ALL ACIDS ARE EQUAL, BUT SOME ACIDS ARE MORE EQUAL THAN OTHERS: (BIO)ACIDIFICATION OF WINES

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Introduction

Acidity is a major quality determinant of each wine. It defines its taste, in particular sourness, and affects is appearance, and chemical and microbial stability (Waterhouse et al., 2016). Cool-climate wines are generally high in acidity, and often undergo partial deacidification to reach balance. In contrast, grapes from warm climates frequently contain insufficient acidity at harvest, therefore requiring acidification.

Acidification is almost routinely carried out through the addition of tartaric acid throughout vinification (i.e., pre-, mid- and post fermentation). Other organic acids (e.g., malic, lactic) are also permitted acidulants under most wine regulatory frameworks (Waterhouse et al., 2016), but are used to a lesser extent than tartaric acid. Tartaric acid is not metabolised during winemaking; however, it can be lost via precipitation. This imposes additional production costs, which are far from negligible. In Australia, they exceed 10 million AUD (6.5 million EUR) on a yearly basis. This is of relevance for a number of warm viticultural regions, including parts of California, Languedoc-Roussillon, La Mancha (incidentally, regions with the largest grape/wine production), and the observed trends are expected to worsen in the context of climate change. Alternative methods to boost wine acidity are therefore in high demand, and biological acidification (i.e., bio-acidification) through the use of acidifying microorganisms during vinification shows particular promise.

One yeast with a remarkable bio-acidifying potential is *Lachancea thermotolerans* (LT), a species commonly found on grape berries and other wine-related environments (Hranilovic et al., 2017). Just like the typical wine yeast, *Saccharomyces cerevisiae* (SC), LT can metabolise grape sugars to ethanol with the release of CO_2 and aroma-active

compounds – a process known as alcoholic fermentation. But unlike SC, LT is during fermentation capable of converting a proportion of sugars into lactic acid instead of ethanol (Figure 1; Hranilovic et al., 2018). Production of lactic acid thus results in acidification accompanied with ethanol decreases. This is an additional benefit, as the grapes that lack acidity often contain excessive sugar, thereby resulting in overly alcoholic wine with compromised chemico-sensory profiles and marketability (Ristic et al., 2016).

The LT strains, however, greatly vary in their lactic acid production (and thus acidification) capacity. For example, concentrations of lactic acid formed by 94 different LT strains while fermenting the same Chardonnay juice ranged between 1.8 to 12.0 g/L, and significantly affected the pH of wines (3.2 – 3.8; Figure 1; Hranilovic et al., 2018). Moreover, as all non-*Saccharomyces* yeasts, LT strains have to be used with either simultaneously (co-inoculation) or subsequently added (sequential inoculation) SC to ferment to dryness. In such mixed-culture fermentations, acidification depends on the LT strain, but also on the inoculation regime, with a more pronounced LT contribution in sequential inoculations than in co-inoculations (Hranilovic et al., 2021).

In our previous work, we acquired a large collection of LT isolates (~200) from around the globe, and developed molecular methods to characterise their diversity at the genetic level (Hranilovic et al., 2017). We then compared a subset of 94 strains for their oenological performance in pure culture Chardonnay fermentations (Hranilovic et al., 2018). The best-performing LT strains were next characterised in mixed culture fermentations with SC in a wide range of oenological conditions in Australia and France (Favier et al., 2021), delivering an LT strain capable of lowering wine pH by ~0.5 units, and ethanol by ~1 % v/v, relative to the SC control (Hranilovic et al., 2021). The current study aimed to i) compare the chemical and sensory profiles of the bio-acidified LT and acid-adjusted SC wines, and ii) evaluate the use of LT wines as blending components.

