FUNGAL CHITOSAN vs BENTONITE IN 'SOLARIS' WHITE WINE UNDER REDUCED SO_2. PART 1: EFFECTS ON TURBIDITY, COLOUR AND POLYPHENOLS

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Abstract

Reducing sulfur dioxide (SO_2) while maintaining clarity and compositional stability remains a key challenge in white winemaking. This study evaluated fungal chitosan (CHIT) derived from Aspergillus niger as an alternative fining/stabilising agent for 'Solaris' (Vitis vinifera L.) white wine, compared with sodium bentonite (BENT), under two free-SO_2 regimes. After fermentation, wines were subjected to one of three treatments: no fining (NF/CTRL), bentonite, or chitosan, followed by SO_2 adjustment at 30–35 mg/L (SO_2_std) or 15–18 mg/L (SO_2_low), cold stabilisation, and bottle storage. Across variants, pH remained stable, whereas acetic acid concentration increased during storage, reaching the highest value in NF/CTRL-SO_2_low. Both fining agents markedly improved clarity. Bentonite caused the greatest reduction in total polyphenols, whereas chitosan-treated wines retained higher levels, particularly CHIT-SO_2_std. Chitosan was also associated with more favourable SO_2 retention under reduced dosing. Overall, fungal chitosan provided effective clarification, better preservation of total polyphenols than bentonite, and more favourable SO_2 retention, supporting its potential use in reduced-SO_2 white wine production.

Keywords: *wine clarification, turbidity, CIE L*a*b*, polyphenol retention, SO_2 management*

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1. Introduction

Chitosan (CHIT), a biopolymer obtained through the deacetylation of chitin, is considered one of the most promising substances currently used in modern oenology [1–3]. Sourced from safe biotechnological materials, including *Aspergillus niger* and *Agaricus bisporus*, it was formally authorised for winemaking applications by the International Organisation of Vine and Wine (OIV) in 2009 [4]. Its increasing popularity is driven by biodegradability, biocompatibility, low toxicity, and natural origin, which align with the growing trend to reduce synthetic additives in wine production [3]. Chitosan is of particular interest as a potential alternative or complementary tool to sulphur dioxide (SO_2), which has long remained the principal stabilising agent in winemaking.

Sulphur dioxide fulfils a dual function: it acts as a preservative through antimicrobial activity and as an antioxidant that protects wine against browning and loss of fresh aroma [5]. The effectiveness of SO_2 depends strongly on pH, as lower pH values favour a higher proportion of the molecular form, which exhibits the greatest antiseptic efficacy [6]. Despite its widespread use, SO_2 may trigger adverse reactions in sensitive individuals, particularly in patients with asthma. In response to health-related and consumer concerns, wine producers are increasingly seeking technologies that allow SO_2 reduction without compromising microbial, oxidative and sensory stability [7, 8].

Fining is an essential stage of winemaking aimed at removing suspended particles and unstable colloidal compounds that may cause haze and compromise the sensory, visual and microbiological stability of the final product [9]. The most frequently used fining agents include bentonite and protein-based preparations such as gelatine, casein and egg white. Bentonite (BENT), a negatively charged mineral, binds positively charged grape and yeast proteins and is still considered the conventional reference treatment for improving the clarity and protein stability of white wines. However, its use may be associated with wine volume losses, the removal of aroma-relevant compounds and changes in the sensory profile [10, 11].

White wine clarification and visual stability are influenced not only by protein removal but also by the aggregation and sedimentation of suspended particles, polysaccharides and phenolic-colloidal complexes. Protein haze caused by grape pathogenesis-related proteins, particularly chitinases and thaumatin-like proteins, is an important aspect of white wine instability; however, turbidity and visual quality during processing and storage also depend on a broader set of colloidal and phenolic interactions [9, 10, 12]. Therefore, in technological studies focused on white wine fining, turbidity, colour and phenolic composition are key practical indicators of treatment effectiveness and storage stability.

Chitosan, owing to its positive charge under acidic conditions, can interact with negatively charged molecules, including selected proteins, polysaccharides and phenolic compounds, forming complexes that may sediment during clarification. This property makes fungal chitosan particularly relevant not only for protein-related stabilisation but also for practical turbidity reduction and visual quality management in white wines. Its oenological effects are not limited to fining, as previous studies have also reported metal-binding, antimicrobial, antioxidant-related and contaminant-removal potential, although the effectiveness of these mechanisms depends strongly on chitosan origin, molecular characteristics, dose, contact time and wine matrix [3, 13–15]. Such matrix-dependent behaviour is especially important when evaluating colour and phenolic changes, because fining agents may remove or modify phenolic and colloidal fractions that contribute to browning, yellowing and optical stability.

Several published studies have directly addressed the use of fungal chitosan in white wine fining and stabilisation. Colangelo *et al.* [16] evaluated chitosan as an alternative to

bentonite for fining an aromatic white wine and assessed its effects not only on heat stability and proteins, but also on organic acids, colour and volatile compounds. Arenas *et al.* [17] compared fungal chitosan, bentonite and κ-carrageenan in Albariño white wine and showed that different fining agents may differently affect pathogenesis-related proteins, polysaccharides and phenolic composition. These results indicate that fungal chitosan may influence several wine-quality components at the same time, but its effectiveness cannot be generalised across wine matrices or technological conditions.

In the context of reduced-SO_2 winemaking, the assessment of turbidity and colour stability becomes particularly important, because lower SO_2 protection may increase the susceptibility of white wines to oxidative changes, including shifts in CIE L*a*b* parameters, yellowing/browning and changes in phenolic composition. However, available studies have rarely combined a direct comparison of fungal chitosan and bentonite with reduced SO_2 regimes and follow-up across consecutive technological stages. In particular, limited information is available on whether fungal chitosan can reduce turbidity and preserve colour-related parameters in cool-climate white wines during stabilisation and storage, while maintaining total polyphenol balance.

The novelty of the present study, therefore, lies in the integrated technological comparison of fungal chitosan derived from *Aspergillus niger* and bentonite in 'Solaris' white wine produced under standard and reduced SO_2 regimes, with a primary focus on turbidity, colour and total polyphenols. Unlike previous studies focused mainly on protein stability, antimicrobial effects, contaminant removal or selected compositional endpoints, this work tracks changes in turbidity, CIE L*a*b* colour parameters, colour difference (ΔE) values, and total polyphenols across successive stages after fining and during storage. This approach enables evaluation of whether fungal chitosan can serve as a practical alternative to bentonite in reduced-SO_2 white wine production without compromising visual quality or phenolic stability.

The following hypotheses has been stated:

H_1. Fining treatment affects wine turbidity after fining (T1) and during subsequent stabilisation and storage (T2-T3), with the magnitude and persistence of this effect depending on the treatment applied: untreated control, bentonite or fungal chitosan derived from *Aspergillus niger*.

H_2. Fining treatment influences CIE L*a*b* colour parameters and ΔE values during stabilisation and storage (T2-T3), indicating treatment-dependent changes in the visual characteristics of 'Solaris' white wine.

H_3. The SO_2 dose modifies the extent of turbidity and colour changes over time, and this effect may depend on the fining treatment applied, as reflected by treatment × SO_2 level × time interactions.

