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Alternatives for replacing or reducing the content of added sulfites in Tannat red wines elaborated with commercial yeasts or with minimal intervention

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Abstract

Sulfite reduction in wines represents a significant challenge in the current wine industry given its widespread use as an additive and the potential health risks for consumers. This study evaluates alternatives to reduce sulfites in Tannat red wines made with minimal intervention or with selected yeasts, focusing on microbiological stability, color, and physicochemical composition. In the 2023 vintage, vinifications were carried out with native yeasts and selected yeasts using reduced SO_2 (SR: 30 mg/L), chitosan (Q: 100 mg/L), combinations of SO_2 and chitosan (SR+Q), lysozyme (SR+L: 5 mg/L) (SR+QL), and fumaric acid (SR+AF: 6 mg/L) that were compared with a Control (125 mg/L of sulfites) and a treatment without additives (SA). Microbial counts in minimal intervention wines and those obtained by sulfite substitution or reduction did not show differences during fermentation. The minimal intervention fermentations rates were slower than the treatments with selected yeasts. From the minimal intervention treatments, the SR+Q wines showed higher malic acid content, color intensity, phenolic compounds, anthocyanins, and tannins compared to the other treatments and similar to the Control. On the other hand, the SR+Q wines from the sulfite substitution or reduction trial also presented values of color intensity and polyphenolic and anthocyanin content similar to the Control. Consequently, the combination of reduced doses of sulfites and chitosan seems to be a viable option for producing Tannat wines with characteristics similar to those made with conventional doses of sulfites, at least, when grape soundness is good, as in the vintage analyzed in the present study.

Keywords: native yeast, sulfur dioxide, polyphenols, wine preservation, Uruguayan wines

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Alternativas para reducir el contenido de sulfitos añadidos en vinos Tannat elaborados con levaduras nativas o con levaduras seleccionadas

Resumen

La reducción de sulfitos en vinos es un desafío para la industria vitivinícola debido a los posibles riesgos para la salud del consumidor. Este estudio evaluó alternativas para disminuir los sulfitos en vinos Tannat elaborados con mínima intervención o levaduras seleccionadas, analizando estabilidad microbiológica, color y composición fisicoquímica. Durante la vendimia 2023, se realizaron vinificaciones con levaduras nativas y seleccionadas, utilizando SO2 reducido (SR: 30 mg/L), quitosano (Q: 100 mg/L), combinaciones de ambos (SR+Q), lisozima (SR+L: 5 mg/L), ácido fumárico (SR+AF: 6 mg/L), un control (125 mg/L de sulfitos) y un tratamiento sin agregados (SA). Los recuentos microbianos en vinos de mínima intervención y los obtenidos con sulfitos reducidos o sustituidos no mostraron diferencias durante la fermentación. Las cinéticas de fermentación de mostos de mínima intervención fueron más lentas que con levaduras seleccionadas. Entre los tratamientos de mínima intervención, los vinos SR+Q destacaron por su mayor contenido de ácido málico, intensidad colorante, compuestos fenólicos, antocianos y taninos, similares al Control. En los ensayos de sustitución o reducción de sulfitos, SR+Q también mostró valores comparables al Control en intensidad colorante, contenido polifenólico y antociánico.La combinación de dosis reducidas de sulfitos con quitosano parece una opción viable para producir vinos Tannat con características similares a los elaborados con sulfitos convencionales, especialmente en vendimias de excelente calidad sanitaria de la uva, como la analizada en este estudio.

Palabras clave: levaduras nativas, anhídrido sulfuroso, polifenoles, conservación del vino, vinos del Uruguay

Alternativas para substituir ou reduzir o teor de sulfitos adicionados em vinhos tintos Tannat elaborados com leveduras comerciais ou com intervenção mínima

Resumo

A redução de sulfitos nos vinhos é um desafio fundamental na indústria vinícola devido à sua ampla utilização como aditivo e aos potenciais riscos para a saúde do consumidor. Este estudo avaliou alternativas para reduzir os sulfitos em vinhos tintos Tannat elaborados com intervenção mínima ou leveduras selecionadas, com foco na estabilidade microbiológica, cor e composição físico-química. Na safra de 2023, foram realizadas vinificações com leveduras nativas e selecionadas, utilizando SO2 reduzido (SR: 30 mg/L), quitosano (Q: 100 mg/L), combinações de SO2 e quitosano (SR+Q), lisozima (SR+L: 5 mg/L), ácido fumárico (SR+AF: 6 mg/L), além de um Controle (125 mg/L de sulfitos) e um tratamento sem agregados (SA). As contagens microbianas nos vinhos de intervenção mínima e nos tratamentos de substituição ou redução de sulfitos não apresentaram diferenças significativas durante a fermentação. As fermentações de intervenção mínima foram mais lentas em comparação com as realizadas com leveduras selecionadas. Entre os tratamentos de intervenção mínima, os vinhos SR+Q destacaram-se por apresentar maior teor de ácido málico, intensidade de cor, compostos fenólicos, antocianinas e taninos, semelhantes aos valores do Controle. Da mesma forma, os vinhos SR+Q dos ensaios de redução ou substituição de sulfitos também exibiram valores de intensidade de cor, polifenóis e antocianinas comparáveis ao Controle. Assim, a combinação de doses reduzidas de sulfitos e quitosano surge como uma alternativa viável para a produção de vinhos Tannat com características similares aos vinhos elaborados com doses convencionais de sulfitos, especialmente em safras de uvas com excelente sanidade, como a analisada neste estudo.

Palavras-chave: leveduras nativas, anidrido sulfuroso, polifenóis, conservação do vinho, vinhos do Uruguai

1. Introduction

Sulfur dioxide is one of the most widely used additives in enology[1]. Due to its antioxidant activity, it protects wine from oxidations that could alter the chemical composition of phenolic and aromatic compounds, causing the loss of specific sensory characteristics. Its use before alcoholic fermentation has an antioxidant action, inhibiting the polyphenol oxidase enzymes naturally present in grapes and lacassa (in grapes affected by *Botrytis cinerea*). It also has an antiseptic action, inhibiting the development of yeasts, lactic acid bacteria, and, to a lesser extent, acetic acid bacteria that can have a negative effect on wine[2].

However, high sulfite content in wines can affect consumers' health by causing allergic reactions, headaches, and nausea[3][4]. The European Food Safety Agency[5] determined that up to 70 mg/kg of body weight/day of sulfites do not produce adverse effects on consumers health. Even so, it is estimated that exposure to sulfites is higher than the acceptable daily intake in a large part of the population. The most critical challenge for the wine industry is to reduce the content of added sulfites due to the reasons mentioned above, along with the fact that these compounds accumulate in the body and that sulfites are added to various food products[4].

Recently, the wine industry has incorporated alternatives to increase sustainability of its practices and quality of the products[6], motivated by the change in consumers' behavior, who make more conscious decisions based on the characteristics of the product, its effect on health, and its influence on the environment and the society[7]. Some alternatives implemented seek to replace or reduce the addition of sulfites during winemaking, while others promote the development of new products, such as “natural” or minimal intervention wines.

Chitosan, lysozyme, and fumaric acid are among the options to substitute or replace sulfur dioxide in winemaking. Chitosan is a natural polysaccharide of fungal origin with authorized use in wines to reduce the presence of undesirable microorganisms, recommending a maximum dose of 10 g/hl[8]. Its use in wines inactivates the development of yeasts and lactic and acetic acid bacteria[9], even during storage[10]. Furthermore, it has been shown that chitosan can perform an antioxidant function in white wines due to its ability to eliminate the ions that promote the Fenton reaction in the presence of tartaric acid, inhibiting the browning of the wine[11].

