

Article

Impact of the Thermovinification Practice Combined with the Use of Autochthonous Yeasts on the Fermentation Kinetics of Red Wines

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Abstract

Thermovinification has emerged as a rising alternative method in red wine production, gaining popularity among winemakers. The use of autochthonous yeasts isolated from grapes is also an interesting practice that contributes to the creation of wine with a distinctive regional character. This research investigated how combining thermovinification with autochthonous yeast strains influences the fermentation dynamics of Syrah wine. Six treatments were conducted, combining the use of commercial and two autochthonous yeasts with traditional vinification (7-day maceration) and thermovinification (65 °C for 2 h) processes. Sugars and alcohols were quantified during alcoholic fermentation by high-performance liquid chromatography with refractive index detection. Cell viability and kinetic parameters, such as ethanol formation rate and sugar consumption, were also evaluated. The Syrah wine's composition was characterized by classical wine analyses (OIV procedures). The results showed that cell viability was unaffected by thermovinification. Thermovinification associated with autochthonous yeasts improved the efficiency of alcoholic fermentation. Thermovinified wines also yielded a higher alcohol content (13.9%). Future studies should investigate how thermovinification associated with autochthonous yeasts affects the metabolomic and flavoromic properties of Syrah wine and product acceptability.

Keywords: tropical wine; thermomaceration; microbial *terroir*; non-*Saccharomyces* yeasts; *Hanseniaspora opuntiae*



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1. Introduction

Thermovinification (TV) is a nonconventional practice that involves the extraction of grape compounds at elevated temperatures (approximately 65 °C), with a short maceration period (approximately 2 h), followed by alcoholic fermentation (AF), typically in the

absence of grape skins [1]. This technique is steadily gaining traction in the making of red wines [2].

The application of advanced winemaking techniques, such as thermovinification with pre-fermentative heating maceration, has resulted in significantly higher concentrations of phenolic compounds in Teran red wine compared to those obtained using prolonged maceration and pre-fermentative cooling maceration [3]. A recent study demonstrated that applying this technique at 65 °C increases the total phenolic compounds, antioxidant potential, and color intensity in Syrah red wines, without causing the degradation of monomeric anthocyanins observed at 75 °C. The study recommended 65 °C for 2 h as the optimal condition for thermovinification [4].

The purpose of TV is to enhance the enrichment of red wine must, particularly by increasing the concentration of phenolic compounds, reducing the microbial load, and promoting the degradation of oxidative enzymes such as polyphenol oxidase [5]. Another great difference of this practice is that the yeast strain used can significantly affect the aroma and flavor of thermovinified wines because of the prevalence of fermentative aromas, and a greater degradation of primary volatile compounds present in the grape berries has been reported with this process compared to that in conventional maceration [6].

Currently, the inoculation of mixed cultures of autochthonous yeasts, including the incorporation of different strains of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts, has been tested with the aim of diversifying the aroma and flavor of wines. These strains also impart sensory characteristics similar to those derived from wild yeasts during spontaneous fermentation. Moreover, they offer the advantage of reducing the risks of contamination or stuck/arrested fermentation [7].

The use of autochthonous yeasts well adapted to the vineyard microenvironment therefore represents a promising strategy for promoting wines with regional identity while also favoring the expression of differentiated fermentative characteristics. Thus, investigating the behavior of these yeasts under different winemaking conditions can provide relevant insights for their targeted use, expanding the understanding of their technological potential and their contribution to the final quality of wines [8,9].

The predominant microbial species during alcoholic fermentation influence the physicochemical properties and sensory profile of the wine [10,11]. Thus, prospecting for autochthonous yeasts that are genuinely derived from the territory and possess enological aptitude constitutes a relevant alternative for the wine industry. This study aimed to identify autochthonous yeast strains with efficient fermentative performance, high resistance to sulfur dioxide, and nonproduction of acetic acid, among other desirable technological characteristics such as the production of distinctive aromas and flavors and higher concentrations of bioactive compounds [12].

However, past studies on the impact of different alternative winemaking practices on the AF kinetics of yeasts are limited. Through this study, we aimed to evaluate, for the first time, the effect of inoculating autochthonous yeast strains isolated from the grapes from Sub-middle São Francisco Valley on the AF kinetics of red wines prepared from the cultivar Syrah in the region associated with the use of TV.

2. Materials and Methods

2.1. Raw Material

A total of 487 kg of Syrah grapes (*Vitis vinifera* L.) was sourced from an experimental vineyard belonging to Embrapa Semiárida, situated in the Sub-middle São Francisco Valley (coordinates: 09°09' S, 40°22' W; altitude: 365.5 m), in Petrolina, Pernambuco State, Brazil. According to the multicriteria climatic classification system for grape-growing (GMCC) regions worldwide [13], the area falls within the BswH category, denoting a hot semi-arid

environment region, with an average yearly temperature of 26 °C, around 64% relative humidity, and an annual precipitation of 549 mm. The harvest occurred in November 2020, and the grapes presented 22.7° Brix of soluble solids, a pH of 3.64, and a total acidity of 0.6%. The vines were trained using a vertical shoot positioning system, grafted onto Paulsen 1103 rootstock, and maintained through drip irrigation.

2.2. Yeasts

The autochthonous yeasts *Hanseniaspora opuntiae* 4VSFI10 (BRM 044661) and *S. cerevisiae* 45VSFCS10 (BRM 43894) were isolated from grapes grown in the Submédio São Francisco Valley region and provided by the Collection of Microorganisms of Agroindustrial Interest (CMIA) located at Embrapa Uva e Vinho (Bento Gonçalves, RS, Brazil). The yeast strains were identified through mass spectrometry using the matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) technique and molecular biology, employing polymerase chain reaction (PCR) combined with restriction fragment length polymorphism (PCR-RFLP) [14].