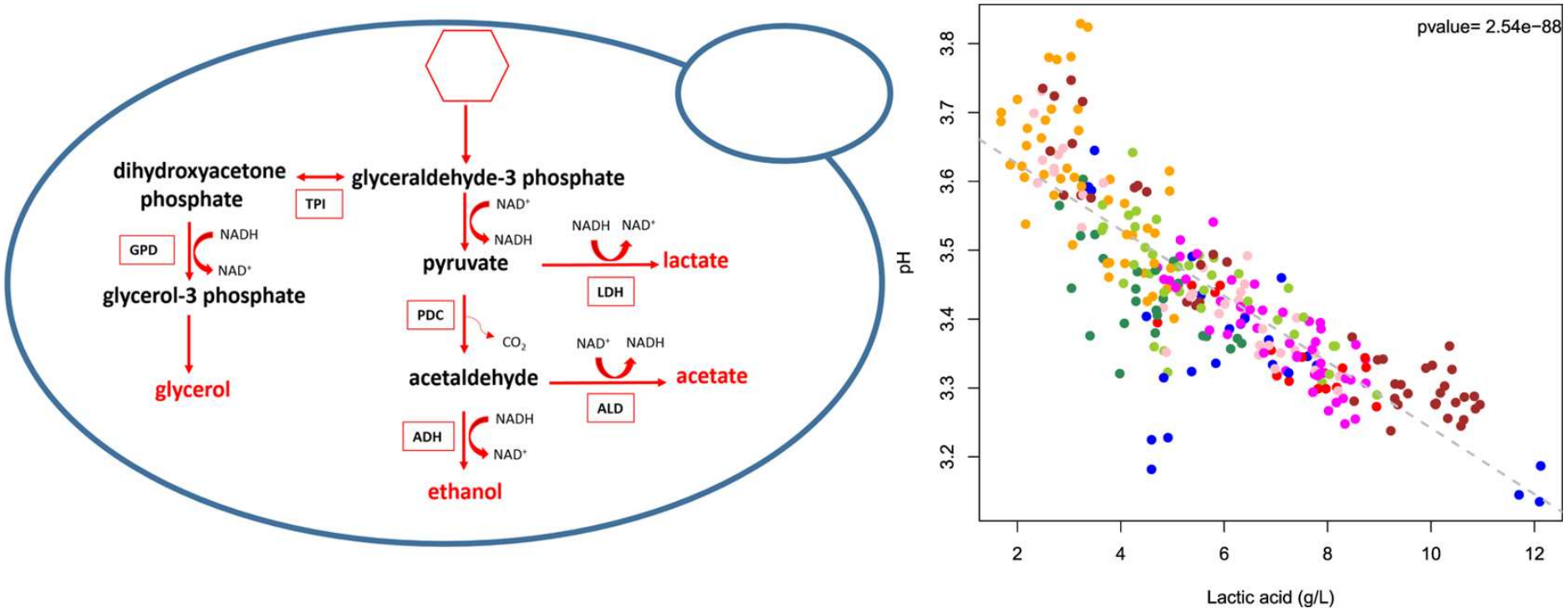

Figure 1. Lactic acid production by *Lachancea thermotolerans*. Metabolic pathway (left) and variation in lactic acid production and resultant pH modulation between different LT strains (right). Adapted from Hranilovic et al., 2018.

Materials and methods

Merlot grapes were handpicked from the experimental Coombe vineyard (Waite Campus, University of Adelaide, AU) on the 7 March 2019. Upon processing, the grapes were split into two fermenters (20 kg plastic bucket with a lid), each containing 12 kg of must at 14.5 ºBaumé and pH 3.9. One batch was inoculated with a culture of LT followed by SC at 48 h, and the other with a monoculture of the same SC strain. The tested LT strain was previously reported as UNIFG 18 and LT2 (Hranilovic et al., 2017, 2018, 2021), while the SC strain is commercially known as ZYMAFLORE Spark (Laffort, France). The liquid inoculation cultures were prepared as described in Hranilovic et al. (2021). The fermentations were carried out at 24 ºC, and the initial yeast assimilable nitrogen (YAN) levels (80 mg/L) were increased to 260 mg/L through multiple additions during fermentation. The cap was plunged once a day with concurrent monitoring of total soluble solids (TSS) and pH. After 8 days of maceration, wines were pressed off the skins using a basket press to finish fermentation in 5 L demijohns.