Lysozyme is an enzyme-based additive derived from egg white, known for its ability to hydrolyze peptidoglycans, the primary structural components of Gram-positive bacteria cell walls. In contrast, Gram-negative bacteria, such as acetic acid bacteria, possess a lower proportion of peptidoglycans, making them less susceptible to lysozyme's action. Consequently, lysozyme selectively inhibits the growth of lactic acid bacteria, effectively reducing the production of volatile acids[1], while preserving the growth and metabolic activity of yeasts[12]. This substitute could be helpful in wines with high pH where sulfur dioxide is less effective. Its use in musts and wines is approved at a maximum accumulated dose of 500 mg/L, with the objectives of controlling the malolactic fermentation and reducing the content of sulfites added to the wine. Lysozyme does not completely replace sulfur dioxide as it does not have antioxidant properties[13].

The International Organization of Vine and Wine (OIV) has recently approved the use of fumaric acid in wines in enology to control the growth and activity of lactic bacteria, reduce the dose of added sulfites and preserve the malic acid content of the wine. Doses of 300-600 mg/L are recommended to control malolactic fermentation, even in large amounts of inoculum. Fumaric acid is a food additive used as an acidifier. Although its effect as an inhibitor of the development of lactic acid bacteria has been known for some time, recent studies have demonstrated its effectiveness in inhibiting malolactic fermentation[14].

Minimal intervention wines refer to production with the least interventions from managing the vineyard, grape harvest, vinification, conservation, and aging[15]. This production develops at the end of the 20th century in the Beaujolais region of France. Since 2005, this country has a definition that allows the incorporation of a distinc-

tive logo on the labeling of wines of this type. In addition, between 2019 and 2020, they established the characteristics and conditions of these wines by adopting the designation "Natural Method Wine" as the first stage to achieve the PDO "product" without geographical delimitation[16]. At the international level, there is no defined regulation for these wines[6][16][17]. It is agreed that for production the grapes must be harvested by hand from vineyards managed using sustainable practices, the alcoholic and malolactic fermentation should be carried out with native microorganisms, the composition of the musts and wines is not corrected, and no chemical substances should be added for stabilization. Additionally, any processes that may accelerate the natural processes of the wine are not allowed. French regulations authorize the post-fermentation addition of up to 30 mg/L of sulfur dioxide, classifying the wines as natural methods wines without added sulfites[17][18]. In addition, chitosan is allowed due to its natural origin.

In Uruguay, replacing or reducing added sulfite content or producing minimal intervention wines have attracted great interest in the last five years. A survey carried out by our research group from 2021 to 2023 revealed that approximately 10% of the wineries registered with the National Institute of Vitiviniculture (INAVI) are exploring these practices (data not shown). However, there are no legal definitions or any clear regulatory framework that certify these products, which generates variability in the production processes and a lack of guarantees for the consumers. This research aims to evaluate alternatives for replacing or reducing the contents of added sulfites in Tannat red wines elaborated with selected yeasts or by minimum intervention, without affecting their microbiological stability and physicochemical and sensory characteristics. Consequently, two experiments were carried out: one focused on replacing or reducing the contents of added sulfites in winemaking with selected yeasts, and another focused on producing minimal intervention wines.

2. Materials and methods

2.1 Grapes and wines

The study was carried out during the 2023 vintage using grapes of Tannat cultivar (Vitis International variety catalog number VIVC 12257) (*Vitis vinífera* L.). Tannat is an emblematic cultivar of Uruguay, recognized for its high productive and oenological potential, as well as its adaptation to the country's agroclimatic conditions. The grapes were harvested from a commercial vineyard in El Colorado, Canelones, south of Uruguay. The variety was grafted on SO4, trained on a trellis with Guyot pruning, and managed under the Sustainable Viticulture Program.

When the grapes reached technological maturity, 330 kg were manually harvested and transported to the School of Agronomy's Experimental Winery (Montevideo, Uruguay). The grapes were destemmed and crushed (Alfa 60 R crusher, Italcom, Piazzola Sul Brenta, Italy), and the must and pomace obtained were distributed in 33 polyethylene containers of 10 L capacity each. The containers were separated into two batches: 21 containers were assigned to the trial of replace or reduction of added sulfite contents in a vinification with selected yeasts, and the remaining 12 containers were assigned to the trial of minimal intervention winemaking.

2.1.1 Experiment 1: Replacement or reduction of added sulfite contents in traditional winemaking

The 21 containers used for this experiment were inoculated with selected yeast (Lamothe Abeit – Z.A. Actipolis, France) and separated into groups of three, to which the following treatments were assigned:

- Chitosan (Q) – Addition of 10 g/hl of chitosan (Bactiless™, Lallemand, Danstar Ferment A.G. – Fredericia, Denmark) at devatting and, before bottling, divided into equal parts.

- Reduced sulfur dioxide (SR) – Addition of 6 g/hl of potassium metabisulphite (Kadifit, Hesse, Germany) (30 mg/L of added sulfite) at devatting and, before bottling, divided into equal parts.

- Reduced sulfur dioxide and chitosan (SR+Q) – Addition of 10 g/hl of chitosan and 6 g/hl of potassium metabisulphite at devatting and, prior to bottling the wine, divided into equal parts.

- Reduced sulfur dioxide and lysozyme (SR+L) – Addition of 6 g/hl of potassium metabisulphite at devatting and, prior to bottling the wine, divided into equal parts, and 500 mg/hl of lysozyme (DelvoZyme, DSM, Delft, Netherlands) added only at devatting.

- Reduced sulfur dioxide and chitosan with lysozyme (SR+QL) – Addition of 6 g/hl of potassium metabisulphite to devatting and, prior to bottling the wine, divided into equal parts, and 20 g/hl of chitosan with lysozyme (Killbact, Lamothe Abeit – Z.A. Bordeaux, France) added only at devatting.

- Reduced sulfur dioxide and fumaric acid (SR+AF) – Addition of 6 g/hl of potassium metabisulphite to devatting and, prior to bottling the wine, divided into equal parts, and 600 mg/hl of fumaric acid added only at devatting.

- Control - Addition of usual doses of potassium metabisulphite (25 g/hl equivalent to 125 mg/L of sulfur dioxide), divided into three moments (10 g/hl at vatting, 10 g/hl at devatting, and 5 g/hl prior to bottling)*

2.1.2 Experiment 2: Minimum intervention winemaking

The 12 containers for this experiment were separated into groups of three to which the following treatments were assigned:

- Chitosan (Q) – Addition of 10 g/hl of chitosan at the time of the devatting and, prior to bottling the wine, divided into equal parts.

- Reduced sulfur dioxide (SR) – Addition of 6 g/hl of potassium metabisulphite (30 mg/L of added sulfites) at the time of the vatting, devatting, and, prior to bottling the wine, divided into equal parts.

- Reduced sulfur dioxide and chitosan (SR+Q) – Addition of 10 g/hl of chitosan at the time of the devatting and, prior to bottling the wine, divided into equal parts, and 6 g/hl of potassium metabisulphite at the time of the vatting, devatting, and, prior to bottling, divided into equal parts.

- No addition (SA) – No additions were made during the entire winemaking process.

- *Control (T) – *Shared with Experiment 1.

The vinification of both experiments underwent a fermentative maceration of 7 days. During vinification, the cap was submerged daily. At the end of the maceration, the wine was separated from the grapes, and the grape skins were lightly pressed using a manual press. The wine from the grapes and the wine from the press were combined and kept in 5-liter polyethylene containers to decant the lees. They were then transferred to 3L polyethylene containers for natural stabilization until they were bottled, three months after devatting, in 375 ml bottles.

2.2 Analytical determinations

2.2.1 Must fermentation kinetics

During the fermentation maceration, musts density (2001FC-20/20, Alla France, Chemille, France) and temperature (2905, Alla France, Chemille, France) were monitored daily. The fermentation temperature was kept controlled between 24 and 28 °C.