2. Materials and Methods

2.1. Study Site and Plant Material

The study used grapes of *Vitis vinifera* L., white-berried cultivar 'Solaris', sourced from the organic Winnica Pałacu Rajkowo vineyard located in north-western Poland near Szczecin (53°21′09.8″ N, 14°26′23.3″ E). The area is classified as USDA (United States Department of Agriculture) hardiness zone 7B, with typical minimum winter temperatures of approximately −12 °C to −15 °C. The mean growing-season temperature (May-October) over 1951–2019 was 15.4 °C, with cumulative precipitation of ca. 350 mm. In 2020–2022, prolonged drought periods (32–46 days) were recorded, interspersed with episodes of

intense rainfall exceeding 100 mm in 24 h (data: Ostoja Meteorological Station, 53°20′35″ N, 14°58′10″ E) [18].

The vineyard was established in 2016. Vines are trained to a single Guyot system with vertical shoot positioning (VSP). Spacing equals 1.01 m within rows and 2.28 m between rows. During the growing season, mechanical weed control was applied within rows, while inter-rows were sown with green-manure cover-crop mixtures (red and white clover, alfalfa, and oilseed radish) to improve soil structure and increase organic matter content. Every four years, magnesium lime was applied at 2 t/ha. No mineral phosphorus or potassium fertilisers were used.

Plant protection relied exclusively on inputs permitted in organic farming:

- sulphur: 12.5 kg/ha;
- copper-based products (hydroxide and oxychloride): 1.75 kg/ha;
- potassium carbonate: 17.5 kg/ha;
- potassium soap: 4 kg/ha.

Grapes were hand-harvested in late September/early October at technological maturity. Fruit was collected from the central part of the vineyard block and pooled to obtain one uniform grape lot for must preparation. This approach was intentionally used to minimise the effect of raw-material variability and to ensure that subsequent differences among experimental variants resulted primarily from the applied fining treatment, SO_2 level and storage time, rather than from differences in grape composition. After crushing and pressing, the resulting must was homogenised and used as a common starting material for the preparation of three independent technological/winemaking replicates.

2.2. Must Preparation and Fermentation

Grapes were destemmed, crushed, and pressed using a basket press. The resulting must was cooled to 10 °C and subjected to 12 h of gravitational settling. After removal of the settled solids, the clarified must was thoroughly homogenised and divided into three equal-volume fermentation batches. All three batches originated from the same uniform must, which was deliberately used to minimise the influence of grape composition variability and to allow the effects of subsequent technological treatments to be assessed under comparable starting conditions.

Each batch was supplemented with the same dose of yeast nutrient and inoculated with the same dose of *Saccharomyces cerevisiae* yeast (20 g/hL, ca. 10^6 cells/mL). The three batches were fermented independently under identical conditions and were treated as technological/winemaking replicates. Fermentation was carried out at 12–14 °C until reducing sugars fell below 2 g/L, as determined by the enzymatic method. Upon completion of alcoholic fermentation, each of the three wines was racked off the lees separately. This stage was designated as T0.

After T0, each independently fermented wine was subsequently divided into six separate tanks corresponding to the six technological variants described in Section 2.3. This design resulted in 18 experimental tanks in total, i.e. three technological/winemaking replicates × six treatment variants.

2.3. Experimental Schedule and Design

The full experimental schedule is provided in Table 1. The experiment was designed to assess the effects of fining treatment (Factor A) and SO_2 level (Factor B) on turbidity, colour and polyphenol-related changes in 'Solaris' white wine during stabilisation and storage.

Factor A comprises:

- NF/CTRL: no fining agent, natural settling only;
- BENT: sodium bentonite, dose 50 g/hL;
- CHIT: fungal chitosan derived from *Aspergillus niger* (an oenological preparation authorised by OIV), dose 10 g/hL;

while Factor B:

- SO_2_std: standard free SO_2 level of 30–35 mg/L (corresponding to 0.5–0.8 mg/L molecular SO_2);
- SO_2_low: reduced free SO_2 level, approximately 50% lower than SO_2_std, i.e. 15–18 mg/L.

In total, six technological variants were obtained:

1. NF/CTRL–SO_2_std;
2. NF/CTRL–SO_2_low;
3. BENT–SO_2_std;
4. BENT–SO_2_low;
5. CHIT–SO_2_std;
6. CHIT–SO_2_low.

The experiment was based on three independent technological/winemaking replicates prepared from the same homogenised must. The use of a single uniform must was intentional and was intended to minimise variability related to grape composition, so that the effects of the applied technological treatments could be assessed under comparable starting conditions.

After alcoholic fermentation and the first racking, each of the three independently fermented wines was sampled at T0 and then divided into six separate stainless-steel tanks, one for each technological variant. Thus, the experiment comprised 18 tanks in total, corresponding to three technological/winemaking replicates × six treatment combinations. Because T0 represented the baseline stage before fining treatment and SO_2-level differentiation, T0 values were determined for each independently fermented wine before differentiation into the final technological variants. These baseline values were used as the starting point for the corresponding variants derived from the same replicate wine.

Each tank represented one experimental unit for a given variant. Analytical and instrumental measurements performed within each tank were treated as technical/analytical replicates. These measurements were averaged before statistical analysis, and the resulting mean value for each tank and time point was used as the experimental observation.

This design allowed evaluation of the effects of fining treatment, SO_2 level, and storage time while limiting the influence of raw-material heterogeneity. The replication strategy, therefore, reflects independent technological/winemaking replicates rather than independent vineyard-derived biological replicates.

2.4. Procedure of Fining Agents Application

Fining agents were applied immediately after sampling at T0 (Table 1). In case of BENT, 10% (w/v) bentonite suspension was prepared in deionised water and allowed to hydrate for 12 h. Then, suspension was added to the wine to achieve a final dose of 50 g/hL, and the wines were mixed for 10 min. For CHIT, a 1% (w/v) chitosan solution was prepared in water acidified with tartaric acid (pH 3.0). The polymer solution was added to the wine to reach 10 g/hL, and the wines were mixed for 10 min. In the case of NF/CTRL, no fining agent was added.

Finally, all wines were stored for 7 days in closed stainless-steel tanks at 10–12 °C. After 7 days (T1), wines were racked off the lees.

Table 1. Full experimental schedule.

Time point/stage	Timing within the process	Actions and analysis
	alcoholic fermentation (ALC)	a single, identical wine base for all subsequent variants (no SO_2 addition, no fining)
T0 baseline	after completion of fermentation and the first racking off the lees	baseline physicochemical analyses performed, determination of selected metals content (no SO_2 adjustment, no fining)
application of fining agents	immediately after T0	the wine divided into variants (i.e. NF/CTRL, BENT, and CHIT), the appropriate fining agent added to the tanks (none in case of NF/CTRL) (no SO_2 differentiation)
contact with the fining agent	7 days at 10–12 °C	wines remain in contact with fining agents (only gravitational settling occurs in case of NF/CTRL), maintained under identical temperature and protected from light
T1 post-fining	after 7 days of contact and racking off the lees	wines racked off the lees/sediment, physicochemical analyses and determination of selected metals content
SO_2 adjustment	immediately after T1	SO_2 added to each variant (SO_2_std or SO_2_low), gentle mixing, measuring of free SO_2, tanks filled to capacity
cold stabilisation	30 days at 12 ± 1 °C	wines mature under conditions of minimal air contact (simulation of pre-bottling stabilisation)
T2 post-stabilisation	after cold stabilisation	comprehensive analyses performed, wines are prepared for bottling
bottling	immediately after T2	wines are poured into bottles and sealed
storage under near-commercial conditions	60 days at 16–18 °C in darkness	bottles stored upright simulating warehouse/shelf conditions
T3 post-storage	after 60 days of storage	stability-related analyses: colour, turbidity, free and total SO_2, evaluation of basic chemical parameters

2.5. Procedure of SO_2 Adjustment and Cold Stabilisation

At T1, free SO_2 was determined, and potassium metabisulfite was added to reach the target levels: (i) SO_2_std: (30–35 mg/L free SO_2) and (ii) SO_2_low (15–18 mg/L free SO_2). Wines were then stored for 30 days at 12 ± 1 °C (cold stabilisation). After this period, samples were collected for T2 analyses.