The isolated yeast strains were cryopreserved in an ultrafreezer at −80 °C. For reactivation, under a laminar flow hood, 20 µL of the cryopreserved content was transferred into a test tube containing YEPD culture medium (10 g/L yeast extract; 20 g/L peptone; 20 g/L dextrose). The tube was incubated in an oven for 72 h to assess growth. Subsequently, the cells were transferred to test tubes containing the same YEPD medium (pH 5.5) so as to prepare the inoculate, which was then incubated for 24 h at 25 °C with agitation at 100 rpm until it reached a cell concentration of 10^8 cells/mL [15]. The autochthonous yeasts were then inoculated simultaneously, but in different concentrations: *S. cerevisiae* at 1×10^5 CFU/mL and *H. opuntiae* at 1×10^6 CFU/mL.

For the control treatment, 200 mg/L of the commercial yeast culture *S. cerevisiae* var. *bayanus* (Maurivin PDM®; Mauri Yeast Pty Ltd., Camellia, NSW, Australia) was used as recommended by the manufacturer.

2.3. Winemaking

In this research, red wine was produced using two methods: TV (65 °C for 2 h) and traditional winemaking to produce the control wines (TW) (control). Traditional winemaking involves simultaneous maceration and AF for 7 days. In both these processes, the different yeast strains mentioned in Section 2.2 were inoculated, as described in Figure 1. Each treatment was conducted in triplicate, resulting in a total of 18 microvinifications.

Syrah grapes were destemmed, lightly crushed, and treated with 0.10 g/L of potassium metabisulfite (Amazon Group Ltd.a., Bento Gonçalves, RS, Brazil) and 0.03 g/L of pectinolytic enzyme (Pectozim Rouge Gr®, Ever Brasil, Garibaldi, RS, Brazil). The grapes were distributed in two batches: TV and TW.

The TV process was conducted in a stainless-steel vat to extract the grape metabolites (65 °C for 2 h) as described by Silva et al. [4]. After extraction, the must was pressed under a hydraulic press, cooled, racked, and then inoculated with yeast to proceed with the AF. In the TW wines, the grapes were initially destemmed and crushed, the the simultaneous fermentation and maceration stage occurred over a period of 7 days at 24 ± 1 °C. After 7 days, the wine was racked (separation of solids), and the AF continued for another 7 days.

The AF lasted 14 days in a temperature-controlled and dark room at 24 ± 1 °C, with the wine must stored in 20-L glass carboys sealed with glass airlock valves. The end of AF was determined by monitoring the density and total reducing sugar content (constant values of ≤ 0.994 g/mL and ≤ 4 g/L, respectively).

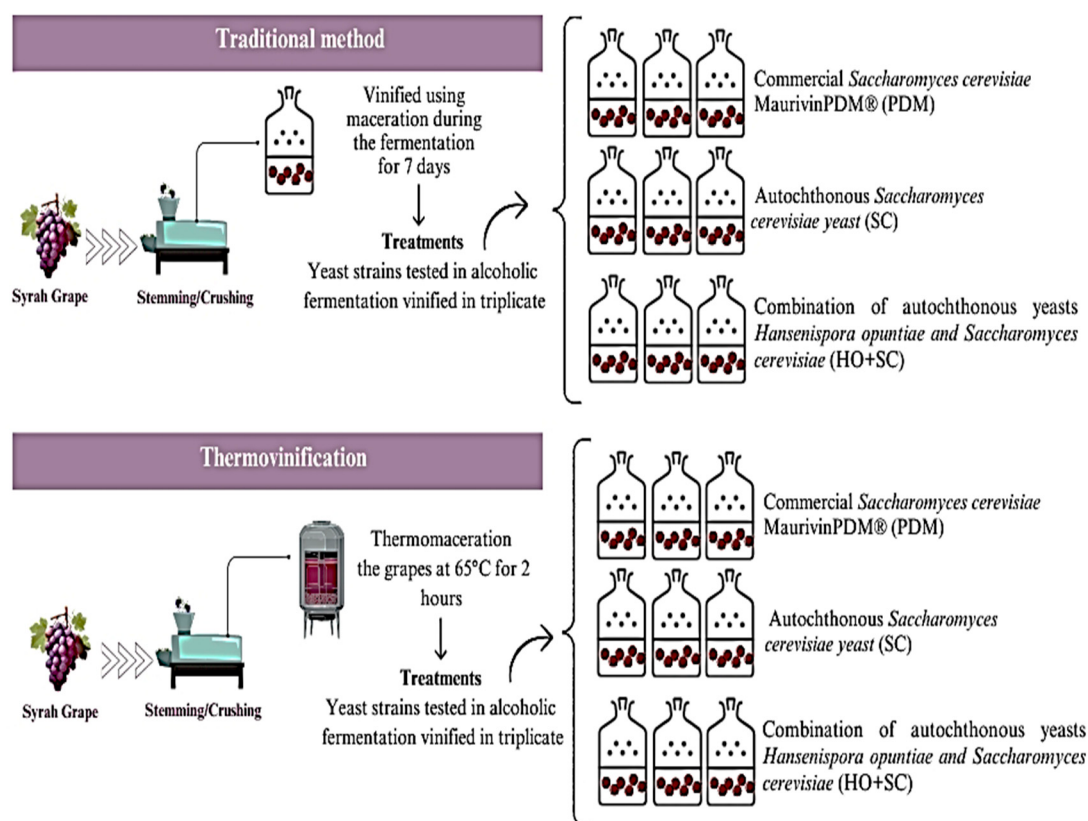


Figure 1. Schematic overview of the enological treatments conducted for Syrah wine production.

Following alcoholic fermentation (AF), malolactic fermentation (MLF) was carried out spontaneously, without the addition of starter cultures, at 18 ± 1 °C until the conversion of malic acid to lactic acid was complete, as confirmed by paper chromatography. Upon completion of MLF, the free SO_2 content was adjusted to 50 mg/L and the wines were transferred to a cold chamber (0 ± 0.5 °C) for tartaric stabilization at -4 °C for 10 days. Subsequently, the wines were bottled in 750 mL dark glass bottles, with nitrogen gas (N_2) used to fill the headspace in order to minimize oxidation. To await further analysis, the bottles were stored horizontally in a climate-controlled cellar at 18 ± 1 °C and approximately 60% relative humidity.

