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Comparative Study of the Sensory Impacts of Acidifiers for Red Wine Production

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Abstract: Rising temperatures have caused a major shift in wine chemistry, including increased sugar and pH along with decreased acidity. Wines produced from such grapes tend to be microbiologically unstable and are often described as unpalatable. This study looks at treatments to lower pH and enhance sensory characteristics of wines produced from grapes grown at higher temperatures. The four acidification treatments included the following: tartaric acid; verjus—an acidic juice made from unripe grapes; glucose oxidase with catalase enzyme (GOx), which converts glucose to gluconic acid; and ion exchange. All treatments were able to reduce pH to the target pH of 3.6. Sensory analysis was conducted using the Hierarchical Rate-All-That-Apply (HRATA) method and preference testing. Analysis of the HRATA and GCMS data using Principal Component Analysis (PCA) accounted for 78.69% and 70% of the variance observed, respectively. Wines treated with GOx and verjus exhibited the most distinct sensory profiles when compared to each other, the other treatments, and the control group. GOx-treated wines were associated with positive flavor descriptors including caramel, hazelnut, lemon, and fruity which correlated well with the aromatic compounds determined by GCMS. There were no significant differences in consumer preferences of treatments. This study shows how different acidifiers can be utilized by winemakers to affect not just the pH and acidity but also the aromatic and flavor profile of the wine.

Keywords: acidification; climate change; HRATA; sensory; wine

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1. Introduction

With global temperatures on the rise, significant shifts in grape chemistry have been observed. In recent times, grapes are being harvested 2–3 weeks earlier compared to centuries ago [1]. This accelerated berry maturation leads to increased sugar levels and decreased acidity. Wines crafted from Texas grapes exhibit elevated pH levels, reduced acidity, and increased alcohol resulting in wines that are flabby and unpalatable [2]. Wines with high pH levels pose various challenges for winemakers, as they are susceptible to issues related to microbial, color, protein, and tartrate stability. These issues, in turn, have adverse impacts on the appearance, aging potential, aroma, and phenolic content of the wine [3,4].

Currently, chemical acidification is the most common method to overcome high pH. Tartaric acid is naturally formed in grapes and is currently the most popular acidifier for

wine [5]. Other acids including citric, malic, lactic, and fumaric can be used. Fumaric acid is a stronger acidifier than tartaric; it is currently approved for use as an acidifier by the Codex Alimentarius and is under review for acidification purposes by the OIV [6]. Currently, the OIV only approves fumaric acid for use as a malolactic fermentation inhibitor. There are some drawbacks of using these acid additions, such as the occurrence of potassium bitartrates (KHT) when using tartaric acid; this requires cold stabilization or other remediation processes in order to avoid it occurring in bottle [7]. When adding malic acid, it can allow for malolactic fermentation which would increase pH and change the sensory profile of the wine. Lactic acid is a very weak acid and would require a large addition to lower the pH [5].

Verjus is a highly acidic juice made from unripe grapes. This product traces its origins to the Middle East and is traditionally used in cooking in countries like Iran and Turkey. Verjus has a low pH that makes it able to lower the pH of must/juice. Unlike other acidifiers, verjus has the opportunity to operate not only as an acidifier but also enhance the aromatic and nutritional properties of wine. Verjus can be high in phenolic compounds which could lead to a higher antioxidant content in finished wine. Exact phenolic content depends on how early the unripe grapes are harvested [8]. A recent study published in 2024 found that verjus can successfully lower pH and raise titratable acidity to produce a more balanced wine and lower overall alcohol content. The study also found no difference in consumer preference between the wines made with verjus vs. wines made without any acidifiers [9]. Although more expensive as an acidifier than tartaric acid, the "added volume of the verjus could offset the cost, making it desirable" [10].

Glucose oxidase in conjunction with catalase enzyme (Catazyme) works by converting glucose into gluconic acid, which results in a wine that is lower in alcohol due to the decreased amount of sugar and that has higher acidity levels. At a 1 g/L addition, Catazyme was shown to drop pH from 4.6 to 3.8 in a 24 h period. Catazyme was shown to have positive effects on the color and aromatics of the finished wine. Wines treated with Catazyme were described as having "cherry, fig/raisin, fruity, and sour" aromas and flavors [11]. Other studies have shown a 78% decrease in sugar after a 48 h treatment, resulting in decreased alcohol content compared to control [12].