2.6. Bottle storage

After T2, all variants were poured into 0.75 L bottles and filled to approximately 2 cm below the aluminium screw cap (Table 1). Bottles were stored upright for 60 days at 16–18 °C in darkness. After 60 days, the full set of T3 determinations was performed.

2.7. Determination of free and total SO_2

Free (FSO_2) and total (TSO_2) sulphur dioxide in wine were determined using the aeration-oxidation method in accordance with OIV procedures [19] and the relevant updates for free and total fractions [20, 21].

Principle of the method. Sulphur dioxide is stripped from an acidified sample by a stream of air or nitrogen, then captured and oxidised in hydrogen peroxide solution to sulphuric acid, which is quantified by titration with a standardised NaOH solution.

Free SO_2 (FSO_2). Determination was performed at controlled room temperature (recommended range 18–24 °C), as the stripping efficiency of free SO_2 is strongly temperature-dependent. The released SO_2 was carried by an air/nitrogen stream into an absorber containing diluted, neutralised H_2O_2. The resulting sulphuric acid was titrated with NaOH of known concentration. Results were expressed as mg/L.

Total SO_2 (TSO_2). Total SO_2 was extracted under elevated temperature (approximately 100 °C) during aspiration (air/nitrogen) after acidification. The transferred SO_2 was oxidised in H_2O_2 to H_2SO_4 and quantified by NaOH titration (typically 0.01 M). Extraction was performed for the duration specified in the OIV method (typically several to ~15 min), and results were calculated according to the method's equations and reported as mg/L.

2.8. Wine Turbidity

Wine turbidity was measured using a Lovibond TB211IR turbidimeter (Hach, Iowa, USA), which determines turbidity from the intensity of scattered light in the 400–600 nm range.

2.9. Colour Analysis and Oxidation-Related Changes

Colour coordinates were measured under the D65 illuminant and the 10° standard observer using a Konica Minolta CM-700d spectrophotometer and results expressed in the CIE L*a*b* colour space, where L* denotes lightness (L* = 0 black; L* = 100 white), a* represents the green (−a*) to red (+a*) axis, and b* represents the blue (−b*) to yellow (+b*) axis. The total colour difference ΔE^*_{ab} was calculated as:

$$\Delta E^*_{ab} = \sqrt{\left(L^*_{Tx} - L^*_{T0}\right)^2 + \left(a^*_{Tx} - a^*_{T0}\right)^2 + \left(b^*_{Tx} - b^*_{T0}\right)^2} \quad (1)$$

where:

T0 – represents the initial sampling point;

Tx – represents the subsequent sampling points, i.e. T1, T2, and T3.

CIE L*a*b* parameters were determined in 25 independent replicates per sample. Each replicate corresponded to a measurement of a separate aliquot (25 different aliquots), rather than repeated readings of the same portion/cuvette. This approach ensured that

the number of replicates reflected sample variability rather than instrument repeatability alone [22].

For each tank and time point, the 25 aliquot measurements were averaged before statistical analysis. The resulting mean value represented the colour result for that experimental unit, i.e. one tank within a given technological variant and replicate. Thus, the aliquot measurements were treated as technical/analytical replicates describing within-sample variability and were not considered independent technological or biological replicates in the ANOVA. For statistical comparisons, only the averaged values representing individual experimental units were used.

Because T0 was measured before fining treatment and before SO_2-level differentiation, ΔE^*_{ab} values were calculated relative to the corresponding T0 value of the same technological/winemaking replicate. This allowed colour changes during T1-T3 to be interpreted in relation to the initial colour of the same wine replicate, rather than to a separate or unrelated baseline.

2.10. Identification of Phenolic Compounds and Organic Acids

Phenolic compounds and organic acids were analysed using a Waters ACQUITY UPLC-PDA-MS/MS system (Waters, Milford, MA, USA) equipped with a binary solvent manager, sample manager, column manager, photodiode array (PDA) detector, and a tandem quadrupole mass spectrometer (TQD) with electrospray ionisation (ESI). Separation was performed on a BEH C18 column (100 mm × 2.1 mm i.d., 1.7 µm, Waters) maintained at 50 °C. The mobile phases were: (A) 0.1% formic acid in water (v/v), (B) 0.1% formic acid in acetonitrile (v/v). The gradient programme was as follows: 0 min, 5% B; 0–8 min, linear increase to 100% of B; 8–9.5 min, column wash and return to initial conditions. A flow rate of 0.35 mL/min was used, while the injection volume was 5 µL (partial-loop injection with needle overfill).

2.11. Statistical Analysis

All statistical analyses were performed using Statistica 12.5 (StatSoft Polska, Kraków, Poland). Before analysis, all analytical or instrumental replicates obtained for a given tank at a given time point were averaged. The resulting mean value was used as one observation representing that experimental unit. The experimental unit was the individual tank prepared from one of the three independent technological/winemaking replicates. Technical or analytical replicates, including repeated instrumental readings and separate aliquot measurements, were used to obtain representative mean values for each tank and time point, but were not treated as independent experimental units in the ANOVA.

For each response variable, the effects of technological variant, time point and their interaction were assessed using two-way analysis of variance (ANOVA). The technological variant factor included six levels, as given in Section 2.3. The time-point factor included four stages: T0, T1, T2 and T3. The technological variant factor combined fining treatment and SO_2 level, which enabled direct comparison of the six final treatment combinations across successive stages of stabilisation and storage.

Where significant main effects and/or interactions were detected, means were compared using Tukey's HSD post hoc test. Differences were considered statistically significant at $p < 0.05$.

For each analysed parameter, to improve the transparency of the statistical interpretation, ANOVA p-values were reported for (i) the main effect of technological variant, (ii) the main effect of time point, and (iii) the variant × time point interaction.

Results are presented as mean ± SD. The standard deviations reported in the tables were calculated from values for the independent technological/winemaking replicates, after averaging technical or analytical measurements within each experimental unit. Uppercase letters (e.g. A, B, C) indicate statistically significant differences attributable to main effects, i.e. comparisons of marginal means for a single factor irrespective of the other factor. Lowercase letters (e.g. a, b, c) indicate statistically significant differences within the variant × time point interaction, i.e. comparisons among specific variant × time point combinations.

3. Results and Discussion

3.1. pH Stability

Mean pH values across all variants remained within a very narrow range (3.25–3.33) (Table 2). No significant differences were found between fining treatments (NF/CTRL, BENT, CHIT) or between SO_2 regimes (SO_2_std vs SO_2_low). Any between-variant differences were minimal (typically 0.01–0.03 pH units) and therefore of no practical technological relevance.