2.2.2 Microbiological analysis of the fermenting must and the wine

Microbiological analyses of the wines were carried out using serial decimal dilutions of the samples in peptone water by the surface plate method, at the following time points: three days after vatting, after devatting, fifteen days after devatting, and fifteen days after bottling. Culture media and incubation conditions for each microbial group were: Plate Count Agar (PCA, Oxoid) for total aerobic mesophile counting (35 °C, 24-48 h), de Man Rogosa Agar (MRS, Oxoid) for lactic acid bacteria (37 °C, 48 h, microaerophilia), Glucose-Yeast extract-Calcium Carbonate agar (GYC, Condalab) for acetic bacteria (30 °C, 72 h)[19] and -Yeast extract-Peptone-Dextrose agar (YPD, Neogen) for yeasts (28 °C, 3-5 days)[20]. The counts were performed in triplicate and were reported as log c.f.u./mL of sample.

2.2.3 Color and wine composition evaluation

The wines were analyzed by duplicate fifteen days after bottling. The ethanol content (% v/v), titratable acidity, pH, malic and lactic acid contents, and volatile acidity were determined using a Winescan TM Autosampler 79,000 infrared analyzer (Foss, USA) and the Foss Integrator software version 154 (Foss, Denmark).

The color parameters were determined directly on the wine samples placed in a 1 mm optical path cuvette. Color intensity (CI) and hue were estimated using the method described by Glories[21][22]. The CIELAB coordinates, lightness (L*), chroma (C*), hue (H*), red-greenness (a*), and yellow-blueness (b*) were determined following the method described by Ayala and others[23] and data processing was performed using MSCV software[24].

The concentration of total phenols, anthocyanins, and tannins in wines were determined using spectrophotometric methods according to Singleton and Rossi[25], Ribéreau-Gayon and Stonestreet[26], and Sarneckis and others[27], respectively.

2.2.4 Sensorial analysis of wines

Tannat wines were evaluated after 6 months of their respective bottling. The wines were tasted by a group of twelve assessors (8 male and 4 female), ranging from 26 to 60 years old. Assessors were recruited among winemakers and professors from the School of Agronomy (University of the Republic, Uruguay) and selected according to their availability to participate in the study. All the assessors had extensive previous experience in sensory evaluation of wines as part of their regular jobs. Assessors attended a 20-min previous training session to homogenize the evaluation criteria.

Sensory evaluation was carried out under laboratory conditions in the School of Agronomy's Oenology Laboratory. Assessors evaluated the wines blindly and randomly. For each wine, eight sensorial attributes were evaluated on a continuous scale from 1 to 10: color intensity, hue, aromatic intensity, aromatic quality, structure, acidity, astringency, bitterness, and preference. The values indicate the intensity of the sensation for each attribute.

2.2.5 Data processing and statistical analysis

All data were expressed as the arithmetic average ± standard deviation of three replicates. The data were analyzed through an analysis of variance, followed by a comparison test of means by Tukey ($\alpha \leq 0.05$). The program Infostat was used for the statistical analysis of the data.

3. Results

3.1 Experiment 1: Replacement or reduction of added sulfite contents in traditional winemaking

3.1.1 Musts fermentation kinetics

Alcoholic fermentation occurred within the pre-established temperatures for all treatments, without fermentation stops (**Figure 1**). No significant differences were recorded between treatments in the temperature and density of the musts.

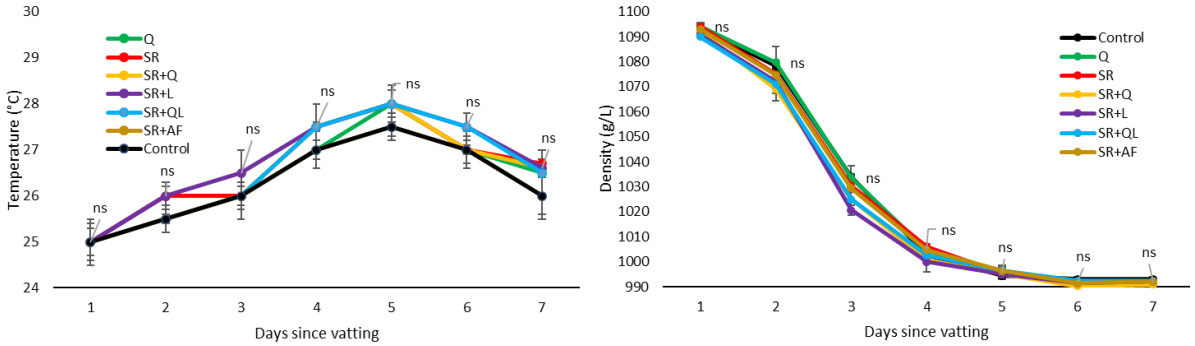

Figure 1. Temperature and density of the musts during alcoholic fermentation according to treatment

NS indicates the absence of significant differences among the means of the different treatments according to Tukey test (α ≤ 0.05). Treatments are chitosan (Q), reduced sulfur dioxide (SR), sulfur dioxide and chitosan (SR+Q), reduced sulfur dioxide and lysozyme (SR+L), reduced sulfur dioxide and chitosan with lysozyme (SR+QL), reduced sulfur dioxide and fumaric acid (SR+AF).

3.1.2 Wine general composition

Alternatives to replace or reduce the added sulfite content modified the basic composition of the wines (**Figure 2**). Regarding ethanol content, Q wines had the highest ethanol content, while the SR+L wines had the lowest. The other treatments had intermediate values. No significant differences were observed between the ethanol contents of the wines elaborated with alternatives to replace or reduce the added sulfite content and the Control wines.

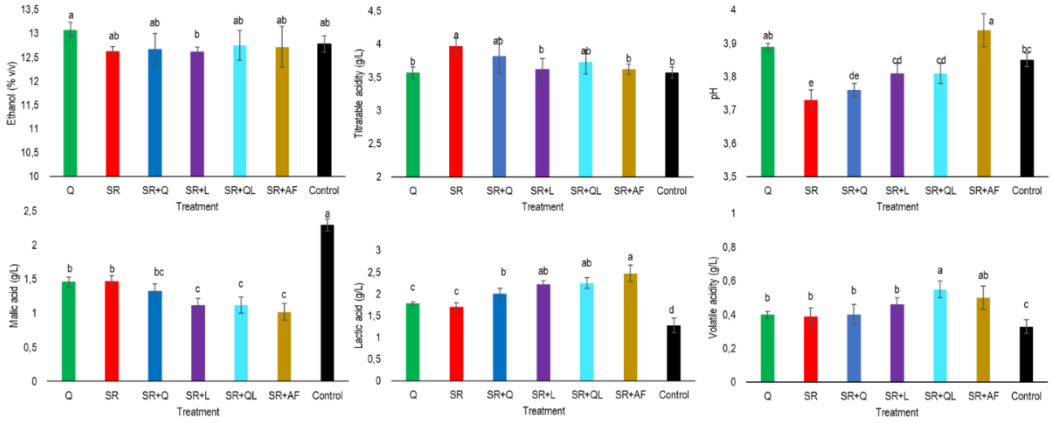

Figure 2. Wine composition fifteen days after bottling according to treatment. Means with different letters indicate significant differences according to Tukey's test (α ≤ 0.05)

Treatments are chitosan (Q), reduced sulfur dioxide (SR), sulfur dioxide and chitosan (SR+Q), reduced sulfur dioxide and lysozyme (SR+L), reduced sulfur dioxide and chitosan with lysozyme (SR+QL), reduced sulfur dioxide and fumaric acid (SR+AF).

Concerning the content of malic and lactic acids, Control wines highlighted for having a higher malic acid and a lower lactic acid content, significantly different from other treatments wines. Among the alternatives for replacing or reducing the added sulfite content, the Q and SR wines had higher malic acid and lower lactic acid than the SR+L, SR+QL, and SR+AF wines. The SR+Q wines had intermediate contents.

The titratable acidity of the SR wines was higher than other treatments' wines, and significantly differed from that of the Control wines. The Q, SR+Q, SR+L, SR+QL, SR+AF, and Control wines did not differ in this parameter. Additionally, the SR+AF wines had the highest pH value, followed by the Q wines, while the SR+Q and SR treatments had the lowest pH. The Control wines had an intermediate pH.

The volatile acidity of the SR+AF and SR+QL wines was higher than that of the Q, SR, SR+Q, and SR+L wines, with no significant differences recorded for the latter. The wines from the treatments to replace or reduce the contents of added sulfur dioxide showed significantly higher volatile acidity values than the Control wines.