2.4. Fermentation Kinetics

2.4.1. Simultaneous Determination of the Sugars and Alcohols

During AF, the levels of sugars and alcohols were simultaneously determined by high-performance liquid chromatography using an Agilent 1260 Infinity LC System (Agilent Technologies, Santa Clara, CA, USA) and a refractive index detector (RID) (model G1362A) following the method developed previously [16] with some modifications [17]. Briefly, 5 mL aliquots were taken from each batch of the six treatments at 2- and 3-day intervals until the end of AF (14 days) and were prediluted with ultrapure water (1:1), filtered through a 0.45- μm nylon membrane (Millex-HA, Millipore, Bedford, MA, USA) for subsequent injection into the HPLC-RID (10 μL). An ion-exchange column (300 $\times$ 7.7 mm Agilent Hi-Plex H) with 8.0- μm internal particles was used for metabolite separation, protected by a PL Hi-Plex H precolumn (5 $\times$ 3 mm) (Agilent Technologies). The separation temperature was set to 60 °C, with a flow rate of 0.8 mL/min and a total run time of 20 min. The mobile phase was composed of a 0.004 mol/L H_2SO_4 (Merck, Darmstadt, Germany) solution in ultrapure water (Martes Scientific Purification System, São Paulo, Brazil). Compound detection was performed by comparing calibration curves obtained with the standards of

ethanol and glycerol (Merck), glucose, fructose, maltose, and rhamnose (Sigma-Aldrich, St. Louis, MO, USA). The methodology was previously validated for linearity, recovery, and the detection and quantification limits as cited by Viana et al. [18].

2.4.2. Monitoring of the Yeast Cell Growth

During AF, yeast viability assessments (exclusion tests) were performed by cell counting in a Neubauer chamber using a light microscope with a total magnification of $400\times$ (Nikon, Eclipse TS 100) (Minato-ku, Minato, Tokyo). Cell viability was determined by staining (1/1 *v/v*) with a 0.1% methylene blue solution [19]. The number of unstained cells divided by the total number of cells $\times 100$ represented the percentage (%) of viable cells. For this analysis, 5-mL aliquots were removed daily from each batch of the six treatments until the end of AF (14 days).

2.4.3. Kinetic Parameters, Productivity, and Efficiency of the Fermentation Process

The kinetic parameters were determined from the data obtained (i.e., initial and final concentrations of sugar and alcohol in the must and wine after AF, respectively) to monitor the fermentation kinetics using the calculations proposed elsewhere [20,21]. To process the data from the reaction kinetics, calculations were made for the reaction rate with respect to ethanol formation (V_p —Equation (1)), the reaction rate measured with respect to sugar consumption (V_s —Equation (2)), the relationship between product and substrate (sugar) concentrations (Y_{PS} —Equation (3)), productivity expressed in $\text{g L}^{-1}\text{h}^{-1}$ (Pr —Equation (4)), and the efficiency of the AF process (expressed as %) (R —Equation (5)), which indicates the proportion of reducing sugars that were effectively transformed into ethanol by yeast during fermentation.

$$V_p = \frac{Pf - P0}{\Delta t} \quad (1)$$

where Pf and $P0$ correspond to the final and initial ethanol concentrations, respectively, and Δt is the time variation ($t_f - t_0$).

$$V_s = -\frac{Sf - S0}{\Delta t} \quad (2)$$

where Sf and $S0$ correspond to the final and initial sugar concentrations, respectively, and Δt is the time variation ($t_f - t_0$).

$$Y_{PS} = \frac{Pf - P0}{S0 - Sf} \quad (3)$$

$$Pr = \frac{P_{exp}}{t} \quad (4)$$

where P_{exp} is the experimentally measured ethanol concentration value and t is the total fermentation process time.

$$R = \left(\frac{P_{exp}}{P_{teo}} \right) \times 100 \quad (5)$$

where P_{teo} is the value of the theoretical ethanol concentration, calculated using a conversion factor of 0.505 ($^{\circ}\text{Brix} \times 0.505$) [22].

2.5. Physicochemical Composition of the Wines

Physicochemical analyses of wines were performed according to the International Organization of Vine and Wine standards [23]. The pH value was determined using a Tec-3MP pH meter (TECNAL[®], Piracicaba, SP, Brazil), and the titratable acidity was determined by titration with 0.1 N NaOH until the pH reached 8.2. The volatile acidity was determined by steam distillation in an enological distiller (Super Dee model, Gibertini[®], Milan, Italy), followed by titration with 0.1 N NaOH. In the same enological distiller, simple

distillation was performed to determine the alcohol content and dry extract by using an electronic hydrostatic balance Super Alcomat (Gibertini®, Italy) to quantify them. This hydrostatic balance was also used to determine the density. It should be noted that, unlike monitoring during fermentation, in which ethanol levels were determined by HPLC, the quantification of alcohol content in the final wine was carried out exclusively by distillation, in accordance with the traditional oenological protocol. The free and total sulfur dioxide contents were determined by using the Ripper method, including the exclusion of polyphenols, which involves titrating the sample with 0.02 N iodine. The total reduced sugar content was determined by using the Lane–Eynon method, which is based on the procedures developed by Ribéreau-Gayon et al. [5]. Physicochemical analyses were performed on the wine after bottling, that is, after the malolactic fermentation and tartaric stabilization stages.

2.6. Statistical Analysis

Statistical evaluation of the physicochemical parameters was carried out through ANOVA followed by Tukey's test ($p \leq 0.05$), using the XLStat software version 2015 (Addinsoft Inc., Paris, France, 2019). Graphs illustrating the variations in sugar and alcohol levels, along with cell viability during fermentation, were generated using OriginLab (v. 2010, Northampton, MA, USA).

3. Results and Discussion

3.1. Evolution of Sugars and Alcohols Throughout the Fermentation

The evolution of the sugars and alcohols during the AF is depicted in Figures 2 and 3 and Table S1 (Supplementary Material). The must initially presented a higher concentration of fructose, followed by those of glucose and maltose. During grape maturation, there is often a greater accumulation of fructose, making its content slightly higher than that of glucose in the grape must [24]. Fraige et al. [25] also observed that the main sugars in Syrah variety grapes were fructose (61.85 g/L), followed by glucose (52.59 g/L). However, products derived from grape processing may also contain sugars from the hydrolysis of polysaccharides through the action of enzymes such as pectinase, maltose [26], and rhamnose [27]. Rhamnose was detected in the must at concentrations below the detection limit of the method in this study. The initial concentrations of maltose in the musts did not exceed 1.74 g/L (Figure 2A,B), with this sugar almost entirely consumed after 14 days of fermentation (i.e., the end of the fermentation process). The lowest residual values found for maltose were in the treatments wherein a combination of autochthonous yeasts (*H. opuntiae* + *S. cerevisiae*) was used, both in TW and TV (0.02 g/L and 0.24 g/L, respectively).