Across time points, pH showed a small numerical increase from T0 to T3 (overall mean: 3.25 to 3.32). However, as indicated by the uniform statistical grouping in Table 2, this pattern was not statistically significant ($p \geq 0.05$). Such a minor numerical change may be associated with normal post-fermentation stabilisation and storage, including limited shifts in acid-base equilibria during cold stabilisation; however, because the effect was not statistically significant, it should be interpreted only as a weak tendency rather than a confirmed process [9]. The magnitude observed here was too small to indicate any meaningful modification of the wine acidity profile.

The lack of a measurable effect of bentonite or chitosan on pH is consistent with reports indicating that fining agents primarily affect the colloidal fraction, including protein stability, particle aggregation and partial sorption of selected compounds, rather than directly modifying wine acidity [3, 15]. Likewise, reduction of the SO_2 dose did not translate into pH changes, indicating that, within the applied range, lower SO_2 level did not disturb the acid-base status of the wine. Overall, pH remained stable during clarification, cold stabilisation and bottle storage, confirming that neither the fining strategy nor the reduced SO_2 regime had a measurable effect on this basic acidity parameter [23].

3.2. Tartaric Acid Content

Tartaric acid concentrations remained within a narrow range throughout the experiment (6.4–6.8 g/L) (Table 2). The between-variant analysis showed no clear technological effect: neither the fining treatment (NF/CTRL vs BENT vs CHIT) nor the SO_2 regime (SO_2_std vs SO_2_low) affected the mean values, which consistently fell between 6.6 and 6.7 g/L.

Over time, tartaric acid concentration showed a small numerical decrease, with the overall mean declining from 6.8 g/L at T0 to 6.5 g/L at T3. Although the main time effect was not statistically significant. The significant interaction between variant and time point indicates that the course of these minor changes differed slightly among treatments. This pattern is consistent with gradual tartrate stabilisation during cooling and subsequent storage, during which tartaric acid may be partially removed from solution via tartrate crystallisation, primarily as potassium hydrogen tartrate and/or calcium tartrate salts [23, 24]. However, given the small magnitude of the observed changes, this should be interpreted as a limited technological effect rather than a substantial modification of wine acidity.

Table 2. Effects of fungal chitosan and bentonite, and of SO_2 level, on acidity-related parameters and SO_2 content in 'Solaris' wine during stabilisation and storage.

Variant / time point	T0	T1	T2	T3	mean
pH					
NF/CTRL–SO_2_std	3.25 ± 0.04^{a}	3.27 ± 0.03^{a}	3.30 ± 0.04^{a}	3.32 ± 0.02^{a}	3.29^{A}
NF/CTRL–SO_2_low	3.25 ± 0.04^{a}	3.27 ± 0.04^{a}	3.30 ± 0.02^{a}	3.33 ± 0.03^{a}	3.29^{A}
BENT–SO_2_std	3.25 ± 0.04^{a}	3.26 ± 0.02^{a}	3.29 ± 0.02^{a}	3.31 ± 0.03^{a}	3.28^{A}
BENT–SO_2_low	3.25 ± 0.04^{a}	3.26 ± 0.03^{a}	3.29 ± 0.03^{a}	3.32 ± 0.02^{a}	3.28^{A}
CHIT–SO_2_std	3.25 ± 0.04^{a}	3.26 ± 0.04^{a}	3.28 ± 0.04^{a}	3.30 ± 0.03^{a}	3.27^{A}
CHIT–SO_2_low	3.25 ± 0.04^{a}	3.26 ± 0.02^{a}	3.28 ± 0.03^{a}	3.31 ± 0.01^{a}	3.28^{A}
mean	3.25^{A}	3.26^{A}	3.29^{A}	3.32^{A}	
variant **p > 0.05**, time point **p > 0.05**, interaction **p > 0.05**					
tartaric acid concentration [g/L]					
NF/CTRL–SO_2_std	6.8 ± 0.1^{a}	6.7 ± 0.1^{ab}	6.6 ± 0.1^{ab}	6.5 ± 0.1^{ab}	6.7^{A}
NF/CTRL–SO_2_low	6.8 ± 0.1^{a}	6.7 ± 0.1^{ab}	6.6 ± 0.2^{ab}	6.5 ± 0.1^{ab}	6.7^{A}
BENT–SO_2_std	6.8 ± 0.1^{a}	6.6 ± 0.2^{ab}	6.5 ± 0.1^{ab}	6.4 ± 0.1^{b}	6.6^{A}
BENT–SO_2_low	6.8 ± 0.1^{a}	6.6 ± 0.1^{ab}	6.5 ± 0.1^{ab}	6.4 ± 0.2^{b}	6.6^{A}
CHIT–SO_2_std	6.8 ± 0.1^{a}	6.7 ± 0.1^{ab}	6.6 ± 0.1^{ab}	6.5 ± 0.1^{ab}	6.7^{A}
CHIT–SO_2_low	6.8 ± 0.1^{a}	6.7 ± 0.1^{ab}	6.6 ± 0.1^{ab}	6.5 ± 0.1^{ab}	6.7^{A}
mean	6.8^{A}	6.7^{A}	6.6^{A}	6.5^{A}	
variant **p > 0.05**, time point **p > 0.05**, interaction **p = 0.032**					
acetic acid concentration [g/L]					
NF/CTRL–SO_2_std	0.25 ± 0.2^{a}	0.28 ± 0.2^{ac}	0.32 ± 0.1^{ae}	0.35 ± 0.2^{ac}	0.30^{A}
NF/CTRL–SO_2_low	0.25 ± 0.2^{a}	0.29 ± 0.1^{ad}	0.35 ± 0.2^{ce}	0.40 ± 0.3^{e}	0.32^{A}
BENT–SO_2_std	0.25 ± 0.2^{a}	0.27 ± 0.3^{ac}	0.31 ± 0.2^{ad}	0.34 ± 0.2^{be}	0.29^{A}
BENT–SO_2_low	0.25 ± 0.2^{a}	0.28 ± 0.2^{ac}	0.33 ± 0.3^{ae}	0.37 ± 0.3^{de}	0.31^{A}
CHIT–SO_2_std	0.25 ± 0.2^{a}	0.26 ± 0.2^{ab}	0.30 ± 0.2^{ad}	0.33 ± 0.1^{ae}	0.29^{A}
CHIT–SO_2_low	0.25 ± 0.2^{a}	0.27 ± 0.1^{ac}	0.31 ± 0.2^{ad}	0.35 ± 0.2^{ce}	0.30^{A}
mean	0.25^{A}	0.28^{A}	0.32^{AB}	0.36^{B}	
variant **p > 0.05**, time point **p = 0.027**, interaction **p = 0.015**					
free SO_2 content [mg/L]					
NF/CTRL–SO_2_std	5 ± 0^{a}	32 ± 2^{ij}	25 ± 1^{gh}	18 ± 1^{ef}	20^{B}
NF/CTRL–SO_2_low	5 ± 0^{a}	16 ± 1^{cde}	10 ± 1^{abc}	6 ± 0^{ab}	9^{A}
BENT–SO_2_std	5 ± 0^{a}	33 ± 2^{ij}	27 ± 1^{hi}	20 ± 1^{efg}	21^{B}
BENT–SO_2_low	5 ± 0^{a}	17 ± 1^{def}	$11 \pm 1^{a-d}$	7 ± 0^{ab}	10^{A}
CHIT–SO_2_std	5 ± 0^{a}	34 ± 2^{j}	29 ± 2^{hij}	23 ± 1^{fgh}	23^{B}
CHIT–SO_2_low	5 ± 0^{a}	17 ± 1^{def}	14 ± 1^{cde}	$11 \pm 1^{a-d}$	12^{AB}
mean	5^{A}	25^{C}	19^{BC}	14^{B}	
variant **p = 0.022**, time point **p = 0.013**, interaction **p = 0.009**					
total SO_2 content [mg/L]					
NF/CTRL–SO_2_std	$45 \pm 2^{c-f}$	65 ± 3^{ij}	55 ± 2^{gh}	42 ± 2^{cde}	52^{B}
NF/CTRL–SO_2_low	$45 \pm 2^{c-f}$	52 ± 2^{fg}	40 ± 1^{cd}	28 ± 2^{a}	41^{A}
BENT–SO_2_std	$45 \pm 2^{c-f}$	66 ± 2^{ij}	58 ± 2^{ghi}	$45 \pm 2^{c-f}$	54^{C}
BENT–SO_2_low	$45 \pm 2^{c-f}$	53 ± 2^{fgh}	42 ± 1^{cde}	30 ± 1^{ab}	43^{A}
CHIT–SO_2_std	$45 \pm 2^{c-f}$	67 ± 3^{ij}	62 ± 3^{hij}	50 ± 2^{efg}	56^{C}
CHIT–SO_2_low	$45 \pm 2^{c-f}$	54 ± 2^{fgh}	48 ± 2^{def}	38 ± 2^{bc}	46^{AB}
mean	45^{AB}	60^{C}	51^{B}	39^{A}	
variant **p = 0.030**, time point **p = 0.024**, interaction **p = 0.011**					

