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Evaluation of bio-acidifying yeast *Lachancea thermotolerans* as a strategy to reduce the effects of climate change in Tempranillo grape must vinification

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Abstract. The effects of climate change are posing major challenges for winemaking, especially in warm regions. Rising temperatures are leading to a considerable increase in sugar content and a reduction in the acidity of grape juices. This has prompted the search for new chemical and biological tools to reduce these effects in winemaking. *Lachancea thermotolerans* represent an interesting tool for wine improvement, due to their effects on lactic acid, ethanol, and volatile acidity. In this study, we evaluated the sequential inoculation of *Lachancea thermotolerans* (AEB Italia) + *Saccharomyces cerevisiae* as an alternative to the addition of water and tartaric acid in the vinification of over-ripened wine must (Tempranillo cv.). To do so, we conducted a micro vinification experiment that included three experimental treatments (replicated four times); AAS = Addition of water + tartaric acid fermented with *Saccharomyces cerevisiae*, ALS = sequential fermentation with *Lachancea thermotolerans* followed by *Saccharomyces cerevisiae* and SAC = Only inoculation with *Saccharomyces cerevisiae* (control). We measured the lactic acid, malic acid, acetic acid, total acidity, pH, glucose + fructose, and ethanol concentration of the resulting wines. We found a higher lactic acid content and total acidity in ALS wines (2.2 g/L y 6.65 g/L) compared with AAS (0.13 g/L y 5.75 g/L) and SAC wines (0.18 g/L y 5.37 g/L). ALS wines showed a significantly lower pH (4.00) than SAC wines (4.19) but did not differ significantly from AAS wines (3.19). Finally, acetic acid was higher in ALS wines (0.89 g/L) than AAS wines (0.64 g/L) but did not differ significantly from SAC wines (1.02 g/L). Our results point out that the use of *Lachancea thermotolerans* in a sequential inoculation with *Saccharomyces cerevisiae* is an alternative to the addition of water and tartaric acid in the vinification of over-ripened Tempranillo musts.

Keywords: *Lachancea termotolerans*, acidity, lactic acid, overripe, tempranillo, climate change

1 Introduction

One of the main challenges that the agricultural sector is facing today is climate change. According to the most recent IPCC report, climate change is already causing widespread impacts in all regions of the world and these effects are expected to be exacerbated as we are failing to limit our carbon and greenhouse gas emissions (IPCC, 2022 [1]). This is especially true for viticulture and enology, as the composition of grape berries (a key determinant of wine quality) is highly affected by environmental variations. The impact of climate on berry quality and wine production is such that it can greatly affect the quality of wine produced and even affect the economic value of a wine region (Hannah et al., 2013 [2]).

Modification of phenology leading to an increased sugar content, acidity deficiencies, changes in phenolic content and berry aroma profile are some of the effects documented in the recent past in different wine regions of the world because of rising temperatures and increased frequency of droughts. Temperature increases lead to unbalanced wines since berry sugar concentration rises at the expense of malic acid and secondary metabolites. Likewise, the rise in sugar content of wine grapes often results in wines with higher alcohol content raising concerns to both policy makers and final consumers (Delrot et al., 2020 [3]).

Such is the impact of climate warning that in recent years some viticultural regions have shifted from the practice of adding sugars to the must to the removal of partial sugar or alcohol by means of physical methods like reverse osmosis. In a similar fashion, the adjustment of must sugar levels with the addition of water is a common practice to ensure a safe fermentation process and to reduce elevated alcohols levels in wines (Schelezki, et.al., 2020 [4]). Likewise, the addition of food-quality acids (*i. e.,* tartaric acid, lactic acid, or citric acid) is used to lower must pH. However, this solution is expensive and presents other problems, such as a lack of chemical stability (Benito, 2018 [5]).

