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Impact of yeast on the characteristics of Sauvignon Blanc wines from the Campanha Gaúcha Region

Impacto de leveduras nas características de vinhos Sauvignon Blanc da Região da Campanha Gaúcha

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Highlights

Different yeast strains produced wines with different characteristics.
The mixed fermentation showed lower levels of reducing sugar.
By inoculating different yeasts, drier wines can be obtained.
One yeast strain was capable of producing medium-dry wines.

Abstract

The objective of this work was to evaluate the effect of different commercial strains *of Saccharomyces cerevisiae* on the characteristics of Sauvignon Blanc wines produced in the Campanha Gaúcha region. The must obtained by mechanical destemming and crushing was treated with 150 mg L of potassium metabisulfite and pectolitic enzymes and subject to enzymatic hidrolisis and débourbage for 24h00 at 4°C. The must was divided into glass fermenters of 4,6 L, in triplicate for each of the four treatments, which were defined by the inoculation of 25g hL^{-1} of dry yeast: T1 - Zymaflore X5® (Laffort, Bordeaux, France); T2 - AWRI 796 (AB Biotek, Sydney, Australia); T3 - Maurivim PDM (AB Biotek, Sydney, Australia); T4 - 50% Zymaflore X5® + 50% AWRI 796. The wines were analyzed, regarding: ethanol; reducing sugars;

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total acidity; volatile acidity; pH; dry matter; and ash. The most significant differences were observed in: ethanol; reducing sugars; pH; dry matter. Among the treatments inoculated with single strains, T1 showed higher ethanol and lower reducing sugars content. T3 presented results similar to T1. T2 resulted in higher content of reducing sugars and consequently lower ethanol levels. The mixed fermentation (T4) achieved the lowest levels of reducing sugars among the treatments. It suggests that mixed fermentations could be an alternative to produce drier wines.
Key words: Commercial strain. Dry wine. Fermentative capacity. *Saccharomyces cerevisiae.* Selected yeast strain.

Resumo

O objetivo do trabalho foi avaliar o efeito de leveduras *Saccharomyces cerevisiae* comerciais nas características de vinhos Sauvignon Blanc, da região da Campanha Gaúcha, Brasil. Após desengace e esmagamento das uvas, o mosto foi sulfitado (150 mg L de metabissulfito de potássio), submetido a 24h00 de *débourbage.* Após, o mosto foi dividido em garrafões de vidro de 4,6 L, para realização da fermentação alcoólica, em triplicata. Os tratamentos constituíram-se pela inoculação de 25 g hL^{-1} leveduras desidratadas: T1 - Zymaflore X5® (Laffort, Bordeaux, França); T2 - AWRI 796® (AB Biotek, Sydney, Austrália); T3 - Maurivin PDM® (AB Biotek, Sydney, Austrália), T4 - 50% Zymaflore X5® + 50% de AWRI 796®. As variáveis analisadas nos vinhos foram: etanol; açúcares redutores; acidez total; acidez volátil; pH; extrato seco; cinzas. As diferenças mais significativas foram observadas para: etanol; açúcares redutores; pH; extrato seco. Dentre os tratamentos com inoculação de cepas únicas, T1 apresentou maior graduação alcoólica e baixo teor de açúcares redutores. T3 apresentou resultados semelhantes a T1. T2 apresentou menor graduação alcoólica e maior teor de açúcares redutores. T4 apresentou os níveis mais baixos de açúcares redutores dentre os tratamentos. Isso sugere que a inoculação de mais de uma cepa pode ser uma alternativa para produzir vinhos mais secos.
Palavras-chave: Capacidade fermentativa. Cepa comercial. Levedura selecionada. *Saccharomyces cerevisiae.* Vinho seco.