Note. ANOVA p-values are given for the technological variant, the time point, and their interaction. Different letters indicate statistically significant differences according to Tukey's HSD test at $p < 0.05$.

Relative to the other variants, bentonite-fined wines showed marginally lower values at T3 (6.4 g/L) than NF/CTRL and chitosan-treated wines (6.5 g/L). However, the difference was small (ca. 0.1 g/L), and did not affect the overall means across the study period. Therefore, although a minor treatment-dependent response was observed at the final time point, the practical relevance of this difference appears limited.

The lack of differences between SO_2 levels indicates that, within the applied dosing range, SO_2 reduction did not directly affect tartaric acid evolution. Overall, the results suggest that tartaric acid remained relatively stable during clarification, cold stabilisation and bottle storage, while the observed variability was mainly associated with small time- and treatment-dependent shifts rather than with a strong effect of either fining strategy or SO_2 dose.

3.3. Acetic Acid Concentration

Acetic acid concentrations across all variants ranged from 0.25 to 0.40 g/L, remaining clearly below levels typically associated with sensory spoilage and within acceptable technological limits for volatile acidity expressed as acetic acid [25, 26]. At the same time, the data indicate a moderate treatment-dependent divergence, most apparent at the final storage time point (T3), suggesting that acetic acid was more responsive to storage conditions and SO_2 protection than to fining treatment alone.

The highest value was recorded for NF/CTRL–SO_2_low at T3 (0.40 g/L), whereas variants maintained at the standard SO_2 dose (SO_2_std) showed lower T3 levels (0.33–0.35 g/L) (Table 2). This suggests that reduced sulphite protection favoured acetic acid accumulation during storage, probably by weakening the protective effect of SO_2 against processes associated with volatile acidity development during storage [25, 27, 28]. Because microbial populations, oxygen uptake and related oxidation markers were not directly analysed in this study, this interpretation should be treated as a plausible explanation rather than a confirmed causal mechanism.

A clear time-related increase was observed (overall mean changed from 0.25 g/L at T0 to 0.36 g/L at T3), with the largest increment occurring between T2 and T3. This is consistent with maturation and bottle storage, during which gradual development of volatile acidity may occur, particularly when SO_2 protection is reduced [28, 29].

Relative to NF/CTRL–SO_2_low, both bentonite and chitosan were associated with slightly lower T3 values (0.37 and 0.35 g/L, respectively), suggesting a limited indirect effect of these treatments. This may be related to improved clarification and, in the case of chitosan, to its potential antimicrobial activity; however, the small magnitude of the differences indicates that these effects were secondary to storage time and SO_2 level [30, 31]. Therefore, the lower acetic acid values in fined wines should be interpreted cautiously and not as evidence of strong microbiological control by the fining agents.

Overall, the acetic acid values indicate that microbiological stability was maintained throughout the experiment, as no concentrations suggestive of spoilage were recorded. The results also indicate that the storage phase (T2-T3) was the most sensitive period for volatile acidity build-up, particularly under a reduced SO_2 dose [27, 28].

3.4. Changes in Free and Total SO_2 Content

The technological variants showed clear differences in the evolution of free SO_2 at T1 (post-fining and SO_2 adjustment), T2 (post-cold stabilisation) and T3 (post-bottle storage) (Table 2). The adjustment performed at T1 achieved the target free SO_2 concentrations, i.e., 32–34 mg/L in the SO_2_std variants and 16–17 mg/L in the SO_2_low variants. From T1 to T3, free SO_2 declined progressively, and the rate of decline was significantly affected

by the fining treatment applied ($p \leq 0.01$). Across all variants, mean free SO_2 decreased from 25 mg/L (T1) to 14 mg/L (T3), indicating substantial SO_2 depletion during stabilisation and storage.

In NF/CTRL and bentonite-treated wines, the decline in free SO_2 followed a broadly similar pattern. In the SO_2_std group, the T1–T3 decrease amounted to 13–14 mg/L (NF/CTRL: 32 → 18 mg/L; BENT: 33 → 20 mg/L), whereas in the SO_2_low group it reached 10 mg/L (NF/CTRL: 16 → 6 mg/L; BENT: 17 → 7 mg/L). Thus, bentonite did not markedly improve free SO_2 retention compared with the non-fined control. This is consistent with its primary role in protein and colloidal stabilisation rather than in directly limiting SO_2 consumption [9, 27].

Chitosan-treated variants (CHIT) exhibited the lowest losses of free SO_2, particularly under the reduced-dose regime. In SO_2_std wines, free SO_2 decreased by 11 mg/L (34 → 23 mg/L), whereas in SO_2_low wines it decreased by 6 mg/L (17 → 11 mg/L). Relative to NF/CTRL and BENT, this corresponds to an approximately 15–20% reduction in losses in the SO_2_std group and ~40% in the SO_2_low group, indicating better SO_2 retention in CHIT-treated wines under limited sulphite protection. Notably, CHIT–SO_2_low maintained free SO_2 above ≥10 mg/L at T3, suggesting that chitosan may help preserve a residual protective SO_2 fraction during storage. However, the actual protective effect depends not only on the free SO_2 concentration but also on pH, molecular SO_2, oxygen exposure, and microbial load [27, 28].

The improved retention of free SO_2 in chitosan-treated wines may be related to reduced reactivity of the wine matrix and/or to limited processes leading to SO_2 consumption. However, because microbial populations, oxygen uptake, transition metals and carbonyl compounds were not directly quantified in this part of the study, these mechanisms should be considered plausible explanations rather than confirmed causal pathways [27, 32].