This context has triggered the interest in the study of non-saccharomyces yeasts in co-culture with *Saccharomyces* strains as adaptation strategies to cope with the effects of climate warming on chemical composition of wines. Previous studies have found valuable this approach to enhance wine parameters such as glycerol content, to reduce the acetic acid content, or to improve the total acidity of wines (Ciani and Ferraro, 1996 [6]; Gobbi, et al.,2013 [7]; Sainz et al 2022 [8], Rantsiou et al., 2012 [9]). *Lachancea thermotolerans* is nowadays an important tool to cope with the effects of climate change during winemaking, due to its ability to reduce pH, ethanol, and volatile acidity while to increasing total acidity (Benito, 2018 [5]; Gobbi et.al., 2013 [7], Peskova et. al., 2021 [10]).

Climate change is expected to have an even stronger effect in viticultural regions where water supply is limited (Hannah et al. 2013 [2]). Such is the case of Ensenada Baja California, where 70-80% of wine production of Mexico occurs (Acosta-Zamorano et al., 2013; Muñoz and Sánchez, 2017 [11]). Climate change models of the region indicate that current wine regions of Baja California will experience a significant increase in the temperature in the next 30 years, posing a challenge to sustain the production of high-quality wines in the region (Valenzuela-Solano, et. al., 2014 [12]). This study aimed to explore experimentally the sequential inoculation of the yeast *Lachancea thermotolerans* (AEB Italia) + *Saccharomyces cerevisiae* as an alternative to the addition of water and acid in the vinification of over-ripened Tempranillo grape must, one of the most important grape varieties in Ensenada, Baja California (Ruíz-Carvajal et al., 2018 [13]). To do so, we compared seven wine analytical parameters (lactic acid, malic acid, total acidity, acetic acid, pH, ethanol content and glucose/fructose) of wines resulting from three different inoculation treatments, Inoculation with *Saccharomyces cerevisiae* (SAC, as a control), Addition of water + tartaric acid fermented with *Saccharomyces cerevisiae* (AAS), and sequential fermentation with *Lachancea thermotolerans* followed by *Saccharomyces cerevisiae* to end the fermentation (ALS). We expected that wines inoculated in the ALS treatment would show higher lactic acid and total acidity and lower pH, ethanol content, acetic acid, and glucose + fructose.

2 Methods

240 kg of Tempranillo grapes from Valle de Guadalupe, Baja California, Mexico were harvested into 20 kg bins by hand. 15 kg of fruit were destemmed and crushed into twelve 20-liter containers, and additioned with 35 ppm sulfites as potassium metabisulfite. After 2 days of cold maceration at 16°C the average degree Brix was 31 by densimeter, pH = 4.15, total acidity = 3.49 gr/lt, yeast assimilable nitrogen (YAN) =126 ppm.

We conducted a microvinification experiment that included three experimental treatments. SAC = Only inoculation with 60 gr/hl *Saccharomyces cerevisiae* (Zymaflore RX60, Laffort, as a control), AAS = Addition of water (2L + tartaric acid (2 g/l of must) fermented with 60 gr/hl of *Saccharomyces cerevisiae* (Zymaflore RX60, Laffort) and ALS = sequential fermentation with 30 gr/hl of *Lachancea thermotolerans* followed by 30 gr/hl *Saccharomyces cerevisiae* 4 days later to end the fermentation (*L. thermotolerans* only tolerates up to 9% alcohol). All treatments were replicated four times. 249 ppm of YAN was added as Fermoplus Integrateur (AEB) for a total YAN of 375 ppm (based on 12.5 ppm YAN per degree Brix). Degree Brix and temperature were measured daily and grape must was punched down by hand twice daily during fermentation. After fermentation was completed or it stopped, the must was pressed by hand using a mosquito net, and 50 ppm of sulfites as KMBS was added to the resulting wines.

Six chemical parameters (lactic acid, malic acid, acetic acid, total acidity, pH, glucose + fructose) were spectrophotometrically determined for the resulting wines using the Y-15 Enology Autoanalyzer (Biosystem, Barcelona, Spain). In addition, ethanol concentration was obtained by means of an ebulliometer (Dujardin-Salleron).

To detect multivariate differences between treatments in wine parameters we performed a one-way MANOVA. Subsequently, univariate one-way ANOVAs were performed for each wine parameter variable independently. Whenever an ANOVA model result was significant, the respective Tukey-Kramer was performed as a post hoc test to detect differences between treatments. All analyses were conducted using JMP 14 (SAS).