Following cold-stabilisation and racking, two aliquots of the SC wine (pH 4) were acidified with either tartaric (SC+TAR) or lactic acid (SC+LAC) to reach the pH of the LT wine (pH 3.6). The initial LT and SC wines were also blended in three proportions (i.e., 3:1, 1:1, 1:3). The wines were bottled and stored at room temperature ahead of further analyses. Chemical analyses included pH and TA measurements, and quantification of ethanol, residual sugars, glycerol, organic acids and volatile compounds, were performed as described in Hranilovic et al. (2021). Sensory analysis was conducted via Rate-All-That-Apply (RATA) profiling with a panel of 30 experienced wine tasters. The panellists were presented with a list of 46 attributes, and instructed to rate those that are relevant for the tasted sample using a seven-point scale (1 = extremely low, 4 = moderate intensity, 7 = extremely high). Wines (25 mL) were served in opaque ISO-standard glasses coded with four-digit numbers and covered with a glass lid in a random order. Data was collected using RedJade online software (Redwood City, USA), and analysed using a two-way ANOVA (panellists as random and samples as fixed factors) and principal component analysis (PCA) in XLSTAT (Addisoft, Paris, FR).

Results and discussion

High sugar/pH Merlot grapes (14.5 ºBé; 3.9 pH) were fermented with two different yeast treatments, i.e. sequential culture of LT strain pre-selected as a superior bio-acidifying wine starter, and the SC control. The LT treatment resulted in a 0.4 unit lower pH than the SC control wine (3.6 and 4.0, respectively; Table 1), with acidification occurring at the early stages of fermentation. This corresponded to increases in TA in the LT wine (9.4 g/L) as compared to the SC control (4.9 g/L; Table 1). The acidification was induced by the production of >10 g/L of lactic acid (data not shown), and also accompanied with 0.9 % v/v lower ethanol content (Table 1). Lower ethanol yields in LT fermentations are in line with partial diversion of grape sugars from ethanol to lactic acid (Figure 1), and the observed acidity and ethanol modulations were consistent with our previous oenological characterisation of the same LT strain (Hranilovic et al., 2021).

The SC control was next acid-adjusted to match the pH of LT wine with either tartaric acid, as per common winemaking practice, or with lactic acid, which drives bio-acidification in LT. At the same pH (i.e., 3.6) the two wines differed in their TA, which was higher in the wine acidified with weaker lactic acid (9.3 g/L), than in wine acidified with stronger tartaric acid (7.1 g/L; Table 1). The two initial wines were also blended in three ratios, and the acidity parameters and ethanol content depended on the proportion of the bio-acidified LT wine in blends (Table 1). For example, the pH and TA of the LT-predominant blend (3 LT + SC) were 3.7 and TA 9.1 g/L, whereas the SC-predominant blend had pH 3.9 and TA 6.5 g/L (Table 1). The former blend also had 0.5 % v/v less ethanol than the latter one, while the LT + SC had intermediary pH/TA and ethanol content (Table 1).

Table 1. Main oenological parameter of dry bio-acidified (LT) and control (SC) wines, acid-adjusted treatments (SC + TAR, SC + LAC) and three blends.

Wine	pH	TA (g/L as tartaric acid)	Ethanol (%v/v)
SC	4	4.9	16.3
LT	3.6	9.4	15.4
SC + TAR	3.6	7.1	16.3
SC + LAC	3.6	9.3	16.3
3 LT + SC	3.7	9.1	15.6
LT + SC	3.8	7.1	15.8
LT + 3SC	3.9	6.5	16.1

Besides chemical analysis, the wines were blind-tasted by the panel of 30 wine experts via RATA methodology, as described above. The panel detected significant differences in 13 out of 46 attributes (Supplementary Table 1). On the nose, the wines differed in 'overall aroma intensity' and 'cooked vegetables' aroma, and retronasally, in 'red fruit', 'jammy', 'earthy' and 'oxidised' flavour. Two taste attributes were also affected (i.e., 'acidity' and 'bitterness'), alongside three mouthfeel ('hotness', 'body', 'astringency') and length-related descriptors ('acidity length' and 'non-fruit flavour length'; Supplementary Table 1). Interestingly, differences were not detected in 'lactic' (dairy/creamy) aroma and flavour, with low scores across all wines.

