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ORIGINAL RESEARCH ARTICLE

Effects of verjus acidification on muscat canelli grape juice and wines

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ABSTRACT

Our study aimed to investigate the use of verjus (green grape juice) as an acidifying agentfor improving the overall acid profile and balance of wine, lowering the pH and alcohol content and creating a sustainable winemaking practice that can be utilised in warm grape-growing regions, such as Texas, where pH levels range from 3.5 to over 4.0 in white juice and wine. Muscat Canelli wines were produced using two different treatments for pH and acidity balance. Verjus was produced by pressing under-ripe grapes resulting from crop thinning practices and added at two different rates of 2 % and 11 % to juice obtained from ripe grapes prior to fermentation. For all the wines produced, pH, titratable acidity (expressed as g/L Tartaric Acid), alcohol, glucose-fructose, l-malic acid, tartaric acid, potassium, polyphenols were analysed, and preference testing was performed via consumer sensory panels. Results obtained indicate that verjus can be used to balance pH and acidity as well as lower alcohol content. Consumer preference ranking indicated no significant differences between any of the treatments, while acidity rankings correctly distinguished the wines according to their acid levels. This work shows that the use of verjus as a pre-fermentation acidification agent could be a potential tool for winemaking to help mitigate the negative effects associated with climate change.

KEYWORDS: verjus, sustainability, acidification, climate change, grapes, Muscat Canelli, winemaking

INTRODUCTION

High pH, low acid wines are typically made from grapes grown in warmer climate regions (such as Texas, as opposed to Oregon, Canada, and New York state, for example). Typical pH levels in Texas range from 3.50 to over 4.00 in white juice and wine. Increasing temperatures in warmer climate regions cause a higher accumulation of sugars and degradation of acidity due to malic acid consumption, and impacts the synthesis of polyphenols, lowering anthocyanin concentrations and increasing flavonol levels. (Pereira *et al.*, 2022; Hussain Shah *et al.*, 2022). Jones *et al.* (2005) state that projections for future climates indicate an anticipated average warming of 2°C over the next 50 years in global wine-producing regions. For regions already producing high-quality grapes at the limits of their climatic suitability, these forecasts imply that future climate changes may surpass critical thresholds. This could lead to increasing challenges in achieving the balanced fruit ripening necessary for current grape varieties and wine styles.

As temperatures increase across the globe due to climate change, grape-growing regions will most likely start to encounter grape and wine quality issues related to high pH and low acidity (Venios *et al.,* 2020). Some of the wine quality issues attributed to high pH are increased potential for microbial spoilage, oxidation and a shorter shelf life (Payan *et al.*, 2023; Pereira *et al.*, 2022). Another issue related to high pH wines is the effectiveness of sulfur dioxide as an antimicrobial (Payan *et al.*, 2023). This makes pH the main target for acidification, rather than titratable acidity, which mainly influences the palate of the wines. The observed impacts of climate change on the grape and wine industry, along with the emerging wine quality concerns, underscores the imperative for finding effective solutions to the potential wine quality issues.

The most common method for adjusting pH and raising acidity is the addition of tartaric acid (Pereira *et al.*, 2022). Tartaric acid is an organic acid found in grapes which is the most stable acid and has the highest impact on pH (Pereira *et al.*, 2022). Some limitations to adding tartaric acid are the amount needed to adjust the pH to the desired level (especially in wines with pHs of 4 or higher), costs associated with the practice, and the risk of tartaric acid precipitatating out as potassium hydrogen tartrate (Pereira *et al.*, 2022). Other acidification methods include ion exchange resins, glucose oxidase enzyme (GOX) (Pickering *et al.*, 1998; Botezatu *et al.*, 2021), electrodialysis (Bonorden *et al.*, 1986; Walker *et al.*, 2004), addition of acids other than tartaric (fumaric and citric), and the use of *Saccharomyces* and non-*Saccharomyces* yeast (Martínez-Moreno *et al.*, 2023; Payan *et al.*, 2023).

Some research has been conducted to find an innovative, environmentally friendly and sustainable approach to acidification that involves utilising unripe grape juice (verjus) (Kontoudakis *et al.*, 2011; Teslić *et al.*, 2018; Piccardo *et al.*, 2019; Pereira *et al.*, 2022; Martínez-Moreno *et al.*, 2023). This avenue of investigation has multiple motivations, including the fact that it addresses the challenges posed by climate change and rising temperatures in grape-growing regions. It also seeks ways to reduce alcohol content, enhance acidity, lower pH levels and intensify colour in wines (Kontoudakis *et al.*, 2011; Teslić *et al.*, 2018; Piccardo *et al.*, 2019; Pereira *et al.*, 2022; Martínez-Moreno *et al.*, 2023). Moreover, the use of unripe grape juice has gained recognition in the broader food and beverage industry as a valuable food additive and preservative (Karapinar and Sengun, 2007; Tinello and Lante, 2017; Dupas De Matos *et al.*, 2018; Dupas De Matos *et al.*, 2019; Gutiérrez-Gamboa *et al.*, 2021).

