception and design of study, acquisition of data. **Maria Cristina Mazzaglia**: acquisition of data. **Aldo Todaro**: conception and design of study, interpretation of data. **Fabiola Pesce**: interpretation of data. **Biagio Fallico**: revising the manuscript critically for important intellectual content. **Rosa Palmeri**: interpretation of data, revising the manuscript critically for important intellectual content. All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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