The affected sensory attributes were further analysed using PCA, which helped resolve the wine profiles and their relationship to specific attributes (Figure 2). The LT wine was related to an increased intensity and length of acidity, and red fruit flavour. In contrast, the SC wine acidified with tartaric acid was less acidic, but more bitter, hot, astringent and full-bodied, and its flavour was shifted towards jammy and earthy character (Figure 2). The lactic acid-adjusted SC wine, conversely, resembled the bio-acidified LT wine more. It was seen as comparatively less 'ripe' (i.e., hot, bitter, astringent and jammy) and 'fresher' (i.e., red fruit, acidity) compared to the tartaric acid-adjusted wine. The two wine wines had identical pH, ethanol content, aroma and polyphenolic profile, but they differed in TA, which is in fact a better proxy for wine acidity than its pH (Waterhouse et al., 2016). Accordingly, SC + LAC with higher TA was perceived as more acidic than SC + TAR, and the increased acidity in turn affected the perception of the remaining sensory attributes (Figure 2). The three blends also had distinct sensory profiles, which were, just like their chemical composition, modulated depending on the blending ratio (Figure 2). The LT-predominant blend was similar to the LT wine, and the SC-predominant blend to the tartaric acid-adjusted SC wine (Figure 2). This confirmed the potential of bio-acidified LT wines to be used as a blending component to fine-tune chemical and sensory profiles of the wines.

In conclusion, this study confirmed the exceptional bio-acidifying capacity of the pre-selected LT strain, and compared its wine profile to the pH-adjusted SC control. Interestingly, pH adjustment with tartaric and lactic acid resulted in differences in a range of wine sensory attributes, which were driven by higher TA (and thus acidity perception) in lactic acid-adjusted wine. The lactic-acid adjusted wine, in fact, largely resembled to the LT bio-acidified wine, but in the LT wine, acidification occurred without external inputs and was accompanied by decreases in ethanol content. Moreover, as highlighted by our data set, such wine lends itself for use as a blending component to boost 'freshness' and differentiate styles in response to changing climate and consumer acceptance.