Typically, unripe grapes are harvested from the period following flowering until veraison, coinciding with a vineyard technique known as cluster thinning (Carmona-Jiménez *et al.*, 2021). Cluster thinning serves the dual purpose of controlling yield and improving grape quality, albeit at an additional labour cost (Preszler *et al.*, 2013). Grapes from this thinning process are often deemed to be waste, being discarded and left to decay on the ground of the vineyard (Kontoudakis *et al.*, 2011; Carmona-Jiménez *et al.*, 2021). During the herbaceous growth phase, these berries are small, green, and firm (Coombe, 1995). However, unripe grapes are known for their rich antioxidant potential and as a source of bioactive compounds, such as flavonols, flavan-3-ol, cinnamic acids and benzoic acids. (Carmona-Jiménez *et al.*, 2021). Derived from these unripe grapes, Verjus is distinguished by its high acidity, low sugar content, and a tart/sour flavour profile (Nikfardjam, 2008; Hayoglu *et al.*, 2009; Dupas De Matos *et al.*, 2017).

The aim of this research was to investigate the use of verjus as an acidifying agent in order to improve the overall acid profile and balance of wine, lower its pH and alcohol content, and create a sustainable winemaking practice in which waste generated by cluster thinning is minimised. This is the first study of its kind in the United States, and the proposed method could be key to increasing wine quality in Texas and other warm growing regions in the country and abroad.

MATERIALS AND METHODS

1. Chemical reagents and standards

Acetic Acid, Glucose-Fructose, L-Malic Acid, Polyphenol, Potassium and Tartaric Acid reagents were purchased from Thermo Fisher Scientific (Vantaa, Finland). Glucose-Fructose, L-Malic Acid, and Tartaric Acid standards were purchased from Thermo Fisher Scientific (Vantaa, Finland). Spectrum Anhydrous Gallic Acid (Gardena CA, USA) for the Polyphenol standard was purchased from The Lab Depot (Dawsonville, GA, USA). Glacial Acetic Acid for the Acetic Acid standard was purchased from Fisher Chemical (Fair Lawn, NJ, USA). For the Potassium standards, Potassium Chloride was purchased from Sigma Aldrich (St. Louis, MO, USA). 20-degree brix solution, 0.1 N Sodium Hydroxide, 0.01 N Sodium Hydroxide, 25 % Phosphoric Acid, 30 % Hydrogen Peroxide, Tartaric Acid, Potassium Metabisulfite, Sulfur Color Indicator Solution, pH 4 and pH 7 buffer solutions were purchased from Vintner's Vault (Paso Robles, CA, USA). Yeast, yeast nutrient and yeast starter were purchased from Scott Labs (Petaluma, CA, USA). The water used in all experiments were purified by Mega-Pure System MP-6A (Barnstead - Corning, Dubuque, Iowa, USA).

2. Experimental setup

The treatments examined the use of verjus as an acidification method for winemaking. There were two treatments for the acidification of juice obtained from ripe grapes with verjus and a control, performed in duplicate or triplicate. In total, there were six carboys for the trial treatment set up. The treatments were determined based on pH, as this is an important factor affecting the quality of grapes and wines in Texas. Treatment 1 targeted a low pH range that represents a typical white wine pH (3.3). Treatment 2 was set up in order to asses how much verjus it would take to drop the pH 0.1 units, which, ultimately represented a middle pH range between Treatment 1 and Control as shown in Figure 1.

Figure 2 outlines the steps in the winemaking process for this research. The unripe grapes were harvested, destemmed and pressed to make fresh juice. Once the fresh unripe grape juice had been pressed the juice was sulphited (50 ppm addition) and allowed to settle for twenty-four hours. After the twenty-four hour settling period the juice was racked and sulphited again (50 ppm addition). The juice was returned to cold storage at 5 °C until the ripe grapes had been harvested. Three separate actions were then carried out: chemical analysis was performed in triplicate on the unripe grape juice, part of the unripe grape juice was blended with the juice obtained from ripe grapes as needed for the Treatments, and the remaining unripe grape juice was filtered and bottled as verjus.

FIGURE 1. Verjus trial set up with treatments.

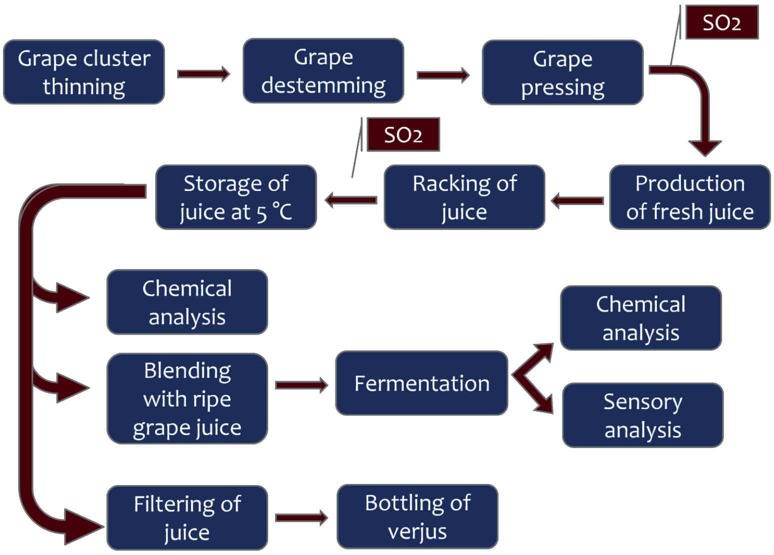

FIGURE 2. Winemaking process flow for the verjus trial.