3.1.3 Evolution of yeasts and lactic acid bacteria populations

A high yeast population was observed three days after vatting, which remained constant until devatting in all treatments with no significant differences (**Figure 3**). Fifteen days after devatting, the yeast population decreased in all treatments, remaining at high levels only in the Q wines. Fifteen days after bottling, the yeast population remained low in all treatments, with significant differences between them, SR, SR+QL, and Control wines presented the lowest yeast population.

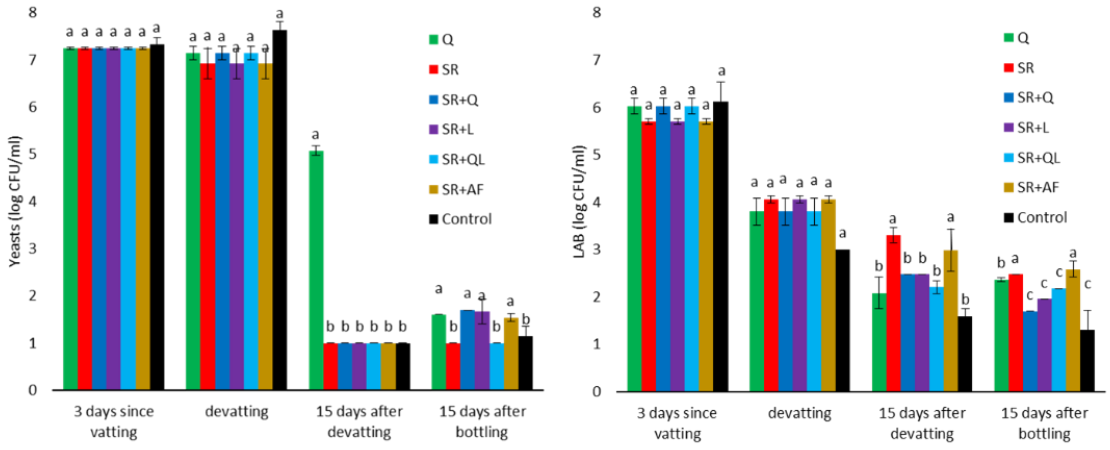

Figure 3. Yeasts and lactic acid bacteria count according to treatment

Means with different letters indicate significant differences by Tukey test (α ≤ 0.05). Treatments are chitosan (Q), reduced sulfur dioxide (SR), sulfur dioxide and chitosan (SR+Q), reduced sulfur dioxide and lysozyme (SR+L), reduced sulfur dioxide and chitosan with lysozyme (SR+QL), reduced sulfur dioxide and fumaric acid (SR+AF).

A high population of lactic bacteria was recorded three days after vatting, which decreased at devatting, with no significant differences between treatments. Fifteen days after devatting, lactic acid bacteria count continued to decline; however, the SR and SR+AF wines presented significantly higher values than the other treatments. These differences remained until fifteen days after bottling, when a slight increase in the population of lactic acid bacteria was recorded in the Q wines.

Acetic acid bacteria were detected in the sampling conducted three days after the start of fermentation (data not shown). The average population recorded was approximately 3.6 log CFU/ml, with no significant differ-

ences observed among the vinifications of the different treatments. In subsequent samplings, the population of acetic acid bacteria remained below the detection limit of the technique (≤ 1 log CFU/ml) across all treatments.

3.1.4 Color and phenolic composition of wines

The color intensity showed that SR wines had the highest value (16.3 ± 1.1), followed by Q wines (15.5 ± 0.3) (**Table 1**). In contrast, treatments combining sulfur dioxide with other additives, such as SR+Q (13.7 ± 0.6), SR+L (13.8 ± 0.5), and SR+QL (14.1 ± 0.8), showed color intensities similar to Control wines (13.9 ± 0.5). Wines treated with SR+AF showed the lowest color intensity value (11.1 ± 0.3). Regarding hue, SR+AF wines had the highest value (0.71 ± 0.02) compared to the other treatments. Instead, SR (0.64 ± 0.01), SR+Q (0.65 ± 0.01), and Control (0.64 ± 0.01) wines had the lowest hue, with no significant differences between them.

Table 1. Color and phenolic composition of wines fifteen days after bottling according to treatment

Analitical parameter	Treatment						
	Q	SR	SR+Q	SR+L	SR+QL	SR+AF	Control
Color intensity	15,5 ± 0,3 a	16,3 ± 1,1 a	13,7 ± 0,6 b	13,8 ± 0,5 b	14,1 ± 0,8 b	11,1 ± 0,3 c	13,9 ± 0,5 b
Hue	0,68 ± 0,01 b	0,64 ± 0,01 c	0,65 ± 0,01 c	0,68 ± 0,02 b	0,68 ± 0,01 b	0,71 ± 0,02 a	0,64 ± 0,01 c
L*	36,9 ± 0,5 c	35,9 ± 2,6 c	41,7 ± 1,1 b	40,9 ± 0,9 b	40,6 ± 2,8 b	48,1 ± 0,5 a	41,2 ± 1,2 b
a*	48,3 ± 0,5 b	50,5 ± 0,6 a	47,6 ± 1,5 bc	46,3 ± 1,1 cd	45,6 ± 0,8 d	41,9 ± 0,5 e	49,1 ± 0,3 ab
b*	11,0 ± 0,6 a	9,6 ± 0,3 ab	9,8 ± 1,0 ab	9,8 ± 1,3 ab	10,3 ± 2,9 ab	7,6 ± 2,2 b	9,4 ± 0,8 ab
Total phenolic compounds (mg/L)	1368 ± 256 ab	1373 ± 214 ab	1338 ± 255 ab	1203 ± 207 ab	1005 ± 180 b	1013 ± 217 b	1519 ± 161 a
Anthocyanins (mg/L)	305 ± 17 bc	373 ± 44 ab	332 ± 27 b	283 ± 39 c	268 ± 31 c	288 ± 68 c	379 ± 15 a
Tannins (mg/L)	632 ± 39 ab	557 ± 77 bc	527 ± 36 c	549 ± 56 bc	509 ± 30 c	518 ± 23 c	672 ± 44 a

Means with different letters indicate significant differences by Tukey test (α ≤ 0.05). Treatments are chitosan (Q), reduced sulfur dioxide (SR), sulfur dioxide and chitosan (SR+Q), reduced sulfur dioxide and lysozyme (SR+L), reduced sulfur dioxide and chitosan with lysozyme (SR+QL), reduced sulfur dioxide and fumaric acid (SR+AF).

Regarding the luminosity parameter, Control wines had an intermediate luminosity (41.2 ± 1.2) compared to the other treatments. SR+AF wines were the lightest (48.1 ± 0.5), while Q (36.9 ± 2.5) and SR (35.9 ± 2.6) were the darkest. On the a* axis (red-greenness), the SR+AF (41.9 ± 0.5), SR+QL (45.6 ± 0.8), and SR+L (46.3 ± 1.1) wines showed the lowest values, while the SR (50.5 ± 0.6), Q (48.3 ± 0.5), and SR+Q (47.6 ± 1.5) treatments showed the highest values, although no significant differences were found compared to Control (49.1 ± 0.3). On the b* axis (yellow-blueness), the SR+AF treatment showed the lowest value (7.6 ± 2.2) and Q (11.0 ± 0.6) the highest, with a significant difference between them.

Total phenolic compound concentrations were higher in Control wines (1519 ± 161), followed by Q (1368 ± 256), SR (1373 ± 214), SR+Q (1338 ± 255), and SR+L (1203 ± 207) treatments. The SR+QL (1005 ± 180) and SR+AF (1013 ± 217) wines showed significantly lower values. The concentration of anthocyanins was higher in the SR (373 ± 44) and Control (379 ± 15) wines compared to the remaining treatments. The Q (305 ± 17) and SR+Q (332 ± 27) wines had slightly lower anthocyanin concentrations than the Control, while the lowest values were recorded in the other treatments. The tannin contents of the Control (672 ± 44) and Q (632 ± 39) wines were the highest, while the SR+Q (527 ± 36), SR+AF (518 ± 23), and SR+QL (509 ± 30) wines had the lowest values.