Changes in total SO_2 largely mirrored the trends observed for the free fraction. Over the T1-T3 period, the greatest decreases were recorded in the NF/CTRL and BENT variants, amounting to 21–24 mg/L (NF/CTRL–SO_2_std: 65 → 42 mg/L; NF/CTRL–SO_2_low: 52 → 28 mg/L; BENT–SO_2_std: 66 → 45 mg/L; BENT–SO_2_low: 53 → 30 mg/L). In contrast, total SO_2 losses in the chitosan-treated variants were smaller, at 16–17 mg/L (CHIT–SO_2_std: 67 → 50 mg/L; CHIT–SO_2_low: 54 → 38 mg/L), indicating slower depletion of the total SO_2 pool during storage.

The similar behaviour of changes in free and total SO_2 suggests that CHIT variants retained both fractions more effectively during storage, rather than showing only a redistribution between free and bound forms. Nevertheless, this interpretation should be approached with caution, as the specific compounds responsible for SO_2 binding were not directly analysed. It should also be noted that the magnitude of total SO_2 decline was strongly time-dependent (overall mean: 60 mg/L at T1 → 39 mg/L at T3), confirming the dominant influence of stabilisation and storage on the sulphur balance.

Overall, the results indicate that chitosan application helped maintain higher levels of both free and total SO_2 during storage, particularly under the reduced-dose regime. The CHIT–SO_2_low variant retained 11 mg/L free SO_2 and 38 mg/L total SO_2 at T3, whereas the corresponding NF/CTRL–SO_2_low and BENT–SO_2_low variants retained only 6–7 mg/L free SO_2 and 28–30 mg/L total SO_2. Together with stable pH and low acetic acid concentrations, these results suggest that SO_2 reduction in the presence of chitosan did not compromise the basic chemical stability indicators measured in this study. In this context, chitosan can be viewed as a supportive tool in reduced-SO_2 winemaking, although its protective role should be interpreted primarily on the basis of the measured SO_2, pH

and acetic acid responses rather than on unmeasured microbiological or oxidation mechanisms [27].

3.5. CIE L*a*b* Colour Parameters

At the reference point T0, colour was characterised by very high lightness, with L* = 97.3 (Table 3). This provided a common, fully comparable baseline, allowing subsequent changes to be attributed to storage time and to the combinations of SO_2 dose and fining treatment applied. At later sampling points, L* decreased systematically (overall L* mean: from 97.3 to 95.8), with the greatest decline occurring at T3. At the final stage, the most pronounced darkening was recorded in the reduced-SO_2, unfined variant (NF/CTRL–SO_2_low: L* = 94.2), whereas the use of bentonite or chitosan was associated with higher final lightness (e.g., BENT–SO_2_std: 96.8; CHIT–SO_2_std: 96.7). Thus, colour darkening during storage was most pronounced when SO_2 protection was reduced and no fining agent was applied. This trend is consistent with typical colour evolution in white wines during storage [27, 33].

Chromatic coordinates at T0 were likewise uniform (a* = −1.7; b* = 9.4), indicating a colour with a distinct green component (negative a*) and moderate yellowness (b*). During the experiment, the colour point shifted consistently towards less negative a* values and higher b* values, i.e., towards a "warmer" hue (reduced greenness and increased yellowness): on average, a* changed from −1.7 to −0.7 and b* increased from 9.4 to 12.2, with the most pronounced changes occurring between T2 and T3. This shift indicates progressive yellowing of the wine, particularly under reduced antioxidant protection [33, 34].

The greatest shift in the a*-b* plane occurred in the case of NF/CTRL–SO_2_low, where values at T3 showed both the highest yellowness (b* = 14.3) and a transition of a* into positive values (a* = 0.6). This indicates marked warming of the colour and the emergence of a red-brown component at the final storage stage. Bentonite and chitosan limited the magnitude of this shift under the SO_2_low regime (T3: BENT–SO_2_low: a* = −0.7, b* = 12.4; CHIT–SO_2_low: a* = −0.8, b* = 12.6), whereas the highest colour stability was observed under the standard SO_2 dose (e.g., BENT–SO_2_std: a* = −1.2, b* = 11.5; CHIT–SO_2_std: a* = −1.2, b* = 11.7). From a technological perspective, these differences suggest that reducing the SO_2 dose increased susceptibility to storage-related colour changes, while fining partially limited this effect, especially at T3 [33, 35].

The lower colour change observed in fined wines may be associated with the removal or aggregation of selected matrix components involved in colour development. However, because oxygen uptake, transition metals and specific oxidation products were not directly quantified, the underlying mechanisms should be treated as plausible explanations rather than confirmed causal pathways.

The calculation of ΔE^*_{ab} provided an integrated assessment of colour stability by combining changes in lightness, red-green balance and yellow-blue balance (Table 4). In all variants, ΔE^*_{ab} increased with storage time, confirming progressive colour modification. However, the magnitude of this change depended strongly on the treatment and SO_2 level. At T3, BENT–SO_2_std and CHIT–SO_2_std showed the lowest total colour differences, with ΔE^*_{ab} values of 2.22 and 2.43, respectively, indicating comparatively high colour stability. In contrast, NF/CTRL–SO_2_low showed the highest ΔE^*_{ab} value, reaching 6.24, which reflected the greatest colour instability under reduced SO_2 conditions. The reduced-SO_2 variants treated with bentonite or chitosan showed intermediate ΔE^*_{ab} values, confirming that fining partially limited colour changes compared with the untreated reduced-SO_2 control.

Table 3. Effects of fungal chitosan and bentonite, and of SO_2 level, on colour parameters, total polyphenol content, and turbidity in 'Solaris' wine during stabilisation and storage.