Ion exchange is a physical acidification method that involves the use of a polymerized resin that allows for protons to be exchanged with cations in wine, thus reducing the pH. This method is approved by the OIV [13]. A study performed in 2013 showed a decrease in anthocyanin and tannin composition along with a decrease in aromatic composition when wines were treated post fermentation. However, the wines treated with ion exchange performed better in sensory evaluation than untreated wines. Ion exchange has also been used to "improve its tartaric and oxidative stability" [7].

The present study compares the use of tartaric acid, verjus, GOx, and ion exchange in the making of red wine and their impacts on the finished sensory profile of the wine. As the demand for effective acidification methods increases, it is paramount to examine these acidifiers in tandem to discern their implications for wine chemistry, flavor profile, and consumer preference.

2. Materials and Methods

2.1. Reagents and Enological Products

Reagents for the Gallery enzymatic analyzer were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Potassium metabisulfite (KMS), tartaric acid, antifoaming agent, yeasts, and nutrients were obtained from The Vintner Vault (Hye, TX, USA). Catazyme was obtained from Novozymes (Copenhagen, Denmark) and Tulsion Thermax Resin (H+ ion) was supplied by Wedding Oak Winery (San Saba, TX, USA).

2.2. Chemical Analyses of Verjus, Must, and Wine

Verjus was analyzed post pressing and racking. Must was analyzed pre-acidification and post-acidification, and wine was analyzed after fermentation was complete. pH and titratable acidity (TA) were measured using an Orion Star A321 portable pH meter (Thermo Fisher Scientific, Waltham, MA, USA). The TA method utilized is as described in Iland, 2013 [14] by titrating to pH 8.2. SO2 was analyzed using the aspiration–oxidation method as described in Iland, 2013 [14]. Brix was measured using an Anton Parr DMA 35 basic (Ashland, VA, USA). Alcohol was measured using an Anton Parr Alcolyzer M for wine (Ashland, VA, USA). Tartaric, malic, and lactic acid, as well as potassium and polyphenols, were determined by the Gallery Discrete Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) utilizing methods provided by Thermo Fisher Scientific.

2.3. Verjus Making

Verjus was made using Tempranillo grapes that were thinned at E-L growth stage 32–33, where bunch closure has begun but the berries are still very hard, version has not begun, and the berries are still green [15]. Vines were thinned to 8 clusters per vine. Grapes were destemmed then pressed using a hydraulic press. After the first press, the pomace was then stirred and pressed a final time. The resulting yield was 43.39%. KMS was added at a rate of 50 mg/L for stability and storage purposes. Verjus was stored in a cooler at 5 degrees Celsius until harvest of ripe wine grapes. Table 1 shows the final verjus chemistry.

Table 1. Tempranillo verjus chemistry.

Brix	pH	Titratable Acidity	Glucose/ Fructose [1]	Malic Acid [1]	Tartaric Acid [1]	Potassium [2]	Polyphenol [2]
3.9 (±0.0)	2.7 (±0.0)	24.5 (±0.0)	4.7 (±0.0)	16.5 (±0.1)	7.5 (±0.1)	1452 (±8.8)	488 (±5.3)

Concentrations are mean values (n = 3) expressed in g/L [1] and mg/L [2]. Titratable Acidity expressed as tartaric acid.

2.4. Red Wine Making

Syrah grapes were supplied by Messina Hof Winery in Bryan, TX, USA. The grapes were sourced from Lepard Vineyard in the Texas High Plains AVA and were machine harvested. Grapes were treated with a 30 mg/L KMS solution and sprinkled with approximately 2 g/gallon of Gaia (Metschnikowia fructicola, Scott Laboratories, Petaluma, CA, USA) to prevent any unwanted fermentation, bacterial growths, or off odors developing. Grapes were cold soaked for 2 weeks at 5 degrees Celsius. Grapes were then crushed, homogenized, divided into 5-gallon buckets, and treated with 30 mg/L of KMS. Table 2 shows the Syrah must chemistry before any adjustments. The starting pH of the must was 4.16 and the target pH for treatments was 3.60. The must chemistry indicates that malolactic fermentation had begun before alcoholic fermentation due to the presence of lactic acid. No inoculation of malolactic bacteria occurred.