3.1.5 Sensorial analysis of the wines

Figure 4 illustrates the intensity of sensory parameters evaluated in wines from the different treatments, as well as consumer's preference for these wines, measured on a continuous scale ranging from 0 to 10. The sensory analysis of the wines was conducted six months after bottling. Significant differences between treatments were found only in the bitterness attribute. SR+Q wines were the least bitter, with a significant difference compared to SR+AF wines, which showed the highest value. No significant differences were observed among the other treatments. Additionally, the assessors did not find differences in the preference of the wines from the different treatments in this trial.

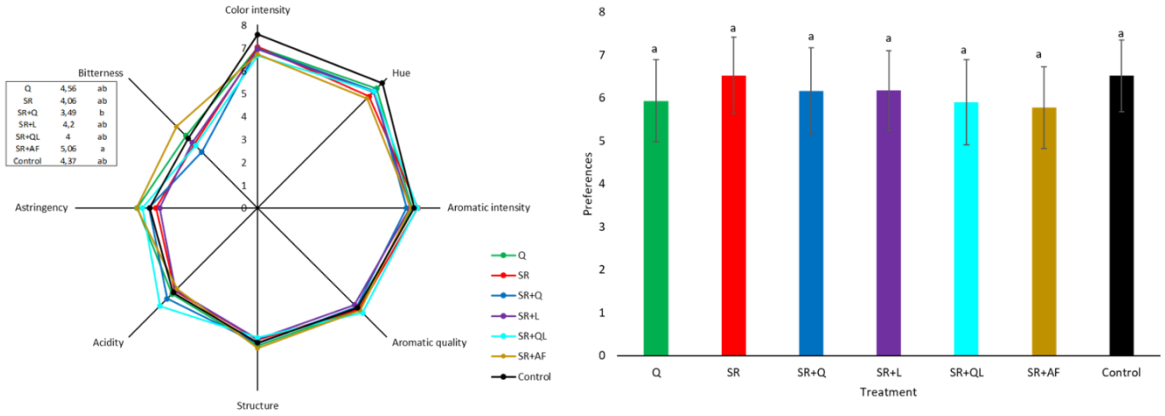

Figure 4. Sensory analysis of wines six months after bottling according to treatment

Means with different letters indicate significant differences according to Tukey's test ($\alpha \leq 0.05$). Treatments are chitosan (Q), reduced sulfur dioxide (SR), sulfur dioxide and chitosan (SR+Q), reduced sulfur dioxide and lysozyme (SR+L), reduced sulfur dioxide and chitosan with lysozyme (SR+QL), reduced sulfur dioxide and fumaric acid (SR+AF).

3.2 Experiment 2: Minimum intervention winemaking

3.2.1 Musts fermentation kinetics

Alcoholic fermentation took place within the temperature range defined for all treatments, without fermentation stops (**Figure 5**). No significant differences were recorded between treatments in the temperature and density of the musts.

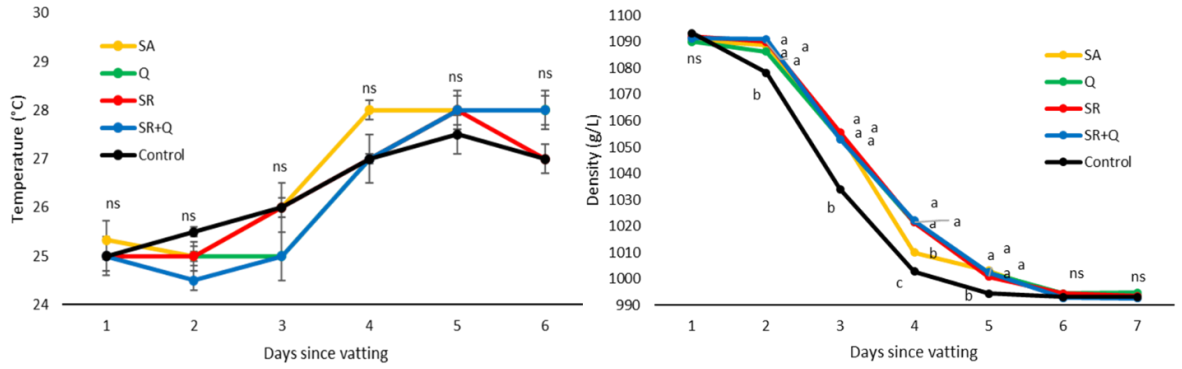

Figure 5. Temperature and density of the musts during alcoholic fermentation according to treatment

Means with different letters indicate significant differences by Tukey test ($\alpha \leq 0.05$) while ns indicates the absence of significant differences. The treatments are SA (no additives), Q (chitosan), SR (reduced sulfur dioxide), SR+Q (reduced sulfur dioxide and chitosan), and the Control.

3.2.2 Wine general composition

The minimal intervention winemaking alternatives modified the elemental composition of the wines (**Figure 6**). The SR+Q wines presented the highest values of ethanol content, which were statistically similar to the Control, while the other treatments presented lower values, with no significant differences between them.

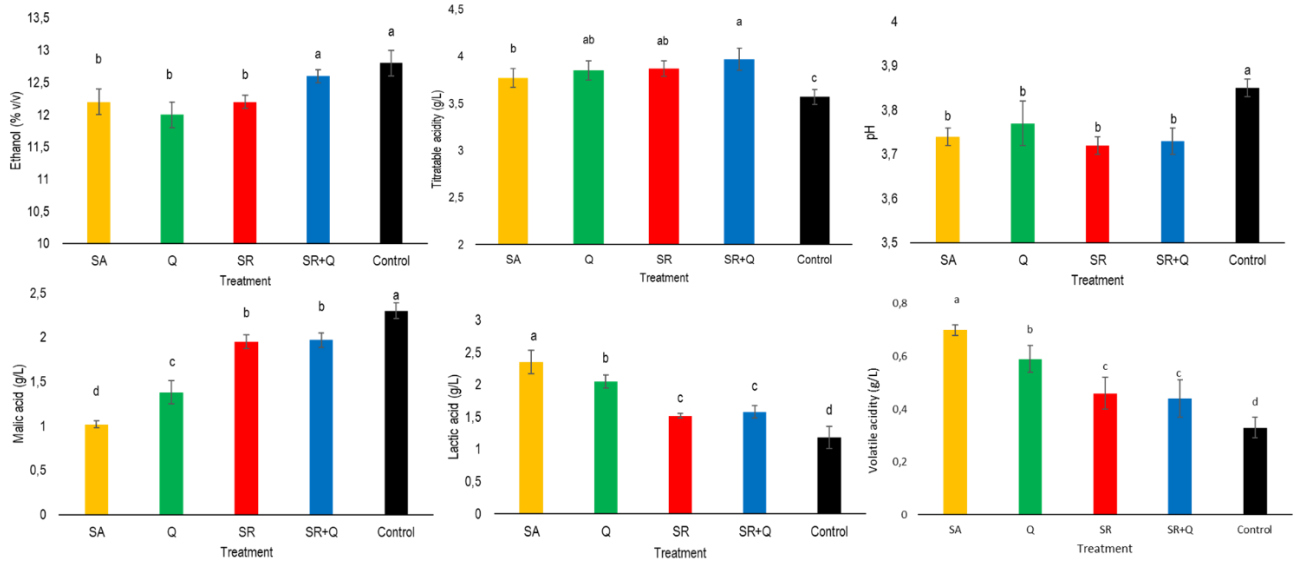

Figure 6. Composition of the wines fifteen days after bottling according to treatment

Means with different letters indicate significant differences according to the Tukey test (α ≤ 0.05). The treatments are SA (no additives), Q (chitosan), SR (reduced sulfur dioxide), SR+Q (reduced sulfur dioxide and chitosan), and the Control.