3 Results

One-way MANOVA revealed a significant multivariate effect for the term inoculation treatment (Wilks' $k = 0.01$, $F = 5.9$, $df = 12$, $p < 0.0084$). Given the significance of the overall test, univariate ANOVAs for each wine parameter were performed. Univariate ANOVA showed significant differences between treatments in pH ($F = 11.81$, $df = 2$, $p < 0.003$). SAC treatment showed a significantly higher pH compared with AAS and ALS treatments, that did not differ from each other (Fig. 1a). Likewise, univariate ANOVA showed significant differences between treatments in total acidity ($F = 15.34$, $df = 2$, $p < 0.0013$). ALS treatment showed a higher total acidity compared with AAS and ALS treatments, that did not differ from each other (Fig. 1b). Univariate ANOVA also showed significant differences between treatments for acetic acid ($F = 9.36$, $df = 2$, $p < 0.0063$). AAS treatment showed a lower acetic acid concentration compared with SAC and ALS treatments, which did not differ from each other (Fig. 1c). Finally, univariate ANOVA showed significant differences between treatments for lactic acid ($F = 15.19$, $df = 2$, $p < 0.0013$). ALS treatment showed a higher lactic acid concentration compared with SAC and AAS treatments, which did not differ from each other (Fig. 1d). Univariate ANOVAs did not find differences between treatments for malic acid, glucose/fructose, or ethanol content (Fig. 1 e-f).

4 Discussion

We found that sequential inoculations with *L. thermotolerans* + *S. cerevisiae* (ALS treatment) showed a significant increase in total acidity and lactic acid concentration compared with the individual inoculation with *S. cerevisiae* (SAC treatment) and the inoculation with *S. cerevisiae* with the addition of water + tartaric acid (AAS treatment). This is in line with previous studies from Gobbi et al. (2013 [7]) and Comitini et al., (2011 [14]), supporting the notion that *L. thermotolerans* represent an option to solve total acidity problems and to