Introduction

During the last decades, the vitiviniculture became expressive in the Campanha Gaúcha region (Southern Brazil), with focus on growing wine grapes (*Vitis vinifera*) for table and sparkling wines (Giovannini & Manfroi, 2009). Worldwide, Sauvignon Blanc is one of most cultivated wine grape varieties and the only white grape with increasing cultivation area between 2000 and 2015 (Organização Internacional da Vinha e do Vinho [OIV], 2017; Chen et al., 2019).

Depending on the terroir and vinification processes, different wines are obtained from the same grape variety. Soil and climate are important drivers of grape quality (Conradie et al., 2002). Along the vinification, and important step is the alcoholic fermentation. Nowadays, it can be conducted in a homogenous and controlled way by the inoculation of selected yeast strains (Shi et al., 2019). Wine quality is closely related to yeast metabolic activity, which affects several sensory characteristics (Wang et al., 2017).

The selected yeast strains should assure rapid fermentation onset, satisfactory breakdown of sugars, and avoid stuck fermentations, while mitigating the production of other undesirable secondary metabolites, such as acetic acid (Capozzi et al., 2015). Additionally, these strains might improve wine sensory quality (Romano et al., 2003). Even though several species of yeasts exist, the most widely used in winemaking is *Saccharomyces cerevisiae,* due to its high ethanol tolerance and dominance throughout the alcoholic fermentation (Petruzzi et al., 2017).

Considering the facts presented above, the objective of this work was to evaluate and compare the physicochemical characteristic of Sauvignon Blanc wines obtained from grapes produced at Campanha Gaúcha and fermented by different commercially available selected yeast strains of *Saccharomyces cerevisiae.*

Material and Methods

The vinification was designed to include all the procedures commonly employed in commercial modern winemaking. First, 171 kg of Sauvignon Blanc grapes were manually harvested in a commercial vineyard located in Santana do Livramento, Brazil. Grapes were transported in 20 kg plastic trays until the experimental winery of UNIPAMPA (Federal University of Pampa), campus Dom Pedrito, RS, where they were stored at 4°C for 24h00, in order to reduce the temperature of the raw materials.

The grapes were mechanically destemmed, crushed, and pressed, without following any additional procedure for sterilization or microbial load reduction (apart from sulfating). That could be considered unnecessary because when all the conventional winemaking procedures and the recommended inoculation rates and procedures are employed, the selected strains of *Saccharomyces cerevisiae* are capable of rapidly dominating the fermentation, largely outnumbering the original inoculum (Marsit & Dequin, 2015; Stein et al., 2018).

Potassium metabisulfite was added to the must at the dose of 150 mg L^{-1}. After 45min, pectolytic enzymes Coavin MXT® (Amazon Group, Bento Gonçalves, RS, Brazil) were added at the dose of 5 g hL^{-1} and the approximately 100L. Enzimatic hidrolysis and settling (*débourbage*) were performed at 4°C, at must pH (3,12), for 24h00. These enzymes, also widely used in modern winemaking, can improve colloidal properties, optimize polysaccharide breakdown, maximize must yield, improve the extraction of aroma compounds, and reduce both the carryover of colloidal particles and turbity (Camargo et al., 2016; Espejo, 2021; Kassara et al., 2019).

After settling, the must was separated into 12 glass bottles of 4,6 L. The four treatments were defined by the inoculation of 25 g hL^{-1} of commercially available dry yeasts: T1 - Zymaflore X5® (Laffort, Bordeaux, France); T2 - AWRI 796 (AB Biotek, Sydney, Australia); T3 - Maurivim PDM (AB Biotek, Sydney, Australia); and T4 - 50% Zymaflore X5® and 50% AWRI 796. Prior to inoculation, the yeast was hydrated with water (10mL/g of yeast) at 37ºC for 15min. Small volumes of must (at 16-18°C) were added every 15min in order to bring the yeast close to the fermentation temperature, avoiding heat temperature shocks. Four days after inoculation, due to the low sugar content of the grapes, 35 g L^{-1} of sucrose were added (chaptalization) to the fermentations in order to increase in 2% (v/v) the final ethanol level. Chaptalization is a common practice in Brazil. The Brazilian legislation determines that up to 3% (volume based) of the alcohol content can derive from chaptalization (Lei nº 7.678, de 8 de novembro de 1988 e Instrução Normativa Nº 49, 2011.)