The minimal intervention wines had significantly higher titratable acidity than the Control wines. The SR+Q wines had the highest values compared to other treatments, while the SA wines had lower titratable acidity. Consequently, the minimal intervention wines had pH values significantly lower than the Control wines, but similar to each other.

Control wines had higher malic acid and lower lactic acid content, significantly differentiating from the other treatments (α ≤ 0.05). Among the minimal intervention alternatives, the SR and SR+Q wines had higher malic acid and lower lactic acid contents than the Q and SA wines. The SA treatment showed lower malic acid and higher lactic acid contents, while the Q wines had intermediate values.

The minimal intervention wines volatile acidity was higher than the Control wines. Among them, SA wines exhibited the highest value, while SR and SR+Q wines had the lowest.

3.2.3 Evolution of yeasts and lactic acid bacteria populations

A high yeast population was observed three days after vatting, which remained constant until devatting, with no significant differences between treatments (**Figure 7**). Fifteen days after devatting, the yeast population decreased in all treatments. Fifteen days after bottling, the yeast population remained low, with significant differences between treatments. The Q wines had a higher yeast count than the others, while SR+Q and Control wines had the lowest counts, and the SA and SR wines had intermediate counts.

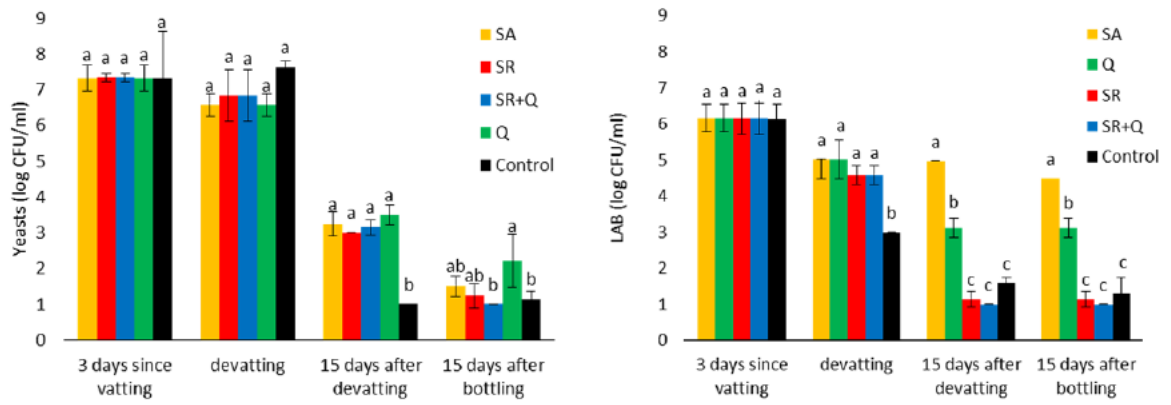

Figure 7. Yeast and lactic acid bacteria count according to treatment

Means with different letters indicate significant differences by Tukey test ($\alpha \leq 0.05$). The treatments are SA (no additives), Q (chitosan), SR (reduced sulfur dioxide), SR+Q (reduced sulfur dioxide and chitosan), and the Control.

Regarding the dynamics of the lactic acid bacteria population throughout the winemaking process, a high bacteria count was detected three days after vatting, which decreased at devatting, with the lowest counts found in the Control wines. Fifteen days after devatting, the population of lactic bacteria continued to decrease, except for the SA wines, which maintained a high bacteria count. The SR, SR+Q, and Control wines had the lowest values, while the Q wines had intermediate values. These differences continued until fifteen days after bottling.

Acetic acid bacteria were detected in the sampling conducted three days after inoculation, with an average population of approximately 4.5 log CFU/mL (data not presented). No significant differences were observed among the fermentations corresponding to the different treatments. In subsequent samplings, the population of acetic acid bacteria remained below the detection limit of the method (≤1 log CFU/mL) across all treatments.

3.2.4 Color and phenolic composition of wines

The color intensity of SR+Q wines (16.3 ± 1.1) was significantly higher than the other treatments, while SA wines showed the lowest values (11.5 ± 0.3) (**Table 2**). The color intensity values of Q (12.7 ± 1.1), SR (14.8 ± 1.0), and Control (13.9 ± 0.5) wines were intermediate. Q wines showed the highest hue (0.72 ± 0.03) compared to the other treatments. While SR (0.66 ± 0.01), SR+Q (0.64 ± 0.01), and Control (0.65 ± 0.01) wines showed the lowest, with no significant differences between them. SA wines (0.68 ± 0.01) showed intermediate values.

Table 2. Color and phenolic composition of wines fifteen days after bottling according to treatment

Analitical parameter	Treatment				
	SA	Q	SR	SR+Q	Control
Color intensity	11,5 ± 0,3 d	12,7 ± 1,1 cd	14,8 ± 1,0 b	16,3 ± 1,1 a	13,9 ± 0,5 bc
Hue	0,68 ± 0,01 b	0,72 ± 0,03 a	0,66 ± 0,01 c	0,64 ± 0,01 c	0,65 ± 0,01 c
L*	46,7 ± 0,8 a	44,1 ± 3,2 ab	38,9 ± 2,3 cd	35,8 ± 2,6 d	41,2 ± 1,2 bc
a*	45,5 ± 0,4 c	44,5 ± 0,7 d	48,7 ± 0,3 b	50,5 ± 0,6 a	49,1 ± 0,3 b
b*	3,5 ± 0,2 b	8,8 ± 1,8 a	8,8 ± 0,7 a	9,8 ± 0,6 a	9,5 ± 0,8 a
Total phenolic compounds (mg/L)	1199 ± 21 c	1241 ± 100 c	1306 ± 253 bc	1701 ± 48 a	1519 ± 161 ab
Anthocyanins (mg/L)	351 ± 11 a	271 ± 42 b	360 ± 26 a	389 ± 52 a	379 ± 15 a
Tannins (mg/L)	363 ± 54 b	407 ± 64 b	402 ± 30 b	620 ± 41 a	672 ± 44 a

Means with different letters indicate significant differences by Tukey test ($\alpha \leq 0.05$). The treatments are SA (no additives), Q (chitosan), SR (reduced sulfur dioxide), SR+Q (reduced sulfur dioxide and chitosan), and the Control.

The luminosity of SA wines was higher (46.7 ± 0.8), whereas SR+Q (35.8 ± 2.6) was the darkest. Q (44.1 ± 3.2), Control (41.2 ± 1.2), and SR (38.9 ± 2.3) wines showed intermediate values. On the a* axis, SA (45.5 ± 0.4) and Q (44.5 ± 0.7) wines showed the lowest values, with significant differences between them. Meanwhile, wines of Control (49.1 ± 0.3) and SR (48.7 ± 0.3) showed intermediate values. The SR+Q wines (50.5 ± 0.6) had the highest red component. On the b* axis, the SA treatment had the lowest value (3.5 ± 0.2), while the other treatments had higher values.

Total phenolic compounds showed the highest concentration in the SR+Q wines (1701 ± 48), followed by Control (1519 ± 161) and SR (1306 ± 253). The lowest values were detected in the Q (1241 ± 100) and SA (1199 ± 21) wines. On the other hand, the concentration of anthocyanins was lower in Q wines (271 ± 42), while treatments SR+Q (389 ± 52), Control (379 ± 15), SR (360 ± 26), and SA (351 ± 11) had higher and similar concentrations of anthocyanins. The tannin content of the Control (672 ± 44) and SR+Q (620 ± 41) wines were similar to each other, while the Q (407 ± 64), SR (402 ± 30) and SA (363 ± 54) wines presented the lowest values.

3.2.5 Sensorial analysis of wines

Of the sensory attributes evaluated (**Figure 8**), significant differences were recorded in the parameters of color intensity, hue, astringency, and bitterness. The SR+Q wines had the highest values of color intensity, being significantly different from the Q wines, which had the lowest values. The rest of the treatments showed intermediate values. Regarding hue, the Control wines had higher values, significantly different from the Q wines. Additionally, the latter was perceived as the most astringent, while the SR+Q wines were perceived as the most bitter. The SR and SR+Q wines generally had sensory characteristics similar to Control. Additionally, no differences were recorded in the consumers' preference for the wines from the different treatments.