Variant / time point	T0	T1	T2	T3	mean
colour L* parameter					
NF/CTRL–SO_2_std	97.3 ± 0.4^{e}	97.1 ± 0.3^{e}	96.8 ± 0.2^{de}	95.5 ± 0.2^{b}	97.0^{A}
NF/CTRL–SO_2_low	97.3 ± 0.4^{e}	97.0 ± 0.2^{de}	$96.6 \pm 0.3^{b-e}$	94.2 ± 0.4^{a}	96.3^{A}
BENT–SO_2_std	97.3 ± 0.4^{e}	97.2 ± 0.3^{e}	97.0 ± 0.3^{de}	96.8 ± 0.2^{de}	97.1^{A}
BENT–SO_2_low	97.3 ± 0.4^{e}	97.1 ± 0.3^{e}	96.9 ± 0.2^{de}	95.6 ± 0.3^{bc}	96.7^{A}
CHIT–SO_2_std	97.3 ± 0.4^{e}	97.1 ± 0.2^{e}	96.9 ± 0.2^{de}	$96.7 \pm 0.2^{c-e}$	97.0^{A}
CHIT–SO_2_low	97.3 ± 0.4^{e}	97.0 ± 0.2^{de}	96.8 ± 0.4^{de}	$95.9 \pm 0.3^{b-d}$	96.8^{A}
mean	97.3^{A}	97.1^{A}	96.8^{A}	95.8^{B}	
variant **p > 0.05**, time point **p = 0.0042**, interaction **p = 0.0026**					
colour a* parameter					
NF/CTRL–SO_2_std	-1.7 ± 0.2^{a}	-1.6 ± 0.1^{ab}	$-1.4 \pm 0.1^{a-d}$	$-1 \pm 0.2^{c-e}$	-1.0^{A}
NF/CTRL–SO_2_low	-1.7 ± 0.2^{a}	-1.5 ± 0.2^{abc}	-0.9 ± 0.1^{de}	0.6 ± 0.1^{f}	-0.9^{A}
BENT–SO_2_std	-1.7 ± 0.2^{a}	-1.6 ± 0.1^{ab}	$-1.5 \pm 0.2^{a-c}$	$-1.2 \pm 0.1^{a-e}$	-1.5^{A}
BENT–SO_2_low	-1.7 ± 0.2^{a}	-1.6 ± 0.3^{ab}	$-1.1 \pm 0.1^{b-e}$	-0.7 ± 0.1^{e}	-1.3^{A}
CHIT–SO_2_std	-1.7 ± 0.2^{a}	-1.6 ± 0.1^{ab}	-1.5 ± 0.2^{abc}	$-1.2 \pm 0.2^{a-e}$	-1.5^{A}
CHIT–SO_2_low	-1.7 ± 0.2^{a}	-1.6 ± 0.2^{ab}	$-1.2 \pm 0.1^{a-e}$	-0.8 ± 0.1^{e}	-1.3^{A}
mean	-1.7^{A}	-1.6^{A}	$-1.3A^{B}$	-0.7^{B}	
variant **p > 0.05**, time point **p = 0.011**, interaction **p = 0.00087**					
colour b* parameter					
NF/CTRL–SO_2_std	9.4 ± 0.3^{a}	9.6 ± 0.2^{a}	$10.5 \pm 0.2^{a-d}$	12.2 ± 0.2^{efg}	10.4^{A}
NF/CTRL–SO_2_low	9.4 ± 0.3^{a}	9.7 ± 0.3^{a}	$11.3 \pm 0.2^{b-e}$	14.3 ± 0.2^{h}	11.2^{B}
BENT–SO_2_std	9.4 ± 0.3^{a}	9.5 ± 0.1^{a}	10.1 ± 0.1^{ab}	$11.5 \pm 0.1^{a-d}$	10.1^{A}
BENT–SO_2_low	9.4 ± 0.3^{a}	9.6 ± 0.2^{a}	10.4 ± 0.2^{abc}	12.4 ± 0.2^{efg}	10.5^{AB}
CHIT–SO_2_std	9.4 ± 0.3^{a}	9.5 ± 0.1^{a}	10.2 ± 0.2^{ab}	11.7 ± 0.2^{def}	10.2^{A}
CHIT–SO_2_low	9.4 ± 0.3^{a}	9.6 ± 0.2^{a}	$10.5 \pm 0.1^{a-d}$	12.6 ± 0.2^{fg}	10.5^{AB}
mean	9.4^{A}	9.6^{A}	10.5^{B}	12.5^{C}	
variant **$p = 3.25 \cdot 10^{-7}$**, time point **$p = 7.83 \cdot 10^{-6}$**, interaction **$p = 5.56 \cdot 10^{-4}$**					
polyphenols content [mg/100 mL]					
NF/CTRL–SO_2_std	54.0 ± 0.8^{m}	52.9 ± 0.7^{lm}	50.3 ± 0.6^{ijk}	46.5 ± 0.8^{fg}	50.9^{B}
NF/CTRL–SO_2_low	54.0 ± 0.8^{m}	51.6 ± 0.7^{kl}	48.4 ± 0.6^{ghi}	$44.3 \pm 0.7^{b-e}$	49.6^{B}
BENT–SO_2_std	54.0 ± 0.8^{m}	45.9 ± 0.7^{def}	44.1 ± 0.5^{bcd}	42.6 ± 0.7^{b}	46.7^{A}
BENT–SO_2_low	54.0 ± 0.8^{m}	$45.2 \pm 0.6^{c-f}$	43.6 ± 0.6^{bc}	40.1 ± 0.6^{a}	45.7^{A}
CHIT–SO_2_std	54.0 ± 0.8^{m}	50.9 ± 0.7^{jk}	50.4 ± 0.7^{jk}	49.5 ± 0.8^{hij}	51.2^{B}
CHIT–SO_2_low	54.0 ± 0.8^{m}	49.5 ± 0.8^{hij}	48.2 ± 0.7^{gh}	46.1 ± 0.6^{ef}	49.5^{B}
mean	54.0^{C}	49.3^{B}	47.5^{B}	44.9^{A}	
variant **$p = 4.06 \cdot 10^{-4}$**, time point **$p = 8.40 \cdot 10^{-6}$**, interaction **$p = 2.78 \cdot 10^{-4}$**					
turbidity [NTU]					
NF/CTRL–SO_2_std	148 ± 5^{g}	120 ± 4^{f}	104 ± 4^{e}	97 ± 3^{d}	117^{B}
NF/CTRL–SO_2_low	148 ± 5^{g}	116 ± 6^{f}	106 ± 3^{f}	92 ± 4^{d}	116^{B}
BENT–SO_2_std	148 ± 5^{g}	5 ± 0^{ab}	3 ± 0^{ab}	2 ± 0^{a}	40^{A}
BENT–SO_2_low	148 ± 5^{g}	8 ± 0^{b}	5 ± 0^{ab}	2 ± 0^{a}	41^{A}
CHIT–SO_2_std	148 ± 5^{g}	17 ± 1^{c}	8 ± 1^{b}	4 ± 0^{ab}	44^{A}
CHIT–SO_2_low	148 ± 5^{g}	14 ± 1^{c}	6 ± 0^{ab}	3 ± 0^{ab}	43^{A}
mean	148^{C}	47^{B}	39^{A}	33^{A}	
variant **p = 0.024**, time point **p = 0.004**, interaction **p = 0.034**					

Note. ANOVA p-values are given for the technological variant, the time point, and their interaction. Different letters indicate statistically significant differences according to Tukey's HSD test at $p < 0.05$.

Table 4. Total colour difference (ΔE^*_{ab}) at T1, T2 and T3 relative to T0.

Variant	ΔE^*_{ab} at point:			ΔL*	Δa*	Δb*
	T1	T2	T3	T3 vs.T0	T3 vs.T0	T3 vs.T0
NF/CTRL–SO_2_std	0.30	1.24	3.40	–1.8	0.7	2.8
NF/CTRL–SO_2_low	0.47	2.18	6.24	–3.1	2.3	4.9
BENT–SO_2_std	0.17	0.79	2.22	–0.5	0.5	2.1
BENT–SO_2_low	0.30	1.23	3.59	–1.7	1.0	3.0
CHIT–SO_2_std	0.24	0.92	2.43	–0.6	0.5	2.3
CHIT–SO_2_low	0.37	1.31	3.61	–1.4	0.9	3.2

Note. ΔE^*_{ab} was calculated descriptively from mean CIE L*, a* and b* values relative to the initial sampling point T0. The values were not subjected to separate statistical analysis because they were calculated from treatment means rather than replicate-level measurements.

3.6. Total Polyphenols Content

At the reference point T0, total polyphenols were 54.0 mg/100 mL (Table 3). This common baseline allows subsequent differences to be attributed to the applied combinations of SO_2 dose, fining treatment, and the duration of stabilisation/storage. Across subsequent stages, total polyphenols declined clearly and consistently (overall mean from 54.0 to 44.9 mg/100 mL). This decrease indicates a progressive reduction in the phenolic pool during processing and storage, most likely related to fining-induced removal and storage-related transformation of phenolic compounds [34, 36].