The initial concentrations of glucose (Figure 2C,D) and fructose (Figure 2E,F) in the must varied according to the winemaking technique employed. The application of thermovinification (TV), which includes a hot maceration step prior to alcoholic fermentation, resulted in lower concentrations of these sugars. It is known that the heat applied during TV can alter the extraction dynamics of grape compounds, favoring the release of phenolics and pigments but potentially affecting the extraction of sugars [28]. A possible explanation for the lower concentrations of glucose and fructose in the TV group is the partial inactivation of the pectinase enzyme at high temperatures, as this enzyme plays an important role in the release of sugars from pulp cells. Although exogenous pectinase was added to the must, its stability and residual activity after thermal treatment were not assessed in this study, which limits the confirmation of this hypothesis.

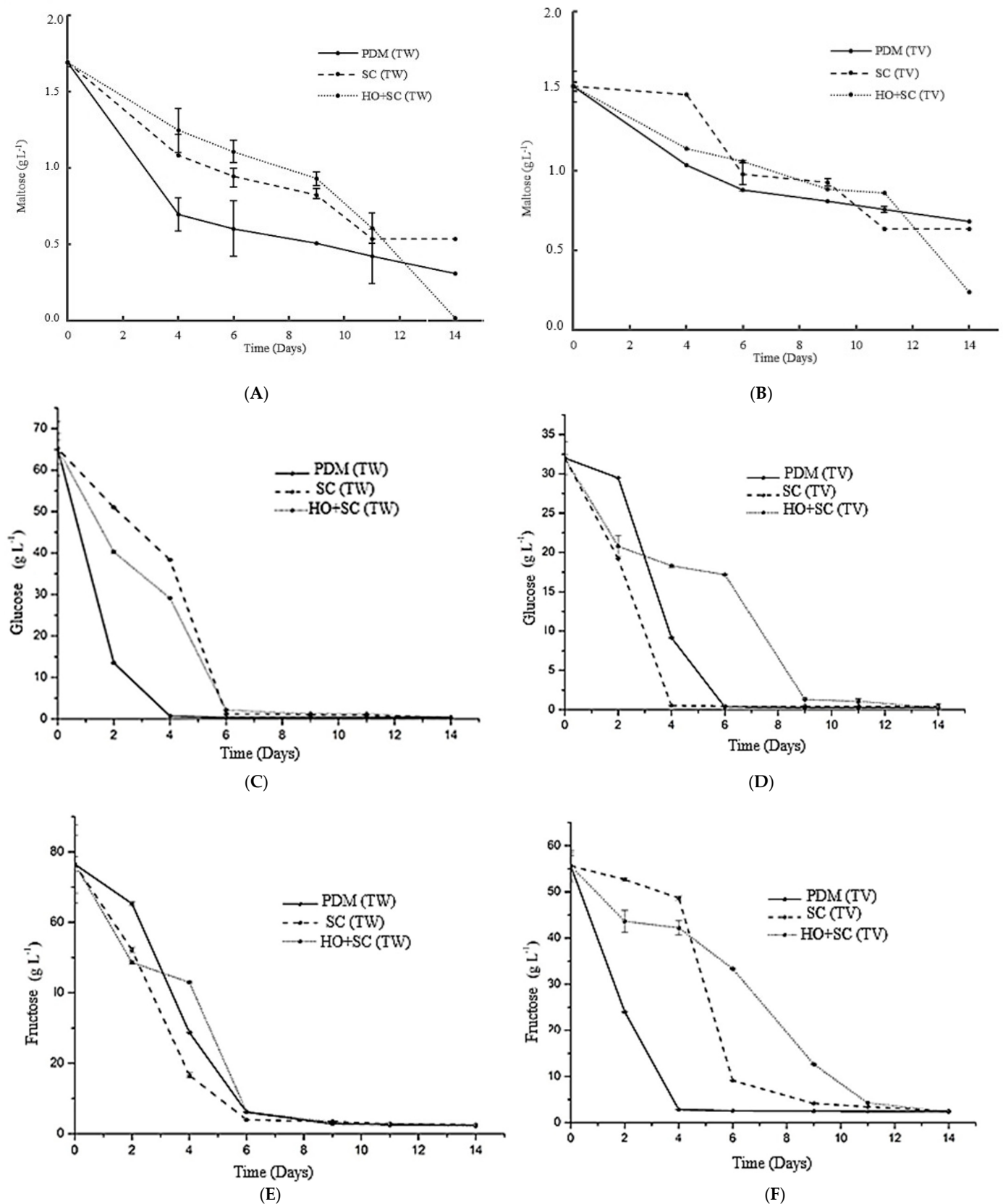


Figure 2. Evolution of sugars during the alcoholic fermentation of cv. Syrah grape must. (A,B) Evolution of maltose in the traditional winemaking and thermovinification treatment, respectively, (C,D) Evolution of glucose in the traditional winemaking and thermovinification treatment, respectively, (E,F) Evolution of fructose in the traditional winemaking and thermovinification treatment, respectively.