3. Grape selection

Unripe and ripe Muscat Canelli grapes were harvested from Lost Draw Vineyards in Brownfield, TX, USA. Unripe Muscat Canelli grapes were hand-harvested (Corona Long Straight Snip, Corona, CA, USA) at growth stage 34 of the modified E-L phenological classification scheme (Coombe, 1995). Ripe Muscat Canelli grapes were harvested at the modified E-L phenological classification growth stage 38 (Coombe, 1995). The grapes were transported back to Texas A&M University in College Station, TX, USA, for weight measurement and processing.

4. Verjus production

A total of 186.2 kg of unripe grapes were destemmed (Enotecnica Pillan Pigia-Diraspatrice, Asti, Italy) and pressed in a hydraulic basket press (Enotecnica Pillan Tico 40, Asti, Italy) This resulted in 97.5 L of unripe grape juice, which was collected in 5-gallon (19 L) carboys (5-Gallon Glass Carboy, Northern Brewer, Milwaukee, WI, USA) and allowed to cold settle for 24 hours at 5 °C. Fifty ppm of potassium metabisulfite (Vintner's Vault, Paso Robles, CA, USA) was added to each carboy. After cold settling, the juice was transferred to four 5-gallon (19 L) carboys (5-Gallon Glass Carboy, Northern Brewer, Milwaukee, WI, USA) to eliminate the lees. A further 50 ppm SO_2 was added to each carboy after transfer of the unripe juice. The total verjus obtained was 80.4 L, which was stored at 5 °C until the ripe grapes had been harvested (28 days). Chemical composition was analysed for brix (Anton Paar DMA-35 Density Meter, Ashland, VA, USA), pH (Orion 8107UWMMD Ross Ultra pH/ATC Triode, Thermo Fisher Scientific, Vantaa, Finland), titratable acidity by titration to pH 8.21 using pH probe (Orion 8107UWMMD Ross Ultra pH/ATC Triode, Thermo Fisher Scientific, Vantaa, Finland), burette (Bomex Chemical, Shanghai, China), and 0.1 N Sodium Hydroxide (Vintner's Vault, Paso Robles, CA, USA). Glucose-fructose, L-malic acid, tartaric acid, potassium, polyphenols were measured by Gallery enzymatic analyser (98611001, Thermo Fisher Scientific, Vantaa, Finland). All analyses were performed in triplicate.

5. Wine production

A total of 277.5 kg of ripe grapes was destemmed (Enotecnica Pillan Pigia-Diraspatrice, Asti, Italy) and pressed in a hydraulic basket press (Enotecnica Pillan Tico 40, Asti, Italy) to collect the juice. A volume of 128.7 L of grape juice was collected in four 5-gallon (19 L) carboys (5-Gallon Glass Carboy, Northern Brewer, Milwaukee, WI, USA) and three 3-gallon (11 L) carboys (3- Gallon Glass Carboy, Northern Brewer, Milwaukee, WI, USA) and allowed to cold settle for 24 hours at 5 °C. Fifty ppm of potassium metabisulfite (Vintner's Vault, Paso Robles, CA, USA) was added to each carboy. After cold settling, 83.3 L of juice was transferred to an Ag Inox (Italy) 100 L stainless steel variable capacity tank. The homogenised juice was transferred to seven 3-gallon (11 L) carboys (3-Gallon Glass Carboy, Northern Brewer, Milwaukee, WI, USA) for the trial treatments. The juice was analysed for its chemical composition after homogenisation: brix (Anton Paar DMA-35 Density Meter, Ashland, VA, USA), pH (Orion 8107UWMMD Ross Ultra pH/ATC Triode, Thermo Fisher Scientific, Vantaa, Finland), titratable acidity by titration to pH 8.21 using pH probe (Orion 8107UWMMD Ross Ultra pH/ATC Triode, Thermo Fisher Scientific, Vantaa, Finland), burette (Bomex Chemical, Shanghai, China), and 0.1 N Sodium Hydroxide (Vintner's Vault, Paso Robles, CA, USA). Glucose-fructose, L-malic acid, tartaric acid, and potassium was measured by Gallery enzymatic analyser (98611001, Thermo Fisher Scientific, Vantaa, Finland). All analyses were performed in duplicate.

Treatment 2 and Control were set up in duplicate replications, while Treatment 1 was done in triplicate due to juice availability. Following benchtop trials, 1.1 L of verjus was blended into 10.2 L of ripe juice for Treatment 1 (11 % verjus addition). Treatment 2 had 0.2 L of verjus blended into 8.5 L of ripe juice (2 % verjus addition). Treatment 2 was set up to gain an understanding of how much verjus it would take to adjust the pH 0.1 units. Treatment 1 aimed to create balance between pH and acidity levels for an aromatic white wine, and to help gain an understanding of how this affects juice and wine quality chemically and sensorially.