Fining treatment was the strongest factor differentiating the results. Bentonite caused the largest reduction in total polyphenols, with values decreasing to 45.2–45.9 mg/100 mL by T1 and reaching 40.1–42.6 mg/100 mL by T3. The T0-T3 losses amounted to 11.4 mg/100 mL for BENT–SO_2_std and 13.9 mg/100 mL for BENT–SO_2_low, indicating that most of the reduction occurred during fining and early stabilisation [37, 38].

By contrast, chitosan-treated variants showed the greatest retention of polyphenols over time. The most stable trajectory was observed for CHIT–SO_2_std, where values decreased only from 54.0 mg/100 mL at T0 to 49.5 mg/100 mL at T3, giving the highest period mean among all variants (51.2 mg/100 mL). In CHIT–SO_2_low, the final value was lower (46.1 mg/100 mL), but still clearly higher than in bentonite-treated wines. Thus, chitosan was associated with better preservation of the total phenolic fraction than bentonite; however, because individual phenolics and oxidation products were not directly quantified, the mechanism should be interpreted cautiously [15, 36].

The effect of the SO_2 level was directional but secondary to the fining effect. Reducing the SO_2 dose was associated with lower polyphenol retention at T3, particularly in NF/CTRL and BENT variants (NF/CTRL: 46.5 vs 44.3 mg/100 mL; BENT: 42.6 vs 40.1 mg/100 mL for SO_2_std and SO_2_low, respectively). This supports the protective role of SO_2 during storage, although the magnitude of the effect depended mainly on the fining agent used [27]. The higher value observed in CHIT–SO_2_low than in BENT–SO_2_low suggests that chitosan partially mitigated the decrease associated with SO_2 reduction.

Overall, retention of total polyphenols depended primarily on the fining agent, with chitosan favouring preservation of the phenolic pool and bentonite causing the greatest reduction. These trends were consistent with the colour results but should be interpreted as associations among measured quality indicators rather than as direct evidence of a specific oxidation pathway.

3.7. Turbidity

At T0, turbidity was identical across all variants (148 NTU), confirming full comparability of the base wine prior to the technological treatments (Table 3). Thereafter, NTU diverged sharply, indicating that the clarification strategy was the primary determinant of optical stability during cold stabilisation and bottle storage.

In the unfined NF/CTRL variants, turbidity remained high throughout the experiment and decreased only slightly over time (NF/CTRL–SO_2_std: 148 → 120 → 104 → 97 NTU; NF/CTRL–SO_2_low: 148 → 116 → 106 → 92 NTU). The persistence of elevated NTU at T3 indicates that gravitational settling alone did not remove the haze-forming colloidal fraction. This is consistent with the known persistence of turbidity-active colloids in white wines, including proteinaceous and polysaccharide-associated material [11, 39]. Although some spontaneous settling occurred during storage, the final NTU values confirmed that passive clarification was insufficient to achieve optical stability.

Bentonite caused the most rapid and extensive clarification: NTU decreased to 5–8 at T1 and reached 2 at T3 under both SO_2 regimes. The magnitude of the drop already at T1 indicates that the principal clarification effect was achieved during the 7-day contact period, with only marginal additional improvement thereafter. This pattern is consistent with the expected action of bentonite, which promotes removal of haze-forming material through adsorption, floc formation and sedimentation [38]. The convergence to very low NTU at T3 under both SO_2 regimes indicates robust clarification and limited dependence on SO_2 dose.

Chitosan addition also resulted in pronounced and durable reduction in turbidity, but with a different kinetic profile. NTU decreased to 14–17 at T1 and continued to decline during stabilisation and storage (T2: 6–8; T3: 3–4 NTU), indicating progressive clarification beyond the initial fining period. This slower but effective response is consistent with the role of fungal chitosan in promoting aggregation and sedimentation of suspended particles in acidic wine matrices [3, 17]. In the present study, chitosan ultimately achieved low single-digit NTU, although turbidity reduction was slightly slower than with bentonite.

The SO_2 regime had only a minor influence on NTU once fining was applied. Differences between SO_2_std and SO_2_low within BENT and CHIT were small and largely disappeared by T3, indicating that the mechanisms governing clarification dominated over indirect SO_2-linked effects. Overall, although mean turbidity decreased over time across the experiment (148 at T0 → 47 at T1 → 39 at T2 → 33 at T3), achieving low and stable turbidity required after fining. Bentonite provided the fastest clarification, whereas chitosan offered a slightly slower but effective alternative, resulting in similarly low turbidity after stabilisation and bottle storage [3, 38, 40].

4. Conclusions

The presented results allow to draw the following scientific conclusions:

1. The use of fungal chitosan derived from *Aspergillus niger* in 'Solaris' white wine significantly improved clarity and optical/colloidal stability (as reflected by NTU) relative to NF/CTRL, as evidenced by a marked reduction in turbidity after treatment and maintenance of low NTU throughout subsequent stages (T1-T3).
2. Bentonite caused the strongest turbidity reduction (the lowest NTU at T3) while chitosan achieved a fining effect broadly comparable to bentonite, although final NTU values remained slightly higher than in bentonite-treated variants.

3. Acidity-related parameters (pH and selected organic acids) varied only marginally between variants, indicating that the quality differences observed were driven primarily by treatment effects on the colloidal fraction and/or stabilisation-sensitive constituents rather than by alterations in acid-base balance.
4. Reducing the SO_2 dose (SO_2_low) was associated with less favourable changes during storage, particularly in the NF/CTRL variant, confirming increased susceptibility to deterioration of selected quality parameters under limited sulphite protection.
5. Bentonite caused the greatest decrease in total polyphenols across processing, whereas chitosan-treated variants showed comparatively better preservation of the total polyphenol pool, suggesting lower losses attributable to technological interactions (e.g., adsorption/coagulation) than with bentonite.
6. SO_2 retention depended on the technological treatment; chitosan-treated variants showed more favourable retention of free SO_2 at the final stages, especially under the reduced SO_2 regime. This finding highlights the potential of chitosan as a supportive tool in low-sulphite strategies; however, based on the measurements performed, the underlying mechanism cannot be determined conclusively.

Based on the presented results, the following practical implications can be enumerated:

1. In the production of 'Solaris' white wine, where effective clarification is required alongside better preservation of the total polyphenol pool, chitosan may be a useful alternative to bentonite.
2. If maximising clarity (i.e., achieving the lowest NTU at the final stage) is the primary objective, bentonite remains the most effective option; however, this should be weighed against a greater reduction in total polyphenols.
3. Under reduced SO_2 dosing (SO_2_low), unfavourable quality changes are most evident in the NF/CTRL variant; therefore, low-sulphite strategies should be combined with stabilising and fining treatments such as bentonite or chitosan.
4. Chitosan may support SO_2 management because, under low-SO_2 conditions, it was associated with more favourable retention of free SO_2 at the final stages than NF/CTRL. In practice, this effect should be verified on a case-by-case basis under the specific process conditions and for the given raw material.
5. In routine practice, it is advisable to monitor NTU and colour parameters after stabilisation and again after the storage period to confirm the persistence of the clarification effect and stability under the selected processing variant.

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6. Author Contributions

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