Table 2. Syrah must chemistry.

Brix	pH	Titratable Acidity	Glucose/ Fructose [1]	Malic Acid [1]	Lactic Acid [1]	Tartaric Acid [1]	Potassium [2]	Polyphenol [2]
23.7 (±0.1)	4.16 (±0.0)	2.7 (±0.1)	861 (±3.6)	0.65 (±0.1)	0.74 (±0.0)	2.3 (±0.0)	2040 (±2.0)	860 (±11.3)

Concentrations are mean values (n = 3) expressed in g/L [1] and mg/L [2]. Titratable Acidity expressed as tartaric acid.

All treatments for red wine were performed in triplicate. Table 3 describes the amount of acidification treatment used to reach the target pH. Bench trials were implemented for tartaric and verjus treatments by incremental addition until the desired pH was reached. The control had no acid adjustment made, but all other additions described for winemaking, including yeast, nutrients, and KMS, were made.

Table 3. Acidification treatments and amount used.

Treatment	Target pH	Amount Used
Tartaric acid	3.60	2.2 g/L
Verjus	3.60	17.5% by volume
GOx	3.60	1 g Catazyme/L for 22.5 h
Ion exchange	3.60	5.4 g resin/L

For the GOx treatment, Catalase 25 L was added at a rate of 1 g/L according to previous literature [11] along with 2 aquarium pumps and 4 sparging stones per treatment vessel. pH was frequently monitored, and when the desired pH was reached, the pumps were pulled from the must, thus stopping the enzymatic reaction which is oxygen-dependent.

Ion exchange was initially attempted prior to fermentation; however, the presence of skins, seeds, and other solid components in the must prevented it from passing through the mesh sieve into the ion exchange column. Consequently, the ion exchange process was conducted post-fermentation.

After acidification treatments of tartaric acid, verjus, and GOx were made, all must was inoculated with Zymaflore RX60 (S. cerevisiae, Laffort, Petaluma, CA, USA) at a rate of 30 g/hL along with Go Ferm (Scott Labs, Petaluma, CA, USA) at a rate of 30 g/hL to aid in starting fermentation. Fermentation occurred at 22 degrees Celsius in 5-gallon food-grade plastic buckets, with punchdowns performed twice a day. A 15 g/hL addition of Fermaid O (Scott Labs, Petaluma, CA, USA) was made when must was at 10–12 Brix to aid in fermentation finishing. Must was fermented to dryness.

Ion exchange was performed after fermentation and first racking. Dr. Misha Kwasniewski from Penn State University provided guidance for the building of the small-scale ion exchange column as well as conducting the ion exchange on wine. A bench trial was conducted to determine the amount of resin needed for ion exchange. Hosing was attached on either end of a Blichmann Engineering RipeTide pump using stainless steel tri-clover clamps, which was then attached to a stainless steel 1-inch tri-clover sight glass with stainless steel mesh sieves on each end (Vinter's Vault, Hye, TX, USA) that had been loaded with the predetermined amount of resin. All wine was pumped through at a rate of 26 L per minute.

Post fermentation, wine was racked off the lees and an addition of KMS at a rate of 50 mg/L was made. Wine was filtered using a Buon Vino Mini Jet wine filter with size #2 pads that are 1.5–2.5 micron filters. Finished wine underwent an addition of 30 mg/L SO_2, was bottled, and was then stored in a 5 degrees Celsius fridge until sensory evaluations.

2.5. GCMS Analysis of Finished Wines

Finished wines were analyzed in triplicate, semi-quantitatively, using Head Space Solid Phase Microextraction Gas Chromatography–Mass Spectrometry (HS-SPME-GC-MS). Analysis was conducted using an Agilent 5977B GC/MSD (Santa Clara, CA, USA) equipped with a PAL autosampler (Zwingen, Switzerland). The method utilized was as described in Hopfer, 2012 [16].