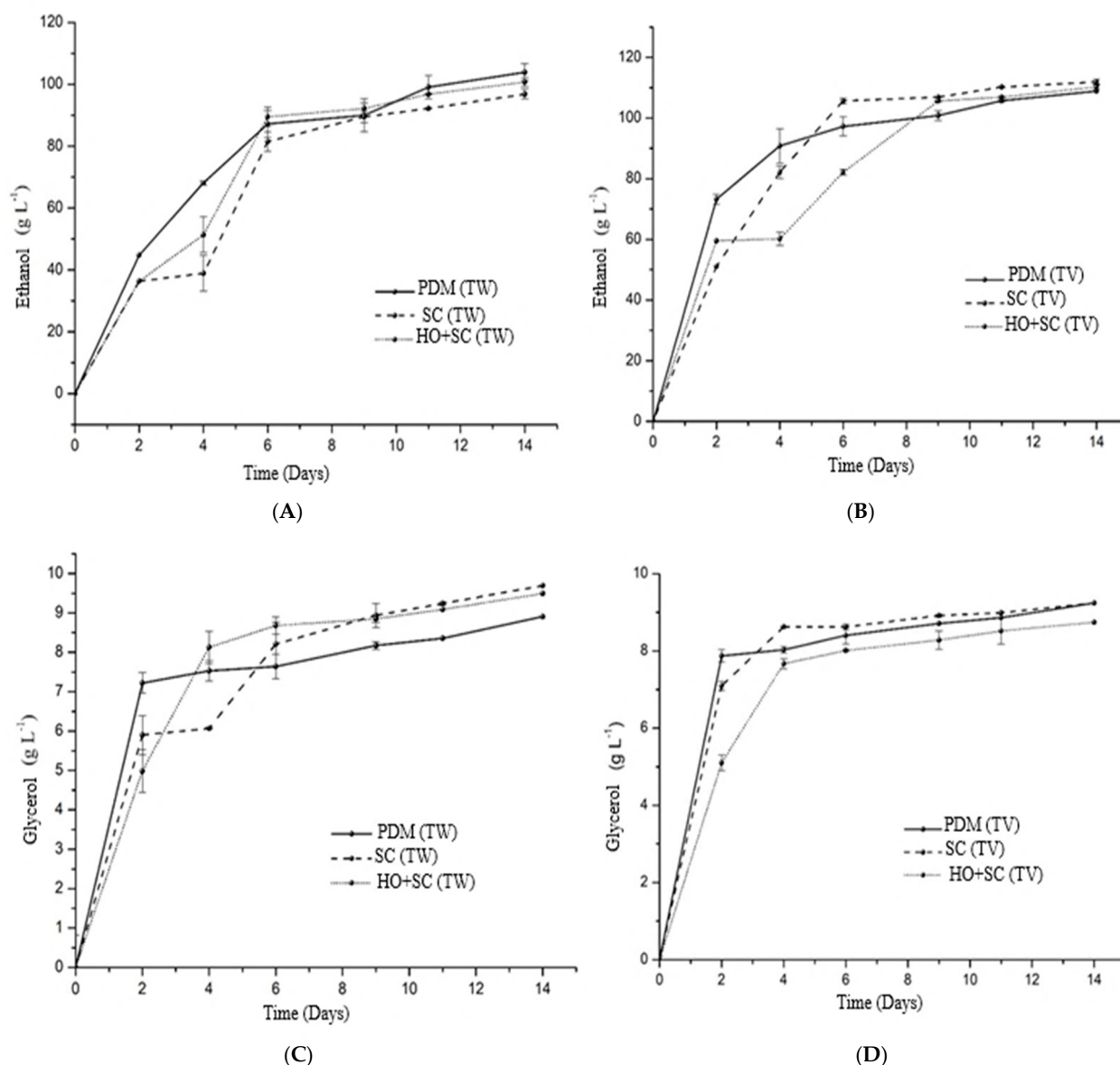


Figure 3. Evolution of alcohols during the alcoholic fermentation of Syrah grape must. (A,B) Evolution of ethanol in the traditional winemaking and thermovinification treatment, respectively, (C,D) Evolution of glycerol in the traditional winemaking and thermovinification treatment, respectively.

The addition of exogenous pectinase played a fundamental role in the extraction of grape compounds, albeit its activity differed between the treatments. In TW, the combined action of endogenous pectinase (naturally present in the grape) and the exogenous enzyme facilitated the degradation of pectin, thereby enhancing the release of sugars and other compounds from the cellular matrix. In contrast, in TV, the heat treatment inactivated both natural and exogenous pectinase, which significantly limited cell wall degradation and consequently sugar extraction. This difference highlights the impact of heat on the enzymatic activity and explains the significant variation observed between treatments.

In the treatment that used TV and the combination of the two autochthonous yeasts *H. opuntiae* and *S. cerevisiae* (HO + SC (TV)), the glucose and fructose took longer to be consumed. The glucose level significantly decreased only after 9 days of fermentation, while the fructose level decreased after the 11th day of the process. This behavior can be explained by the specific metabolic characteristics of the yeasts used. *Hanseniaspora opuntiae* is a non-

Saccharomyces yeast that, although it contributes positively to the sensory complexity of wines, has lower fermentative efficiency compared to *Saccharomyces cerevisiae*, especially with regard to the consumption of sugars. In addition, competition between the two species, especially under thermovinification conditions, may have slowed the fermentative kinetics of HO + SC (TV). Thermovinification, by exposing the must to high temperatures, can alter the composition of the medium, including the availability of nutrients and the concentration of inhibitory compounds, such as volatile fatty acids and phenols, directly impacting yeast performance [2]. Thus, in the treatment HO + SC (TV), the delay in the degradation of glucose and especially fructose probably results from the interaction between the thermal effects of the process of thermovinification and the microbiological dynamics between the yeasts, with an initial predominance of *Hanseniaspora opuntiae* and subsequent more effective action of *Saccharomyces cerevisiae*. This profile suggests a sequential metabolism, in which the adaptation and growth of *Saccharomyces cerevisiae* occur more slowly, postponing the complete consumption of sugars.

In contrast, in the PDM (TV) treatment, the glucose and fructose were almost completely consumed after 6 days of fermentation, and with SC (TV) after only 4 days. Unlike HO + SC (TV), in the treatment using traditional maceration and the combination of the two autochthonous yeasts, the glucose and fructose levels dropped drastically shortly after 6 days of fermentation. Similar behavior to HO + SC (TW) was observed for the treatment wherein traditional maceration was applied using only the autochthonous yeast *S. cerevisiae* (SC (TW)). In SC (TV), glucose was preferentially degraded by yeasts, while the fructose level drastically decreased only from the 9th day of fermentation.