Each treatment was inoculated with yeast nutrient GoFerm (Lallemand, Montreal, QC, Canada) and *Saccharomyces cerevisiae* & *Saccharomyces cariocanus* hybrid (Anchor Exotics Novello, Capetown, South Africa). The wines were fermented to dryness (<1.0 g/L Glucose-Fructose) over the course of twenty days. After fermentation was completed, the wines were racked and sulfited with 70ppm potassium metabisulfite (Vintner's Vault, Paso Robles, CA, USA). The wines were filtered (Buon Vino Mini Jet Filter, No. 2-3 filter pads, Calgary, AB, Canada) two days after fermentation had ended. After filtration and adjustment to 0.8 ppm molecular SO_2, the wine was bottled and labelled according to treatment. Wines were stored at 13 °C until use. The wine was analysed for chemical composition: alcohol (Alcolyzer Wine M, Anton Paar, Ashland, VA, USA), pH (Orion 8107UWMMD Ross Ultra pH/ATC Triode, Thermo Fisher Scientific, Vantaa, Finland), titratable acidity by titration to pH 8.21 using pH probe (Orion 8107UWMMD Ross Ultra pH/ATC Triode, Thermo Fisher Scientific, Vantaa, Finland), burette (Bomex Chemical, Shanghai, China), and 0.1 N Sodium Hydroxide (Vintner's Vault, Paso Robles, CA, USA). Glucose-fructose, L-malic acid, tartaric acid, potassium, acetic acid was measured by Gallery enzymatic analyser (98611001, Thermo Fisher Scientific, Vantaa, Finland). Free sulfur was monitored by aeration-oxidation using an aeration-oxidation apparatus (Adams & Chittenden Scientific Glass Coop., Berkeley, CA, USA), a burette (Bomex Chemical, Shanghai, China), 25 % phosphoric acid (Vintner's Vault, Paso Robles, CA, USA) and 0.01N sodium hydroxide (Vintner's Vault, Paso Robles, CA, USA). All analyses were performed in duplicate. Polyphenolics results were determined by colorimetric Folin-Ciocalteu method by chromogenic complex on the Thermo Scientific Gallery Analzyer (Vantaa, Finland).

6. Sensory analysis

In a preliminary step, the wines were evaluated by oenologists and viticulturists of the Texas A&M University AgriLife Extension team to investigate possible differences between duplicates within the same treatments. No differences were found.

After the preliminary step, one wine per treatment plus the control were selected for the consumer panel evaluations. The three wines were evaluated in terms of preference and acidity perception by 218 Texas wine industry professionals and consumers taking part in four Texas wine industry conferences. The panel was asked to carry out two tasks: the first was to rank the three wines in order of preference, with 1 being the most preferred and 3 being the least preferred and the second to rank the three wines based on acidity, with 1 being the most acidic and 3 being the least acidic. Wines were coded with a three-digit random number code and distributed in random order to all participants in transparent plastic cups containing 20 mL of wine. The individuals were asked to transfer the wines to ISO wine tasting glasses for the sensory evaluation. Bottled water was distributed to assessors for palate cleansing. This research had the ethics approval of the Institutional Review Board at Texas A&M University (IRB approval number IRB2022-1217M).

7. Statistical analysis

The chemical composition (triplicate analytical values) of the juice and wine treatment (duplicate or triplicate biological replicates) data were statistically evaluated using the one-factor analysis of variance (ANOVA) using JMP (v16.0, SAS Institute, Cary, NC, USA). ANOVA tests were carried out by comparisons between treatments using Tukey HSD test and a paired t-test. A predetermined alpha of 0.05 was used. Treatment and rep were used as fixed effects.

The consumer panel sensory data was statistically evaluated by liking data analysis (hedonic data) in XLSTAT (Addinsoft, New York, NY, USA), followed by Tukey's HSD with a predetermined alpha of 0.05.

RESULTS & DISCUSSION

1. Verjus chemistry & yield

The yield of the verjus juice obtained from cluster-thinned grapes was 43.2 %. This is consistent with findings from Hayoglu *et al.* (2009) who obtained a juice yield of 47 % from two Turkish grape varieties (Kabarcik and Yediveren) that were harvested at 45 days post bloom. In general, juice yield from pressing unripe grapes varies from 40 % to 60 % (Fia *et al.*, 2021). This wide range of juice yield could be attributed to grape cultivar and harvest date.

The chemical composition of Muscat Canelli verjus is shown in Table 1. The soluble solids (brix) were 9.4 degrees brix, which falls within the range of findings from other studies in which soluble solids ranged from 3.55 to 17.7 degrees brix. The measured glucose-fructose level in this study averaged at 60.7 g/L (Fia *et al.*, 2022). This is slightly higher than other data obtained in previous studies using unripe grape products, in which the majority of unripe grape juices were found to have less than 50 g/L of sugar. The high sugar level in the verjus can be attributed to the timing of cluster thinning, as well as the warmer growing season in Brownfield, Texas, USA, in 2021. The grapes were cluster-thinned on 7 July 2021; the recorded temperatures were higher than the historic daily averages in June, and about average to above average in July. Malic and tartaric acids stand out as the predominant organic acids found in green grapes, collectively accounting for 90 % of the total acid content (Fia *et al.*, 2022). During the phase of herbaceous growth, malic acid accumulates rapidly, while the accumulation of tartaric acid occurs at a comparatively slower rate. This pattern aligns with the growth tendencies of grapevines and is in line with previous observations of low pH and high titratable acidity in unripe grape-based products (Fia *et al.*, 2022). Our data fall in line with this, with 16.7 g/L malic acid and 9.6 g/L tartaric acid quantified in our verjus. In the present study, the pH was measured to be 2.60, and titratable acidity 27.3 g/L.

As can be observed in Table 1, the mean potassium level (K+) was 1,124 mg/L. It is worth noting that, to the best of our knowledge, this represents the first recorded data concerning potassium levels in unripe grape juices. In an earlier study by Boulton (1980), it was revealed that a juice could exhibit a pH of below 3.25 while maintaining a relatively high concentration of potassium. To enhance our comprehension of the interplay between potassium and pH in verjus, further exploration is required. in grape juice.