Compounds were identified using the NIST 08 Library (Scientific Instrument Services, Inc., Ringoes, NJ, USA) by matching mass spectra with at least a 90% match factor reported. Compound concentrations are reported semi-quantitatively, as internal standard (IS) equivalents in µg/L. In accordance with Hopfer, 2012, 2-Octanol was used as the internal standard [16]. Relative concentrations were compared only between samples from this study.

2.6. Sensory Analysis of Wines

2.6.1. Affective Test

The wines were evaluated by 28 professionals (winemakers and wine growers) with previous wine evaluation experience during a wine-related conference. No training was provided for this test. Table 4 details the frequency of gender, age, and experience of panelists. Panelists were provided with five 1-ounce samples, served in ISO-standard winetasting glasses at room temperature, and coded with a random 3-digit code. The wines were presented in randomized order. Water and plain crackers were provided to cleanse their palates. Panelists were instructed to taste all wines, rinsing with water and cracker between samples, and then rank wines from most to least preferred. This research was approved by the Institutional Review Board (IRB) at Texas A&M University (IRB2023-1259).

Table 4. Frequency of gender, age, and experience of participants (n = 28).

Characteristic	Modality	Frequency
Gender	Female	14
	Male	14
Age	21–41	15
	41–61	6
	61+	7
Experience	Grape grower	8
	Winemaker	4
	Grape Grower and winemaker	6
	Other	10

2.6.2. Hierarchical Rate-All-That-Apply

80 panelists were recruited for the study. Table 5 details the frequency of gender, age, and experience of panelists. Panelists needed to be "wine drinkers" which we defined as drinking wine at least once a month. Panelists did not receive any training on describing wine but were given a 10 min orientation on how to interact with the web-based wheel. This research was approved by the IRB at Texas A&M University (STUDY2024-0108).

Table 5. Frequency of gender, age, and experience of participants (%, n = 80).

Characteristic	Modality	Frequency
Gender	Female	63.75
	Male	35.0
	Non-binary	1.25
Age	21–24	38.750
	25–34	31.250
	35+	30.0
Experience	Wine drinkers	83.75
	Experience in viticulture and enology	16.25

The Hierarchical Rate-All-That-Apply (HRATA) method [17] was used to evaluate the red wines. This method allows for an aromatic assessment of wines with varying

levels of precision depending on the judges' sensitivity and perception. Therefore, it can be used with tasters of varying levels of expertise. The Ann C. Nobel wine aroma wheel was used in conjunction with Fizz Biosystems software (v3.8) for this project. The wheel is structured with the hierarchies that allow panelists to score the families (fruit, floral, wood, etc.) or more general terms, the categories (red fruit, black fruit, citrus, etc.) or intermediary terms, or the specific attributes (cherry, raspberry, strawberry, etc.). Since mouthfeel is an important characteristic in the context of wine acidification, a "Mouthfeel" category was added to the wheel with 4 mandatory attributes to score. Two printed sheets of paper were provided to all participants to assist in visualizing the hierarchical categorization of the attributes. A chart format listed all the attributes, as does a wheel that matched the participants' computer screens, which is shown in Figure 1.