During AF, the glucose/fructose ratio in the wine must be around approximately 0.25, since most yeasts preferentially ferment glucose [29], which justifies the higher residual fructose levels in the wines from all the tested treatments (2.22–2.51 g/L) when compared to glucose residues (0.24–0.47 g/L). Thus, as seen in Figure 2, the highest rate of glucose consumption in the wine must occur when the commercial yeast and the TW practice were used, and a drastic drop in the sugar content was observed as early as the 4th day of fermentation. In contrast, the autochthonous yeast *S. cerevisiae* consumed glucose more quickly when the TV practice was used instead of in the conventional maceration process.

The treatments with TV yield high ethanol formation values as early as the 2nd day of the fermentation (Figure 3), especially with the PDM yeast, which reached 73.27 g/L, followed by the combination of autochthonous yeasts HO + SC with 59.53 g/L and the autochthonous SC with an ethanol concentration of 51.09 g/L. For the treatments with TW, values above 59.3 g/L were reached only after 4 days of fermentation. At the end of fermentation, the highest ethanol concentration was observed in PDM (TV) (117.37 g/L), and the lowest was found with the same yeast (PDM) in the TW treatment (110.67 g/L). Geffroy et al. [30] also reported higher levels of ethanol in the thermovinified wines when compared to those made using the TW method. The authors found values ranging from 112 to 153 g/L of alcohol for TW and from 125 to 156 g/L for the thermovinified wines.

TV generally promotes better conditions for yeast activity, which gives faster and more complete AF. In addition, heat can reduce the presence of undesirable microorganisms in the must that could compete with the yeasts or consume sugars [28,31,32]. Furthermore, the grapes that underwent thermomaceration promoted the degradation of their polysaccharides, allowing the release of some reducing sugars to the wine must. Although glucose and fructose are related, the presence of other sugars in the must, such as arabinose, xylose, and galactose, may have contributed to the increased wine alcohol content. Despite being present in lower concentrations, these sugars can be fermented by non-*Saccharomyces* yeasts or *S. cerevisiae* [27].

The ethanol content produced by yeast depends on factors such as the concentration of available sugars, fermentation temperature, oxygen availability in the medium, the quality and quantity of micronutrients, and the specific metabolism of the yeast strain used. *S. cerevisiae* yeasts have different capacities to convert the energy obtained from sugars into biomass formation. This factor can either decrease or increase the ethanol concentration in the wine, even with the use of the same strain, depending on the winemaking technique selected [33].

Glycerol is, after ethanol, the most important alcohol in wine, typically found in quantities ranging from 5 to 10 g/L [34]. Regarding the formation of this alcohol in the tested treatments, all treatments showed concentrations > 4 g/L on the 2nd day of fermentation, ranging from 4.99 (HO + SC-TW) to 7.87 g/L (PDM-TV). However, at the end of fermentation, the must with the highest glycerol concentration was the one fermented by the autochthonous SC yeast using TW (9.70 g/L).

Glycerol is a byproduct of AF, and its concentration in wine depends on the initial sugar content in the must as well as on the yeast species and the fermentation conditions such as temperature, aeration, and sulfation [29]. Glycerol plays a very important sensory role in wines by presenting a sweet taste, similar to that of glucose, but mild, such that it only affects the sweetness in dry wines if its concentration is >5 g/L, imparting to the wine a sense of smoothness and body, making it full-bodied and velvety [35]. In this context, a higher glycerol concentration in wine can be beneficial, as this compound influences important sensory aspects.

Based on the evaluation of alcohol formation and sugar degradation throughout the AF process, it was observed that both commercial and autochthonous *S. cerevisiae* yeasts exhibited diverse fermentation behaviors, which varied with the selected winemaking practice and the combined use of the *H. opuntiae* yeast. These differences in the metabolomic profiles of the wine must likely cause a difference in the qualities of the wines.

3.2. Cell Viability of Yeasts During the Fermentation Process

High yeast cell viability is essential for the process to function efficiently. Figure 4 depicts the results of cell viability of the yeast strains during the AF of the studied treatments

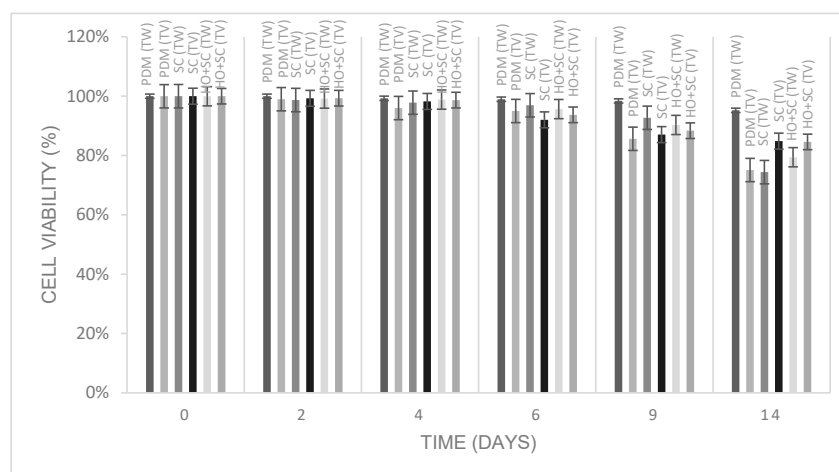


Figure 4. Cell viability during the alcoholic fermentation days of Syrah grape must. Legend: PDM (TW) and PDM (TV)—Commercial yeast Maurivin PDM® (*Saccharomyces cerevisiae* var. *bayanus*) in the traditional winemaking and thermovinification treatment, respectively; SC (TW) and SC (TV)—Autochthonous yeast *Saccharomyces cerevisiae* in the traditional winemaking and thermovinification treatment, respectively; HO + SC (TW) and HO + SC (TV)—Autochthonous yeasts *Hanseniaspora opuntiae* + *Saccharomyces cerevisiae* in the traditional winemaking and thermovinification treatment, respectively.