As can be seen in Table 1, the mean polyphenolic content of Muscat Canelli verjus was found to be 337 mg/L. These results fall into the lower range of polyphenolic content for verjus according to Nikfardjam (2008). Using the colorimetric Folin-Ciocalteu method, Nikfardjam (2008) analysed the chemical composition of various verjus samples from France, Germany and Iran and found that polyphenolics ranged from 315 to 1330 mg/L. The authors highlighted the fact that the concentration of polyphenols was higher in the products with a lower sugar concentration, and our results align with this, given the higher sugar content in the Muscat Canelli verjus. To further support this finding, verjus processed from earlier-harvested grapes would need to be analysed in order to gain a better understanding of the relationship between sugar and polyphenols.

TABLE 1. Muscat canelli verjus chemistry.

	Brix	pH	TA$^{(1)}$	G/F$^{(1)}$	ML$^{(1)}$	H2T$^{(1)}$	K+$^{(2)}$	Polyphenol$^{(2)}$
Muscat Canelli Verjus	9.4 (±0.00)	2.6 (±0.00)	27.3 (±0.22)	60.7 (±0.16)	16.7 (±0.10)	9.6 (±0.05)	1124 (±6.76)	337 (±0.58)

* Concentrations are mean values (n = 3) expressed in g/L (1) and mg/L (2). TA = Titratable Acidity expressed as Tartaric Acid, G/F = Glucose-Fructose, ML = L-Malic Acid, H2T = Tartaric Acid, K^+ = Potassium. (text) = Standard Deviation.

2. Juice treatment chemistry

The acid profile of ripe Muscat Canelli juice that has been acidified with verjus versus the control can be seen in Figure 3. Treatment 1 was acidified with a higher amount of verjus (1.1 L of verjus resulting in a 11 % addition) and the chemical results show a greater change in pH, titratable acidity, malic acid and tartaric acid compared to Treatment 2 and Control. The adjusted pH was 3.3 and titratable acidity 7.1 g/L compared to Control, for which the pH was 3.6 and the titratable acidity was 4.1 g/L. Treatment 2 was conducted to assess the amount of verjus required to induce a 0.1 unit change in pH. This treatment demonstrated that even a minor addition of verjus can exert a noticeable influence on juice chemistry. In comparison to the Control group, Treatment 2 exhibited a pH of 3.5 and a titratable acidity of 4.7 g/L, which was significantly different from Control. The trial results underscore the beneficial impact of verjus acidification on juice composition, as it enhances titratable acidity while reducing pH levels. These findings are consistent with a previous study in which the composition of grape must was analysed before fermentation (Teslić *et al.*, 2018). Since the verjus had higher levels of organic acids, increases in malic acid and tartaric acid were observed in both treatments. These results were expected when adding higher acid and low pH juice to juice of a higher pH and lower acidity. These findings show that it is feasible to use this as an alternate natural, sustainable acidification method for grape juice. When statistically analysing the chemistry results (ANOVA, p-value < 0.05) all of the treatments for each parameter tested were found to be significantly different. This shows that verjus acidification has an impact on the overall composition of grape juice.

3. Wine treatment chemistry

Post fermentation, the chemical parameters of the Control, Treatment 1 and Treatment 2 were analysed to gain a further understanding of how acidifying with verjus affects wine chemistry. The results can be seen in Figure 4 and Figure 5. Looking at Figure 4, the results show positive impacts on the acid profile with regards to pH, titratable acidity, malic acid and tartaric acid. The pH and titratable acidity between the two treatments and Control group were shown to be significantly different, with Treatment 1 having the biggest impact on these parameters compared to the Control group because of the higher rate of verjus used for acidification. The pH and acidity of Treatment 1 were 3.3 and 7.2 g/L respectively, compared to the Control group for which the pH was 3.6 with a titratable acidity of 5.6 g/L. The pH and titratable acidity for Treatment 2 fell into the middle of Treatment 1 and the Control group, with the pH being 3.5 and titratable acidity 5.9 g/L. Previous studies by Kontoudakis *et al.* (2011), Piccardo *et al.* (2019), Pereira *et al.* (2022) and Martínez-Moreno *et al.* (2023) showed positive impacts on pH and titratable acidity when acidifying using verjus. The observed impact of verjus acidification on wine chemistry in relation to pH and titratable acidity indicates that this is a feasible method for use in winemaking.

Between the pre- and post-fermentation stages, increases in titratable acidity were found in the two treatments and Control groups. Control and Treatment 2 had the largest increases in titratable acidity with increases of 1.5 g/L and 1.2 g/L respectively. We hypothesise that this was due to the production of succinic acid during fermentation. According to Coulter *et al.* (2004), the increases in titratable acidity that occur pre to post fermentation can sometimes be attributed to increases in succinic acid, which is a by-product of alcoholic fermentation. Such increases in succinic acid concentrations have previously been linked to the shift in central carbon metabolism of yeast (Larsson *et al.*, 1993; Coulter *et al.*, 2004; Aceituno *et al.*, 2012). There are other factors that could influence the production of succinic acid, including fermentation temperature, aeration, must clarity