After the complete alcoholic fermentation (12 days at 16-18°C) the wines were transferred, to separate them from sediments, potassium metabisulfite was added (80 mg L^{-1}), in order to avoid malolactic fermentation and bentonite (La Elcha®, La Elcha Minera Industrial S.A., Mendoza, Argentina) was added at 0,7 g L^{-1} for clarification. After seven days, another transfer was performed, prior to 30 days at 5°C, for tartaric stabilization. After this, a final transfer was conducted, the molecular SO2 levels were adjusted to 1mg L^{-1}, and the wines were bottled in 750mL glass bottles.

By means of a WineScan™ SO2 (Foss Analytics, Denmark), the following physicochemical parameters were analyzed in the must (after pressing): soluble solids; density; total acidity; pH; reducing sugars; malic acid; tartaric acid; gluconic acid; potassium. Three months of bottling, the wines were evaluated at the labs of EPAGRI (Company of Agricultural Research and Rural Extension of Santa Catarina), Videira, SC, using the methods stablished (Instrução Normativa nº 24, 2005) for: ethanol; reducing sugars; total acidity; volatile acidity; pH; dry matter; and ash.

The experiment comprised the four treatments described above, all in triplicate. The means were subject to analysis of variance (ANOVA) and compared by a Tukey test at 5% of significance. For better visualization, the results showing more significant differences regarding the fermentative capacity were subject to a principal component analysis (PCA). Statistical analyses were runned in the R software (R Core Team [R], 2020) and the RStudio interface (RStudio Team, 2020).

Results and Discussion

The physicochemical characteristics of the must were: soluble solids - 16,8ºBrix; density - 1,069 g mL^{-1}; total acidity - 10,8 g L^{-1} (as tartaric acid); pH - 3,12; reducing sugars - 165g L^{-1}; malic acid - 6,5g L^{-1}; tartaric acid - 5,7 g L^{-1}; gluconic acid - 0,1 g L^{-1}; and potassium - 970 g L^{-1}. The physicochemical characteristics of the wines are presented in the Table 1.

Table 1
Physicochemical characteristics of wines

Variables	T1 (Zymaflore X5®)	T2 (AWRI 796®)	T3 (Maurivin PDM®)	T4 (Zymaflore X5® + AWRI 796®)	CV%
Ethanol (% v/v)	12.27 a	11.55 b	11.94 ab	12.13 a	3.31
Reducing sugars (g L^{-1})	3.83 b	6.37 a	2.57 c	1.88 c	26.23
Total acidity (meq L^{-1})	139.23 a	140.84 a	143.12 a	139.56 a	5.05
Volatile acidity (meq L^{-1})	4.31 b	4.32 b	4.97 a	4.39 ab	10.77
pH	3.32 b	3.55 a	3.41 b	3.31b	2.91
Dry matter (g L^{-1})	22.02 b	24.08 a	21.14 bc	19.56 c	6.53
Ash (g L^{-1})	1.91 a	2.04 a	2.04 a	2.05 a	7.31

Means followed by the same letter did not differ significantly in a Tukey test at 5% of significance.

It is possible to observe that T1 and T4 presented the highest levels of ethanol and T2 the lowest. It suggest a slightly higher fermentative capacity of Zymaflore X5®, used in both T1 and T4. T3 presented intermediate values. Yeast strains showing more intense aerobic metabolism tend to produce less ethanol while those with more anaerobic metabolism tend to yield higher ethanol levels (Figueira et al., 2021). Yeasts producing less ethanol convert a larger part of the sugars into other molecules such as glycerol, pyruvic acid, as well as biomass (Benito et al., 2015).