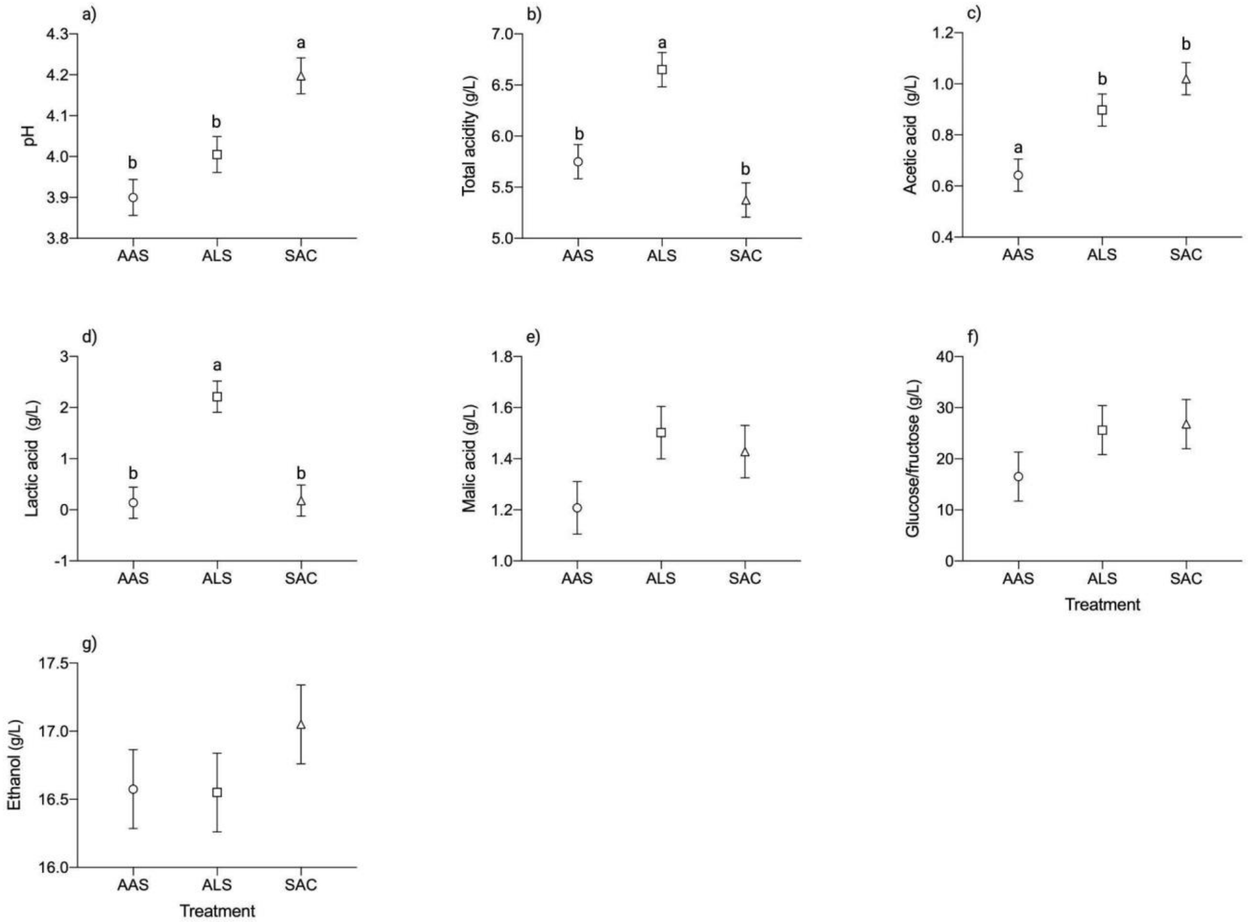

Figure 1. a) pH, b) total acidity, c) acetic acid, d) lactic acid, e) malic acid, f) glucose/fructose and g) ethanol content of wines resulting after the vinification of overippened tempranillo grapes under three different inoculation treatments. AAS = Addition of water + tartaric acid fermented with *Saccharomyces cerevisiae,* ALS = sequential fermentation with *Lachancea thermotolerans* followed by *Saccharomyces cerevisiae* to end the fermentation, SAC = Inoculation with *Saccharomyces cerevisiae.* Levels that do not share the same letter differ significantly after a Tukey-Kramer LSD *post hoc* test ($p \leq 0.05$).

increase lactic acid levels of red wines from warm viticultural areas such as Baja California.

On the other hand, the inoculation with *S. cerevisiae* with the addition of water + tartaric acid (AAS treatment) showed the lower values of pH and acetic acid, whereas sequential inoculations with *L. thermotolerans* + *S. cerevisiae* (ALS treatment) did not differ from the individual inoculation with *S. cerevisiae* (SAC treatment). All treatments showed acetic acid values under the OIV International Code of Oenological Practices limit of 1.2 g/l (OIV, 2021 [14]). *Lachancea thermotolerans* (ALS) was able to lower the must pH from 4.2 to 3.9, a level not significantly different to the addition of water and tartaric acid (AAS, pH 4.0). Nevertheless, the pH of the must in ALS treatment resulted significantly lower compared with the inoculation with *S. cerevisiae* only (SAC).

Furthermore, we did not find significant differences between treatments for ethanol concentration, malic acid, and glucose/fructose. However, there was a tendency for lower malic acid (dilution with water) and lower glucose/fructose (completion of fermentation) in the AAS treatment. Likewise, results showed a non-significant tendency to higher alcohol levels in the SAC treatment, due to higher starting degree Brix for alcoholic fermentation.

5 Conclusion

Altogether our results point that the use of *L. thermotolerans* is a feasible alternative to the addition of water + tartaric acid for wine making over severe ripened must (31º Brix) of the Tempranillo variety. This is of particular interest to winemakers of Ensenada Baja California growing Tempranillo grapes, since climate change is expected to increase the intensity of heat strokes and where water availability is a scarce resource. Further research with *L. thermotolerans* and other non-*Saccharomyce*s yeast and lactic acid bacteria in productive conditions is needed to increase the fermentation microorganism options for winemakers in the region.

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