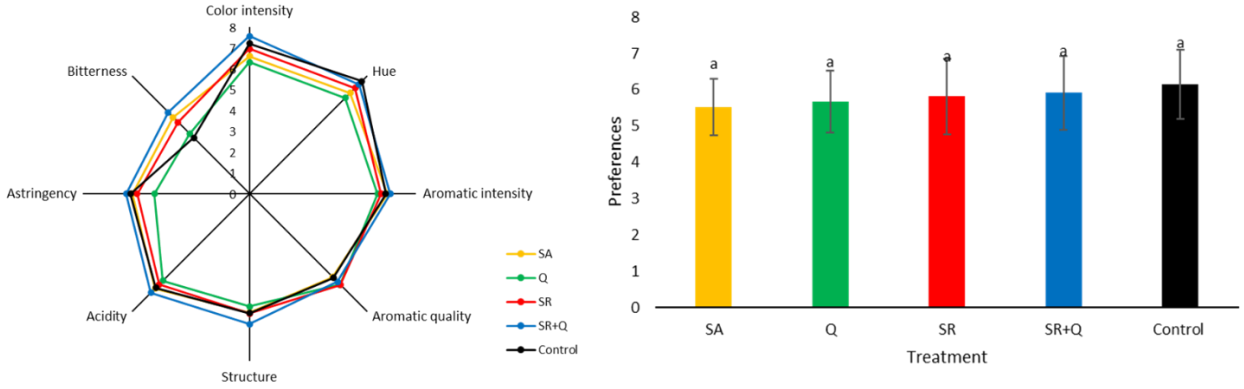

Figure 8. Sensory analysis of the wines six months after bottling according to treatment

The treatments are SA (no additives), Q (chitosan), SR (reduced sulfur dioxide), SR+Q (reduced sulfur dioxide and chitosan), and the Control.

4. Discussion

4.1 Experiment 1: Replacement or reduction of added sulfite contents in traditional winemaking

The alternatives used to replace or reduce added sulfite content did not affect fermentation (**Figure 1**) since these treatments were applied fractionally at the time of devatting (once alcoholic fermentation had finished) and before bottling. Only sulfur dioxide was added to the Control wines at vatting. As it is known, the pre-fermentation addition of sulfur dioxide aims to reduce microbial competition, allowing the subsequent inocula-

tion of selected yeasts responsible for alcoholic fermentation. In addition, sulfur dioxide prevents the risk of oxidation of the must by inhibiting polyphenol oxidase enzymes from the grape or altered harvests[2]. The results obtained in this work show that, regardless of whether sulfur dioxide was added to the must, the fermentation kinetics was not affected, suggesting that the inoculated yeasts rapidly colonized the must and carried out alcoholic fermentation continuously and without interruption.

Although significant differences were found in the ethanol content between treatments for replacing or reducing added sulfite content wines, none differed from the Control wine (**Figure 2**). It should be noted that the differences observed cannot be directly attributed to the treatments since these were made once alcoholic fermentation had finished and before bottling, except for the Control.

The Q wines did not show differences in titratable acidity and pH compared to the Control wines. However, they had lower malic acid and higher lactic acid content. According to studies conducted by Bağder Elmaci and others[10], chitosan has proven to be effective in controlling lactic acid bacteria, particularly *Lactobacillus hilgardii* and *Oenococcus oeni*, at 0.2 mg/mL. The doses used in this experiment (0.1 mg/mL) could not eliminate the lactic acid bacteria population in the wine but kept it at low levels. Even so, the Q wines had a higher population of lactic bacteria than the Control wines fifteen days after bottling.

Regarding the population of yeasts, the results obtained are consistent with those reported by Gómez-Rivas and others[28], who state that chitosan does not affect the *Saccharomyces cerevisiae* population even at high concentrations. SR wines had a lower pH than Control wines, which was explained by a higher titratable acidity. It was observed that wines from this treatment had lower malic acid and higher lactic acid content than Control wines. Free sulfur dioxide in wine is the fraction that has antimicrobial and antioxidant activity[29]. According to Guerrero and Cantos-Villar[30], its use at low doses (30-50 mg/L of free sulfur dioxide) may be ineffective against some microorganisms. The dose of sulfur dioxide used in the SR wines was able to reduce the lactic acid bacteria population. However, Q and SR+AF wines were the least efficient in maintaining the yeast population at similar levels to those of the Control.

The SR+Q treatment showed lower pH levels than the Control, with similar titratable acidity values. However, malic acid contents were lower and lactic acid contents were higher than the Control wines. This treatment combined the effects of reduced doses of sulfur dioxide and chitosan. This combination was more efficient in reducing the lactic acid bacteria population, with values similar to the Control. This was not observed in treatments with the addition of Q and SR separately. However, its efficiency in inhibiting the metabolic activity of yeasts fifteen days after bottling was like the Q treatment.

SR+L wines did not show differences in titratable acidity and pH compared to Control wines. The combination reduced the population of lactic bacteria to levels similar to those of the Control. However, the malic acid and lactic acid contents indicate the development of partial malolactic fermentation. The response of lactic acid bacteria to lysozyme is highly strain-dependent, with *Lactobacillus* species being more resistant than *Oenococcus* or *Pediococcus*[31][32]. Furthermore, it has been reported that lysozyme is less effective in red wines since its antimicrobial activity may be limited by low molecular weight proanthocyanidins[33]. In these trials, no lactic acid bacteria were added, so there was no control over the species that carried out this fermentation. Although Roldan and others[34] suggest that lysozyme can affect yeast growth, the yeast population in SR+L wines was higher than in Control wines fifteen days after bottling.

SR+QL wines did not present significant differences in titratable acidity and pH compared to Control wines. In addition, they presented levels of malic and lactic acid similar to those obtained in SR+L wines. The combination of three additives in the doses used in this experiment allowed the yeast population to be reduced fifteen days after bottling to values similar to those of Control and SR wines. Regarding the effect on lactic acid bacte-

ria, the wines presented a population similar to that obtained in the SR+Q, SR+L, and Control wines fifteen days after bottling.

The SR+AF wines presented a higher pH than the Control wines without differing on titratable acidity. Although several investigations suggest that fumaric acid added to wine can have an acidifying effect[14], the doses used in this experiment did not support this. The higher pH of these wines can be explained by their lower malic acid content and higher lactic acid content, which may also be associated with a smaller reduction in the lactic acid bacteria count compared to the other treatments, except for the SR wines, which showed similar counts to the SR+AF wines (**Figure 3**), results that do not agree with those reported by Morata and others[35].

The wines produced in this experiment presented volatile acidity content within the legal limits allowed (maximum 1 g/L). The alternative treatments to replace or reduce the sulfite content presented higher volatile acidity than the Control. In particular, the SR+QL and SR+AF wines presented the highest values. This may be due to an inefficient antimicrobial control, leading to the undesirable growth of yeasts and bacteria, mainly heterolactic acid bacteria, which under certain conditions can metabolize ethanol, residual sugars, or amino acids, producing acetic acid[36].

Although the color was analyzed using different methods, there is a relationship between color intensity and luminosity, where the wines that present greater color intensity are less luminous (**Table 1**). The SR+AF wines were the only ones presenting lower color intensity and greater luminosity and hue than the Control wines. This can be explained by the high pH presented in these wines, causing a shift of the different forms of anthocyanins towards colorless forms[37][38]. According to Payan[39], the addition of fumaric acid to red wines can affect their phenolic composition and, consequently, their color and organoleptic properties. The remaining treatments presented chromatic characteristics equal to or better than the Control wines, which can be observed in the color intensity and luminosity. The Q, SR, and SR+Q wines presented a more significant red component than the other treatments of sulfite content reduction, which can be explained by a higher concentration of anthocyanins, total polyphenols, anthocyanins, and tannins, which agrees with Bağder Elmaci and others[10]. The SR+L and SR+QL wines presented a lower concentration of total phenolic compounds, anthocyanins, and tannins than the other treatments, except for the concentration of tannins for the SR+L wine. According to what was reported by Bartowsky and others[40], the use of lysozyme in the production of red wines can cause undesirable side effects such as loss of color and increased turbidity. In sensorial analysis, no significant differences were recorded between the different parameters evaluated, except for the SR+AF wines, which were the most bitter. Differences between the SR+AF and Control wines were insignificant.