The strain AWRI 796 is characterized by moderate to rapid fermentations (at 20-30 ºC), tolerance to high ethanol levels (between 14,5 and 15,5 %, volume based) and low production of acetic acid. The strain Maurivin PDM is adequate for fermentation at low temperatures (8-15ºC), presenting even higher tolerance to ethanol (up to 15-17%), and low production of acetic acid. The strain X5 presents rapid fermentations and high tolerance to ethanol levels (up to 16%). Therefore, it is possible to conclude that the fermentations of all the treatments should not have been impaired by high ethanol levels, as all the strains could tolerate much higher levels than those potentially achievable by the sugars initially available in the must.

T3 and T4 presented the lowest levels of reducing sugars, T1, the intermediate, and T2, the highest. The strain AWRI 796® showed the lowest capacity to metabolize sugars, when inoculated as single strain (T2). Interestingly, in T4, this yeast strain mixed with Zymaflore X5® resulted in the lowest levels of reducing sugars among all the treatments. This lowest level of unfermented sugars (1,88 g L-1) corresponds to 49 e 29,5% of the levels achieved by the single strain inoculations in T1 and T2, 3.83 e 6.37 g L^{-1}, respectively. In mixed fermentations, the yeast strains can present metabolical interaction, producing wines different from those fermented by single strains (Schneider et al., 2020).

The reducing sugars in wine determine their classification into dry, medium-dry, etc (Negrulescu et al., 2012). Brazilian legislation classifies wines as: dry ('seco') up to 4 g L^{-1};

and as medium-dry ('demi-sec') between 4 and 20 g L^{-1} (Instrução Normativa n. 14, 2018). Therefore, T2 would be medium-dry, while the others would be dry.

The volatile acidity showed a general average of 4,49 meq L^{-1} and only minor variations between the treatments. The Brazilian legislation allows a maximum of 20 meq L^{-1}, or 1,2 g L^{-1} of acetic acid (Instrução Normativa n. 14, 2018). Therefore, all the wines would fit within the Brazilian legal requirements for this parameter.

Significant differences of dry matter were observed between the treatments. The strain AWRI 796® (T2) resulted in the highest means (24,08 g L^{-1}), while its combination with Zymaflore X5®, in T4, resulted in the lowest means (19,56 g L^{-1}). T3 (Maurivin PDM®) presented intermediate values, similar to T1 (Zymaflore X5®). The dry matter in dry white wines is normally under 25 g L^{-1} (Ribéreau-Gayon et al., 2006), what was observed in all the treatments. The ash content was very similar in all the treatments. The four treatments comply with the Brazilian regulation for ash, which requires a minimum of 1g L^{-1} (Instrução Normativa n. 14, 2018).

Principal component analisys (PCA) was performed with the variables ethanol, pH, reducing sugars, and dry matter, which showed more significant differences and are more correlated to the fermentative capacity. The first two principal components (PC) could comprise 99,24% of the variability in this data set. In Figure 1a presents the loading plot, which shows the correlation between the variables with regard to PC1xPC2. Figure 1b shows the score plot with regard to PC1xPC2, which dispose the treatments according to their main characteristics. This PCA was performed with a small data set in order to summarize into two dimensions the major tendencies observed in this experiment, which were already discussed separately.