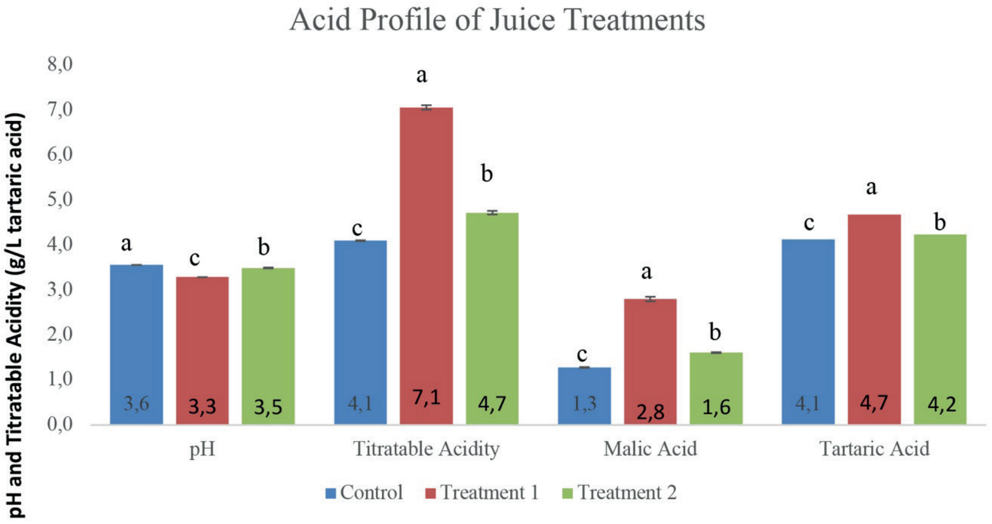

FIGURE 3. Acid profile of verjus acidification treatments vs. control in juice.

[a]different letters indicate significantly different values based on Tukey's HSD test, p = 0.05; pH expressed as pH units. Titratable acidity expressed as tartaric acid, malic acid, and tartaric acid are represented as g/L. Mean values (n = 3) are presented.

and other environmental factors (Coulter *et al.*, 2004). A potential issue related to increased acidity from succinic acid production is the perceived taste associated with succinic acid. Succinic acid can give a salty-bitter taste to wines (Bayraktar, 2013). Further research into succinic acid production from pre to post fermentation with verjus additions is needed in order to understand the increases in titratable acidity during fermentation.

As can be seen in Figure 4, the increases in malic acid content were significant between the treatments and the Control group. These increase can be attributed to the high content of malic acid present in the verjus. In the Control group, the malic acid content was 1.0 g/L, while in Treatment 2 it was 1.2 g/L and in Treatment 1 it was even higher at 2.0 g/L. The increases in malic acid concentrations in the acidified wine treatments are consistent with the results of a similar study on verjus-acidified wine by Teslić *et al.* (2018). We also note that after fermentation the content of malic acid decreased, which could be attributed to the ability of *Saccharomyces cerevisiae* to metabolise malic acid during alcoholic fermentation. The degradation of malic acid during fermentation was first reported in 1966 (Mendes Ferreira and Mendes-Faia, 2020; Rankine, 1966). An alternative potential explanation for the degradation process of malic acid during fermentation is the conversion of malic acid into succinic acid, as suggested by Baroň and Fiala (2012) and supported by Scutarașu *et al.* (2021).

An analysis of the tartaric acid content, as illustrated in Figure 4, revealed that Treatment 1 exhibited the highest mean concentration (3.6 g/L), exceeding both Treatment 2 and Control (3.0 g/L). Statistically, Treatment 1 is significantly different from Treatment 2 and Control. The higher amount of tartaric acid in Treatment 1 can be attributed to the larger addition of verjus before fermentation. After fermentation, tartaric acid content tends to decrease due to the occurrence of various physicochemical mechanisms, including ethanol accumulation or neutralisation via cations, such as Potassium, Calcium, and Sodium (Scutarașu *et al.*, 2021). It is worth noting that tartaric acid remains unmetabolised during winemaking (Rajkovic *et al.*, 2007; Scutarașu *et al.*, 2021). Furthermore, the degradation of tartaric acid is associated with yeast species activity during fermentation (Mendes Ferreira and Mendes-Faia, 2020). These findings are consistent with the Teslić *et al.* (2018) study, in which increased tartaric acid concentrations were observed in acidified treatment versus the control.

Verjus addition affects alcohol concentration by altering sugar content, as depicted in Figure 3. This impact is significant, with the control wine showing 14.3 % alcohol from 23.9 degrees brix, while Treatment 1 exhibits the most notable changes, with 13.2 % alcohol and 22.4 brix. Conversely, Treatment 2 is minimally affected, resulting in 14.1 % alcohol and 23.6 brix, attributed to a smaller verjus addition. These findings align with previous studies, and this indicate that verjus-induced acidification is a potential winemaking technique to mitigate alcohol and sugar levels, which is crucial given the impact of climate change on grape quality.

This proposed practice has previously been shown to be financially sound. Using data generated by our experiments, Marbach *et al.* (2023) investigated the economic feasibility of producing and using verjus as an acidifier or a stand-alone product in Texas vineyards and wineries. They concluded that the use of verjus as an acidifier in the winery could be economically profitable due to the increase in wine volume following verjus additions. Furthermore, the production and sale of verjus as a stand-alone culinary product in wineries was also found to be highly profitable, supporting the adoption of these practices in warm-climate wineries.