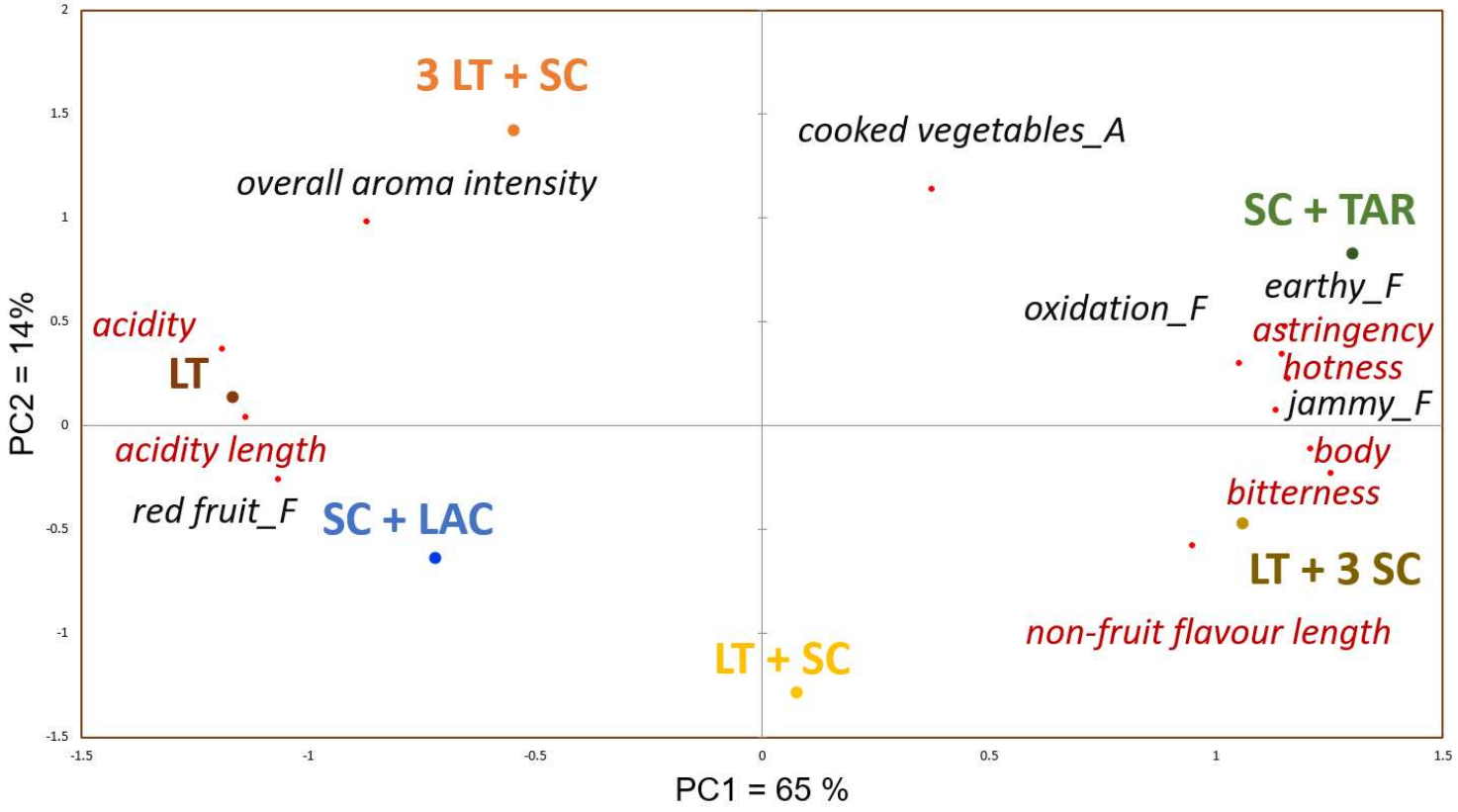

Figure 2. Principal component analysis (PCA) of 13 sensory attributes that significantly different (2-way ANOVA, α = 5 %) between the wines. The attributes in black are related to wine aroma and flavour, and in red to taste, mouthfeel and length. See Table 1 for wine codings.

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Supplementary material

Supplementary Table 1. Sensory attributes for RATA evaluation related to wine aroma (orthonasal perception), flavour (retronasal perception) and taste, mouthfeel and length. The attributes in bold were significantly different (2-way ANVOVA, α = 5 %).

Aroma	Flavour	Taste/mouthfeel/length
overall aroma intensity	overall flavour intensity	sweetness
dark fruit_A	dark fruit_F	**acidity**
red fruit_A	**red fruit_F**	**bitterness**
dried fruit_A	dried fruit_F	**hotness**
jammy_A	**jammy_F**	**body**
confectionery_A	confectionery_F	**astringency**
lactic_A	lactic_F	balance
chocolate_A	chocolate_F	fruit flavour length
cooked vegetables_A	cooked vegetables_F	**non-fruit flavour length**
earth_A	**earthy_F**	**acidity length**
floral_A	floral_F	
herbaceous_A	herbaceous_F	
medicinal_A	medicinal_F	
pepper _A	pepper _F	
savoury _A	savoury _F	
spice_A	spice_F	
oxidation_A	**oxidation_F**	
VA_A	VA_F	