On the 1st day of the fermentation process, all treatments showed 100% cell viability. For the PDM (TW) treatment, the cell viability remained high (>95%) until the 6th day of the fermentation, thereby maintaining the same value on the 14th day (i.e., the end of the fermentation process). This cell viability also remained >95% until the 2nd day of fermentation in the PDM (TV) treatment. However, from then on, it declined more sharply relative to PDM (TW), reaching around 75.10% on day 14—a difference of about 20% between the two treatments. Although the same yeast strains were used, the winemaking technique influenced yeast cell viability. This effect may be partially associated with higher ethanol levels, considering that the total alcohol concentration (ethanol + glycerol) was higher in the TV treatment PDM (TV), (126.6 g/L) than in PDM (TW) (119.6 g/L). However, it is important to note that glycerol does not exhibit toxic effects on yeast cells, and other factors, such as pH variations between treatments (3.89 and 3.60, for PDM (TV) and PDM (TW), respectively), may also have contributed to the observed differences in yeast viability.

The excessive presence of ethanol in the medium is evidenced by a decrease in cell viability and a reduction in yeast growth, as ethanol has a toxic effect on them [36,37]. According to Alves [38], ethanol was the first factor recognized as an inhibitor of AF. The factors that most influence a microorganism's sensitivity to ethanol include temperature, aeration, and the composition of the medium [39]. According to Bai et al. [40], when yeast ferments in stressful environments, in addition to the decrease in cell viability, there may be an increase in glycerol formation and a reduction in biomass formation, which are extremely useful parameters for identifying strains tolerant to a single or combination of stressful factors.

For the SC (TW) treatment, the decrease in cell viability was relatively gradual until the 9th day of AF, when it reached around 92.72%, and then it significantly dropped to 74.39% on the 14th day of the process, showing a reduction of approximately 18% over 5 days of fermentation. For the same yeast with TV (SC (TV)), the decrease was more pronounced after the 6th day of fermentation, reaching 84.85% by the end of the fermentation. For the HO + SC (TW) treatment, after the 6th day of the fermentation process, there was a moderate reduction in cell viability, reaching about 79.43% by the end of fermentation. In contrast, in HO + SC (TV), the decrease in cell viability was more marked after the 6th day of fermentation, reaching approximately 84.60% on day 14. The loss of cell viability in non-*Saccharomyces* yeasts in mixed fermentations is primarily related to ethanol production in the medium [41], although other metabolites produced by the yeasts, such as medium-chain fatty acids and acetaldehyde, may also present inhibitory effects [42].

After the completion of AF, the lowest cell viability detected (74.39%) was for the treatment employing autochthonous *S. cerevisiae* yeast and the TW process (SC (TW)). When this yeast strain was associated with the practice of TV (SC (TV) treatment), the final cell viability was approximately 10% (84.85%) higher. These results indicate that although the autochthonous *S. cerevisiae* yeast exhibited lower cell viability in the TW process, TV favored the survival of the yeast cells.

It is therefore important to highlight that tolerance to ethanol can vary significantly among yeast strains, even within the same species as *S. cerevisiae*. Autochthonous yeasts, adapted to local conditions, may exhibit more pronounced resistance to ethanol in certain regions, but this does not imply that all strains are equally resistant to ethanol, regardless of the winemaking conditions. The interaction between winemaking processes and yeast strains, including the use of practices like TV, can have a direct impact on cell viability and the final wine quality [43].

Therefore, although some autochthonous *S. cerevisiae* strains may tolerate ethanol well in different winemaking processes, as demonstrated by treatments with TW and TV,

ethanol resistance is influenced by a combination of factors, including the yeast strain and specific fermentation conditions.

3.3. Kinetic Parameters of the Fermentation Process

The ethanol formation rates (Vp), substrate consumption rates (Vs), the relationship between them (YPS), and the productivity and efficiency of the fermentation process are expressed in Table 1.

Table 1. Kinetic parameters, productivity, and efficiency of the fermentation process of Syrah grape must.

	Winemaking Technique	Yeasts		
		PDM	SC	HO + SC
VP (Ethanol) g/L·h ¹	TW	0.03 ± 0.01 Aa	0.04 ± 0.01 Aa	0.04 ± 0.01 Aa
	TV	0.05 ± 0.01 Aa	0.05 ± 0.02 Aa	0.06 ± 0.01 Aa
VS (Sugars) g/L·h ²	TW	0.51 ± 0.11 Ba	0.81 ± 0.13 Aa	0.77 ± 0.17 Ba
	TV	1.02 ± 0.16 Aa	0.91 ± 0.06 Aa	1.09 ± 0.01 Aa
YPS (Ethanol/Sugar) g/L·h ³	TW	0.06 ± 0.01 Aa	0.05 ± 0.01 Aa	0.05 ± 0.02 Aa
	TV	0.05 ± 0.01 Aa	0.05 ± 0.02 Aa	0.05 ± 0.01 Aa
Productivity (g/L·h)	TW	0.03 ± 0.00 Ba	0.04 ± 0.00 Ba	0.04 ± 0.00 Ba
	TV	0.05 ± 0.00 Aa	0.05 ± 0.00 Aa	0.06 ± 0.01 Aa
Efficiency (%)	TW	94.67 ± 0.33 Aa	83.97 ± 1.03 Bb	82.75 ± 2.75 Ab
	TV	91.40 ± 1.4 Ba	89.65 ± 0.35 Aa	88.34 ± 3.33 Aa

Results expressed as mean values ± standard deviation. Values followed by different letters indicate significant differences between samples according to Tukey's test ($p \leq 0.05$). Capital letters indicate differences and similarities between wines submitted to different winemaking techniques (Traditional Winemaking (TW) and Thermovinification (TV)). Lower case letters indicate differences and similarities between the yeasts used in fermentation (PDM, SC and HO + SC). ¹ VP: Reaction rate in relation to ethanol production; ² VS: Reaction rate in relation to sugar consumption; ³ YPS: Ratio of product and sugar concentrations.

The results obtained showed significant differences ($p < 0.05$) for the parameters of sugar consumption rate (VS), productivity, and conversion efficiency. The commercial yeast *S. cerevisiae* (PDM) exhibited the highest sugar consumption rate (VS) under thermovinification (TV) conditions (1.02 g/L·h), a performance similar to that observed in the fermentation conducted with the combination of the autochthonous yeasts *H. opuntiae* and *S. cerevisiae* (HO + SC), which reached 1.09 g/L·h under the same condition.