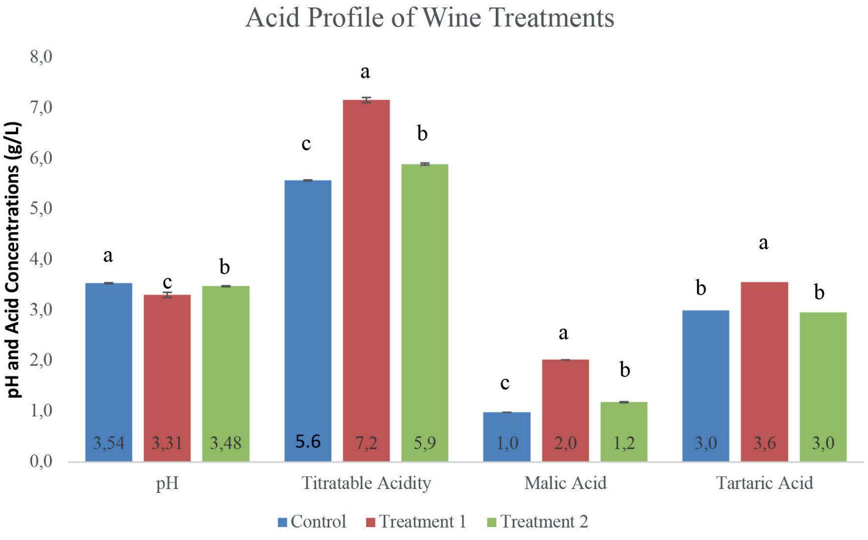

FIGURE 4. Acid profile of verjus acidification treatments vs. control in wine.

[a] Different letters indicate significant differences within each category based on Tukey's HSD test, p = 0.05; pH expressed as pH units. Titratable acidity expressed as tartaric acid, malic acid and tartaric acid are represented as g/L. Mean values (n = 3) are presented.

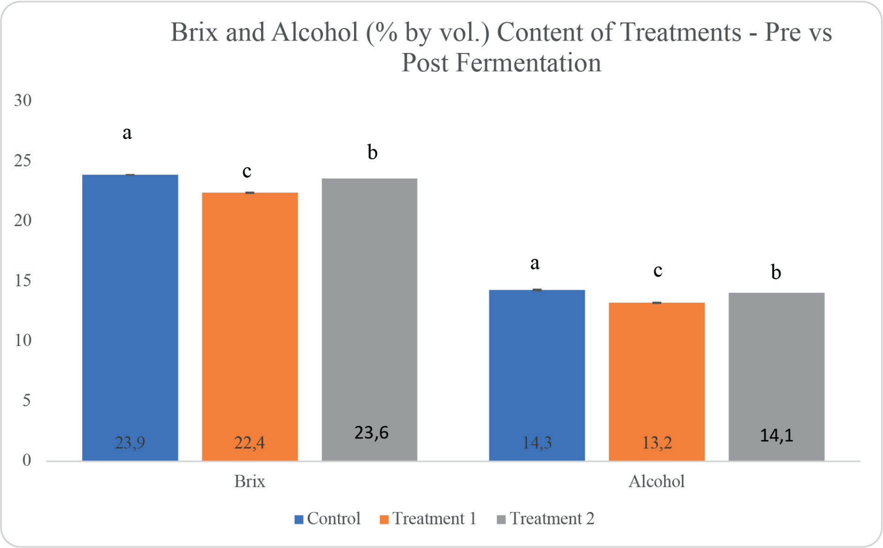

FIGURE 5. Alcohol content of verjus-acidified wines.

[a] Different letters indicate significant differences within each category based on Tukey's HSD test, p = 0.05; sugar is expressed in degrees brix and alcohol expressed as a percentage of ethanol. Mean values (n = 3) are presented.

4. Sensory analysis data

Four different tastings took place at four different industry events in the state. There was a total of 218 assesors over the four sessions. In the first task each assessor rated their wine preference on a scale of 1 to 3. A score of 1 indicated the highest preference, while a score of 3 indicated the lowest. The results of this preference assessment are presented in Table 2 as means of overall evaluations by all participants. No significant differences were found between the means, as shown in Table 3. Figures 6 and 7 display the range of the ratings given to each of the wines based on preference (Figure 6) and acidity (Figure 7).

TABLE 2. Mean values of consumer preference rankings.

	Value
Treatment 1	2.092
Treatment 2	1.968
Control	1.936

* Mean values of preference rankings (1 being the most preferred, 3 being the least preferred) for Treatment 1 (high acidity, low pH), Treatment 2 (intermediate acidity and pH) and Control wines (no verjus addition). Number of subjects = 218, p = 0.05

TABLE 3. Consumer preference rankings – statistical groupings based on mean values.

	LS Means	Standard Error	Lower Bound (95 %)	Upper Bound (95 %)	Groups
Treatment 1	0.093	0.055	-0.015	0.202	A
Treatment 2	-0.031	0.055	-0.139	0.078	A
Control	-0.063	0.055	-0.171	0.046	A

*Statistical groupings based on mean values, according to Tukey's HSD, p = 0.05. Treatments with the same letter are not statistically different from each other.

Furthermore, the analysed data from each individual event also show no significant differences between treatments for any of the events, (data not included). This indicates that the use of verjus had no negative impact on the sensory profiles of the acidified wines.

The second ranking task sought to establish whether the assessors would be able to perceive the difference in acidity between the three wines and rate them accordingly. When asked to rank the wines based on acidity, the assessors correctly identified Treatment 1 as being the most acidic: Treatment 1 had the highest titratable acidity value and the lowest pH. The least acidic wine - also correctly identified by the assessors - was the control wine, which had the lowest titratable acidity value and highest pH. Treatment 2, which had intermediary acidity and pH levels, was ranked between Treatment 1 and Control. The results from the acidity ranking can be found in Tables 4 and 5. These results are derived from the aggregated data from all four tasting events for a total number of 218 assessors, and they indicate a clear difference in perceived acidity levels, as was expected based on the wines' chemistry.