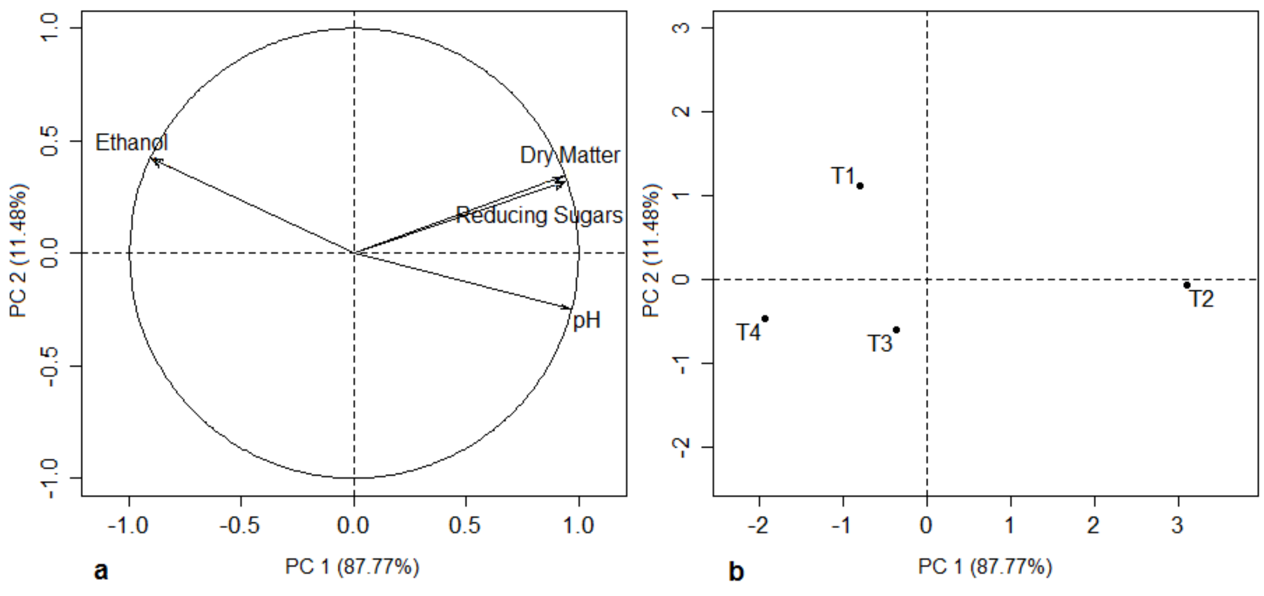

Figure 1. PC1xPC2 of the PCA; a - score plot (physicochemical characteristics); b - loading plot (treatments).

In Figure 1a, the four variables are very well projected. As expected, dry matter and reducing sugars showed great correlation - the variation in dry matter was mainly due to the variation in reducing sugars. The fermentation of these sugars resulted mostly in ethanol production. pH values showed inverse relationship with ethanol values.

In Figura 1b, it is evident that T2 presented the most outlying results, the highest pH, dry matter and reducing sugars means, and lowest ethanol means, being the most distant from T4. T4 was relatively close to T1 but showed the lowest levels of reducing sugars. T3 was close to the graph's origin, with less extreme or more intermediate values. It is also interesting to observe that the results of the mixed fermentation T4 cannot be considered intermediate to the results observed in T1 and T2, as it could be expected. Instead, it is possible to theorize that, as mentioned in the literature (Schneider et al., 2020), a metabolical interaction between strains might have happened, yielding, therefore, a product with distinct characteristics.

In sum, these different outcomes cannot be considered intrinsically neither positive nor negative; it depends on the desired outcome, the type of wine being produced. For example, if a dry and more acidic wine must be produced, the strain used in T2 would not be recommended. Conversely, if the objective is producing a medium dry and less acidic wine, this same strain showed to be capable of leaving enough residual sugars and higher pH, without demanding any extra sugar or base addition before bottling.

Conclusions

The Sauvignon Blanc wines elaborated with different yeast strains showed significant differences with respect to most of the variables analyzed: ethanol, reducing sugars, pH, and dry matter. Among the treatments inoculated with single strains, T1 showed higher ethanol and lower reducing sugars content. T3 presented more intermediate results but more similar to T1. T2 resulted in higher pH and higher content of reducing sugars and, consequently, lower ethanol levels. The mixed fermentation (T4) achieved the lowest levels of reducing sugars among the treatments. It suggests that mixed fermentations (T4) could be an alternative to produce drier wines, while T2 can naturally produce less dry wines.

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