Regarding productivity, the treatments presented significant differences in relation to the use of the vinification technique. The treatments submitted to thermovinification presented the highest values for productivity, ranging from 0.05 to 0.06 g/L·h, with no significant differences between the yeasts used. Tartian et al. [44] and Casassa et al. [45] reported that the alcohol content in wine samples depended only on the harvest year and sugar level and not on the maceration technique, unlike what was found in this study. However, Moldovan et al. [46] observed that the maceration technique employed affects the alcohol content of the wine, with thermovinification (70 °C, 20 min) being the most effective. As shown in Table 2, wines subjected to TV exhibited higher alcohol concentrations, with 13.87% v/v for SC (TV), 13.71% v/v for PDM (TV), and 13.67% v/v for HO + SC (TV).

Table 2. Physical-chemical characteristics of the produced Syrah red wines.

Traditional Winemaking ^{1,2}								
	pH	Total Acidity (g L ^{−1})	Volatile Acidity (g L ^{−1})	Density (g mL ^{−1})	Alcohol Content (% v/v)	Sugars (g L ^{−1})	Dry Extract (g L ^{−1})	Total SO ₂ (mg L ^{−1})
PDM (TW)	3.60 ± 0.0 ^a	5.52 ± 0.02 ^c	0.58 ± 0.01 ^b	0.995 ± 0.00 ^c	12.42 ± 0.06 ^a	2.15 ± 0.02 ^c	29.19 ± 0.22 ^b	91.26 ± 3.36 ^b
SC (TW)	3.44 ± 0.0 ^c	6.08 ± 0.04 ^a	0.67 ± 0.01 ^a	0.997 ± 0.00 ^b	12.20 ± 0.05 ^b	2.56 ± 0.03 ^a	33.93 ± 0.23 ^a	97.11 ± 4.6 ^{ab}
HO + SC (TW)	3.46 ± 0.0 ^b	5.89 ± 0.05 ^b	0.67 ± 0.04 ^a	0.997 ± 0.00 ^a	11.78 ± 0.14 ^c	2.33 ± 0.02 ^b	32.66 ± 0.78 ^a	113.07 ± 6.1 ^a
Thermovinification ^{1,3}								
	pH	Total Acidity (g L ^{−1})	Volatile Acidity (g L ^{−1})	Density (g mL ^{−1})	Alcohol Content (% v/v)	Sugars (g L ^{−1})	Dry Extract (g L ^{−1})	Total SO ₂ (mg L ^{−1})
PDM (TV)	3.89 ± 0.25 ^a	5.88 ± 0.04 ^b	0.47 ± 0.01 ^b	0.996 ± 0.0 ^a	13.71 ± 0.05 ^b	2.18 ± 0.02 ^c	34.66 ± 0.29 ^c	78.40 ± 0.8 ^{ab}
SC (TV)	3.63 ± 0.02 ^a	6.30 ± 0.19 ^a	0.47 ± 0.04 ^b	0.996 ± 0.0 ^a	13.87 ± 0.02 ^a	2.25 ± 0.04 ^b	36.53 ± 0.15 ^b	74.00 ± 1.89 ^b
HO + SC (TV)	3.54 ± 0.0 ^a	5.88 ± 0.01 ^b	0.56 ± 0.0 ^a	0.997 ± 0.0 ^a	13.67 ± 0.01 ^b	2.34 ± 0.03 ^a	37.38 ± 0.06 ^a	80.56 ± 1.1 ^a

¹ Data represent the mean values for each sample ± standard error; different letters in the same column indicate a significant difference ($p < 0.05$). ² Traditional winemaking: PDM (TW)—Commercial yeast Maurivin PDM[®] (*Saccharomyces cerevisiae* var. *bayanus*); SC (TW)—Autochthonous yeast *Saccharomyces cerevisiae*; HO + SC (TW)—Autochthonous yeasts *Hanseniaspora opuntiae* + *Saccharomyces cerevisiae*; ³ Thermovinification: PDM (TV)—Commercial yeast Maurivin PDM[®] (*Saccharomyces cerevisiae* var. *bayanus*); SC (TV)—Autochthonous yeast *Saccharomyces cerevisiae*; HO + SC (TV)—Autochthonous yeasts *Hanseniaspora opuntiae* + *Saccharomyces cerevisiae*.

In the traditional winemaking (TW), particularly when conducted with the commercial yeast PDM, resulted in the highest percentage efficiency of sugar-to-ethanol conversion (94.67%), indicating a more effective substrate utilization for alcohol production. In contrast, the autochthonous yeasts showed better efficiency performance when subjected to thermovinification, reaching 89.65% and 88.34% for SC (TV) and HO + SC (TV), respectively.

Autochthonous *Saccharomyces cerevisiae* and *Hanseniaspora opuntiae* strains exhibited distinct fermentation behaviors depending on the winemaking technique applied. Notably, the combination of both yeasts under TV conditions resulted in efficient sugar consumption, although with slower kinetics compared to the use of commercial yeast. This highlights the complexity of microbial interactions under thermal stress and suggests a sequential fermentative pattern, where *H. opuntiae* initially dominates, followed by *S. cerevisiae*.

4. Conclusions

This study demonstrated that the combination of thermovinification (TV) with autochthonous yeasts significantly influenced the fermentation kinetics of Syrah wines. TV, as a pre-fermentation heat treatment, not only enhanced the fermentation rate—particularly in terms of sugar consumption—but also improved ethanol productivity and yielded wines with higher alcohol content. Additionally, TV positively impacted the viability of autochthonous yeasts, favoring their performance under conditions of elevated ethanol concentrations.

These findings have relevant practical implications for the wine industry, as they indicate that the combination of techniques such as thermovinification and the use of autochthonous yeasts can be strategically leveraged to develop more sustainable wines with greater added value, quality, typicity, and regional identity.

For significant advances in understanding and applying these methods, future research should explore how thermovinification and autochthonous yeasts impact the metabolomic and flavoromic properties of Syrah wine. Furthermore, it is essential that subsequent studies include comprehensive sensory evaluations to assess how these approaches influence the perceived wine quality from the consumer's perspective, thereby consolidating the market potential of the studied practices.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation11080436/s1>, Table S1: Evolution of sugars and alcohols during the alcoholic fermentation process.

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