Meanwhile, the SR+Q wines highlighted as the least bitter wine (**Figure 4**). The results suggest that the analytical differences observed between different treatments were not perceived by assessors. Additionally, no differences were recorded in the overall judgment due to the other treatments evaluated.

4.2 Experiment 2: Minimum intervention winemaking

The minimal intervention wines' fermentation kinetics differed from those recorded in the Control wines (**Figure 5**). Alcoholic fermentation with native yeasts is slower than with selected yeasts conceivably due to the diversity of autochthonous yeast strains, competition between microorganisms, and their lower adaptation to the controlled conditions of the must[41]. The selected yeasts, such as *Saccharomyces cerevisiae*, are optimized to ferment efficiently, tolerate environmental stress better, and convert sugars into alcohol more quickly[42]. In the SR and SR+Q wines, sulfur dioxide is added at three stages: at the time of vatting, at the time of devatting, and before bottling. The initial dose at the time of vatting can delay alcoholic fermentation with native yeasts due to their antimicrobial capacity. Furthermore, native yeasts are more sensitive to sulfur dioxide than the selected strains, which may inhibit their metabolic activity, slowing down their growth and fermentation

rate[43][44]. Even so, the fermentation kinetics of these wines did not differ from those of the SA and Q wines, with alcoholic fermentation ending seven days after vatting. The temperature of the musts during fermentation was kept within the established limits.

The ethanol content of the minimal intervention wines was significantly lower than the Control wines, except for the SR+Q, where no differences were observed (**Figure 6**). These results can be explained by the diversity and variability of the native yeasts with respect to their fermentation efficiency. Some autochthonous yeasts have a lower capacity to convert sugars into ethanol due to their low tolerance to ethanol or reduced fermentative activity[45]. This can result in incomplete fermentation, leaving residual sugars or reducing the total amount of ethanol produced. In addition, competition between different species of native yeasts and other microorganisms can slow down or stop fermentation before reaching the ethanol levels achieved with yeast's selected strains[41].

The minimal intervention wines showed lower pH and malic acid content, as well as higher titratable acidity and lactic acid content compared to the Control wines. The yeast population decreased in all treatments, even in SA wines. This decrease may be due to both the effect of the treatments and the accumulation of ethanol in the medium, which can be toxic to yeasts, inhibiting their metabolic activity. In addition, the decrease in available nutrients, such as sugars and nitrogen compounds, limits their capacity for growth and survival. The environment also becomes less favorable due to increased osmotic pressure, oxygen reduction, and pH changes[41][42]. It is worth noting that Q wines had a significantly higher yeast population than Control wines fifteen days after bottling (**Figure 7**).

With respect to the lactic acid bacteria population, in the SA wines the counts did not decrease, presumably by the fact that lactic bacteria, such as *Oenococcus oeni*, grow better in media with moderate pH and in the absence of sulfur, since they do not face the inhibitory effects that generally control their proliferation[46]. Furthermore, SA wines show that these microorganisms can tolerate ethanol better and survive in low-nutrient media, metabolizing compounds such as malic acid and producing lactic acid[2]. Adding chitosan was less effective for controlling lactic acid bacteria, which explains the differences in malic acid and lactic acid contents. The susceptibility of lactic acid bacteria to chitosan shows differences between species. Generally, *Oenococcus oeni*, *Pediococcus* spp. and some *Lactobacillus* species such as *Lactobacillus hilgardii* are sensitive to chitosan[10]. SR and SR+Q wines were the treatments that presented malic acid and lactic acid contents most similar to the Control wines, probably because they showed the same composition on lactic acid bacteria population. The minimal intervention wines' volatile acidity was higher than the Control wines. In particular, the highest values for this parameter were recorded in SA wines, followed by Q wines. The absence of control over the microbial population or poor control, observed in the case of the use of chitosan, increased the volatile acidity of the wine, deteriorating its sensory characteristics. The specific effects of the additives used were previously discussed in Experiment 1, where the results obtained are comparable to those observed in minimal intervention winemaking.

The total polyphenol, anthocyanin, and tannin content of the SA wines were lower than Control wines, negatively affecting their color (**Table 2**). The differences in extraction protocols and stabilization efficiency can explain these results. The selected yeasts are optimized for faster fermentation, which enhances the extraction of polyphenols, anthocyanins, and tannins from the skins during maceration, as well as the production of enzymes that aid in the release of these compounds and the wine color stabilization. In contrast, native yeasts, being more diverse and less efficient, might generate slower and less effective fermentations in terms of extraction and stabilization of these compounds[47][48]. The total polyphenol, anthocyanin, and tannin contents of the SR+Q wines did not differ significantly from the Control, suggesting that the combination of both additives does not modify the phenolic composition of the wine.

The Q and SR wines presented lower values without differentiating between them, except for the anthocyanin content. Consequently, the SR+Q wines presented greater color intensity and lower luminosity, with a higher proportion of red. The SR wines presented intermediate chromatic characteristics between the SR+Q and the Control wines, while Q wines presented lower color intensity, explained by a reduced concentration of anthocyanins. Chitosan is a polysaccharide that can interact with anthocyanins, causing precipitation and affecting color intensity[49]. This effect was only observed when chitosan was used as the only additive.

Consequently, the results indicate that the combination of reduced doses of sulfur dioxide and chitosan promotes a positive interaction that enhances the effectiveness of both additives. The differences in wine composition across the different treatments determined the sensory perception of the wines, where the SR+Q wines presented greater color intensity, astringency, and bitterness, closely resembling the Control wines in most sensory attributes, except for bitterness, where they presented a significantly higher value. On the other hand, the Q wines presented lower color intensity, tonality, and astringency, while the SA and SR wines showed intermediate ratings. No significant differences were found between Control and treatments' wines at the level of global judgment made by the evaluators.

5. Conclusions

The experiments on replacing or reducing added sulfites and minimal intervention winemaking indicate that the tested alternatives can influence both the fermentation process and the final characteristics of the wine.

The replacement or reduction of added sulfite, along with the use of chitosan and lysozyme, showed better control over the lactic acid bacteria population. However, this was not reflected in the contents of malic and lactic acids. Treatments that combined reduced sulfites and chitosan did not affect the color and phenolic composition of the wine, maintained the acidity, and effectively controlled lactic acid bacteria. This suggests that this combination could be the most suitable approach for producing Tannat red wines with lower sulfite content, without compromising their sensory quality. In this research, the combination of sulfur dioxide with fumaric acid did not show the effects reported by other authors, so further investigation is needed to assess the effectiveness of this treatment.

The wines produced by minimal intervention were characterized by lower ethanol content and higher volatile acidity. The combination of reduced sulfites and chitosan allowed the production of wines with a phenolic composition similar to the Control wines, being the most promising alternative for producing Tannat red wines with minimal intervention.

Ongoing research aims to identify the yeast and bacteria species present during fermentation and conservation of wines produced with these treatments. This will enable the optimization of winemaking, provide a deeper understanding of the results obtained, and support the transfer of these techniques to an industrial scale.

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Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

Diego Piccardo: Conceptualization; Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing-original draft, Writing-review and editing

Marcela González: Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing-review and editing

Guzmán Favre: Investigation, Methodology, Resources, Validation, Writing-review and editing

Alejandro Cammarota: Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing

Florencia Pereyra: Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing

Jorge Olivera: Formal analysis, Investigation, Methodology

Gustavo González-Neves: Investigation, Methodology, Resources, Validation, Writing-review and editing

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