TABLE 4. Mean values of consumer acidity rankings.

	Value
Treatment 1	1.642
Treatment 2	2.064
Control	2.294

* Mean values of acidity rankings (1 being the most acidic, 3 being the least acidic) for Treatment 1 (high acidity, low pH), Treatment 2 (intermediate acidity and pH) and Control wines (no verjus addition). Number of subjects = 218.

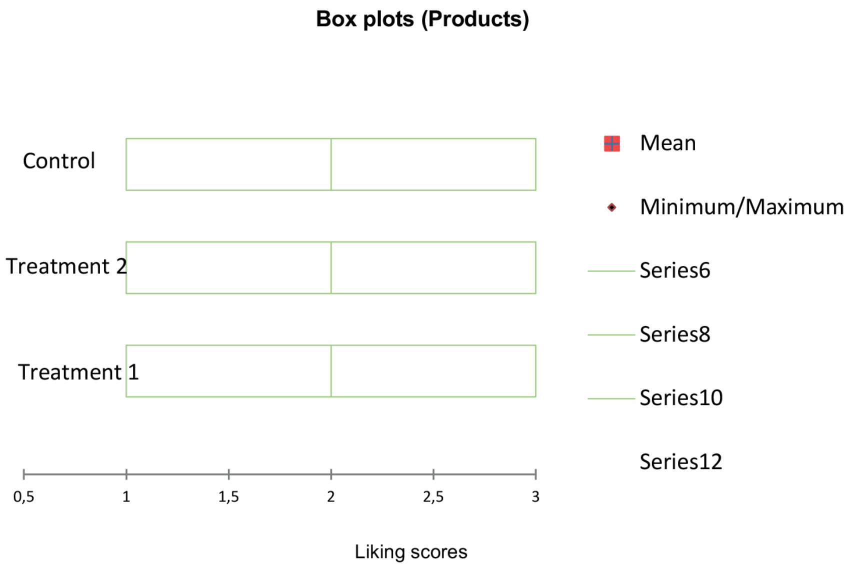

FIGURE 6. Box plots of the liking scores by product.

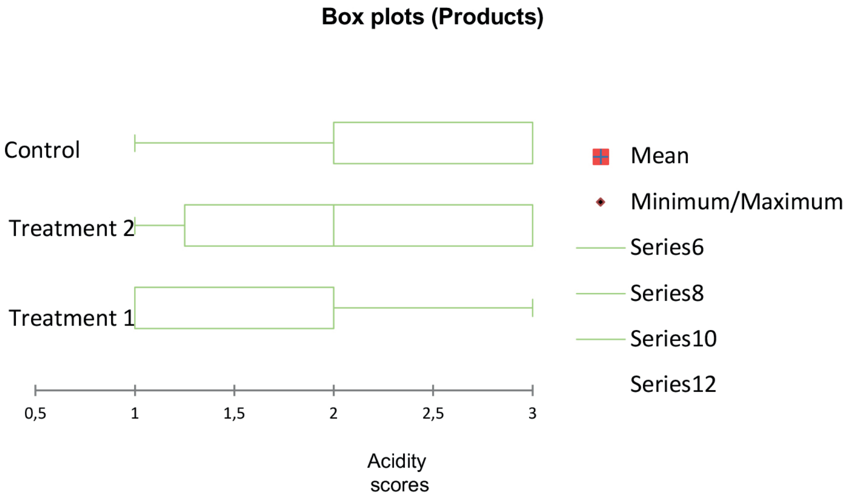

FIGURE 7. Box plots of the acidity scores by product.

TABLE 5. Consumer acidity preference rankings – statistical groupings based on mean values.

	LS Means	Standard Error	Lower Bound (95 %)	Upper Bound (95 %)	Groups		
Control	0.294	0.052	0.191	0.396	A	-	-
Treatment 2	0.064	0.052	-0.039	0.167	-	B	-
Treatment 1	-0.358	0.052	-0.461	-0.255	-	-	C

*Statistical groupings based on mean values, according to Tukey's HSD, p = 0.05. Treatments with different letters are statistically different from each other.

While our sensory data were not used to investigate specific organoleptic descriptors for the three treatments, they showed that the use of verjus as an acidifier did not have a negative impact on the sensory profiles of treated wines, and that verjus has the capacity to modify acidity, both chemically and sensorially. Further descriptive work on this acidification method would be beneficial.

CONCLUSION

The results obtained from this study indicate that the utilisation of verjus for acidifying grape juice and wine has a significant impact s it reduced pH, increased titratable acidity and reduced alcohol content. These findings demonstrate that verjus had favorable effects on the chemical composition of both juice and wine and no negative effects on the sensory profile of the wines. Consequently, this method could prove to be a sustainable and practical approach to addressing issues related to high pH in grapes grown in warmer climates. Furthermore, it opens up a potential avenue for decreasing alcohol levels in wines made from grapes that undergo excessive sugar accumulation during ripening; it is thus a potentially valuable tool in the wine industry for addressing the adverse effects of climate change. Importantly, implementing this acidification method in most wineries would be straightforward, as it does not require any additional equipment.

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