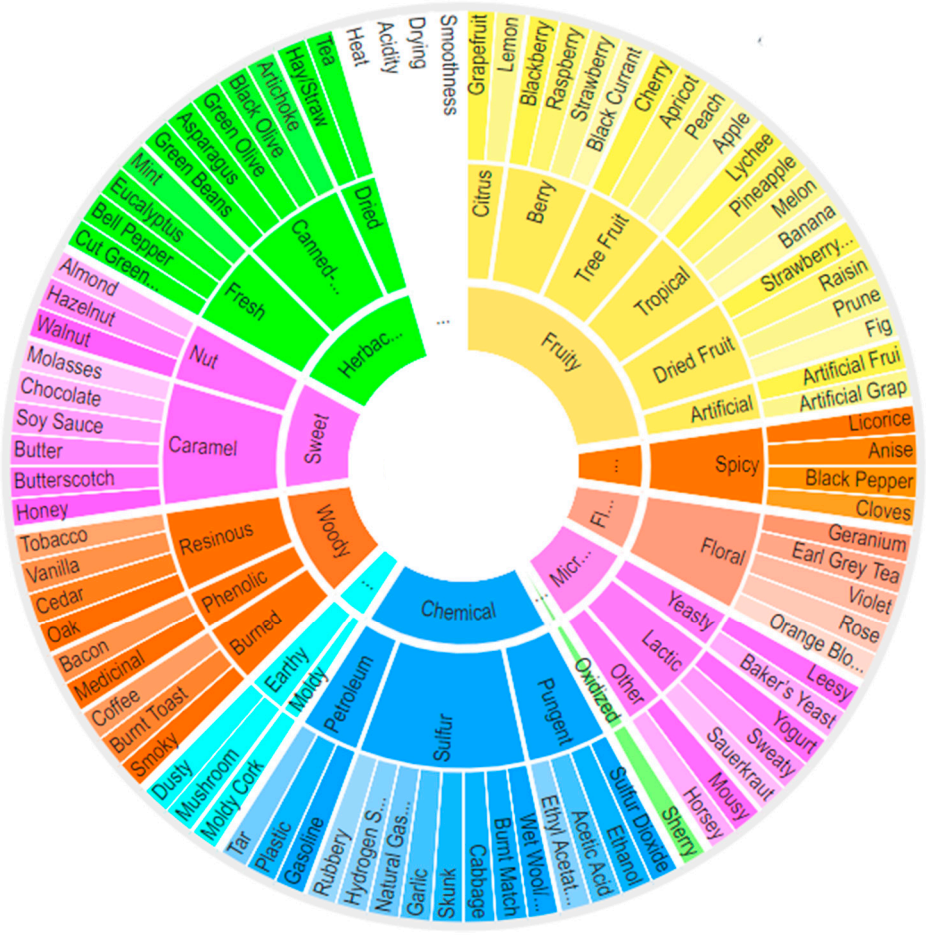


Figure 1. Attribute Wheel for HRATA.

Wines were brought to room temperature, and five 1.5 oz samples were served in ISO-standard wine tasting glasses, each coded with a random 3-digit code. Water and plain crackers were provided for palate cleansing. Samples were presented in randomized order. The panelists were instructed to smell, taste, and expectorate the wine samples and then select and score on a three-level scale (low, medium, high) as many attributes as they wanted from the family, category, or specific terms. They were also instructed to score all the "mouthfeel" family terms that included "heat, acidity, smoothness, and roughness" on a discrete 11-point scale, ranging from low to high. The panelists were required to wait 2 min between samples and were asked to rinse their mouths with water and plain crackers while they waited. Along with the online interactive wheel, panelists also had a printed version of the wheel in table format to be able to visualize all attributes available to rank.

2.7. Statistical Analyses

Chemistry (triplicate analytical values) of the verjus, must, and wine treatments (duplicate or triplicate biological replicates) data were statistically analyzed by one-factor analysis of variance (ANOVA) with Tukey's HSD at a significance level of 0.05 using XLSTAT (Addinsoft, New York, NY, USA). Alcohol data was reported as percent by volume and log transformed before performing one-way ANOVA using the Lme4 [18] package in R (v4.4.0) with Tukey's HSD at a significance level of 0.01 using the eemeans [19] package in R.

The GCMS data was log transformed before performing a one-way ANOVA using Lme4 [18] in R at a significance level of 0.01. Of the 141 compounds detected and measured, 55 compounds were identified with significant differences among treatments. Principal Component Analysis (PCA) was performed on the significant compounds and a bar plot of the variables' cos2 was created using the FactoMineR and factoextra R packages [20,21]. While all 55 significant compounds were utilized in the PCA, only compounds with a cos2 greater than 0.9 (25 compounds) were plotted to increase the readability of the plot.

Affective sensory data was reported using Qualtrics surveying and analyzed using Friedman's test [22] at a significance level of 0.05 using XLSTAT (Addinsoft, New York, NY, USA).

The HRATA data was analyzed by separating the aromatic attributes from the mandatory "Mouthfeel" attributes. For the 4 "Mouthfeel" attributes, two-way analysis of variance (ANOVA) with a Fisher LSD test at a significance level of 0.05 was carried out using lmerTest package [23] in R.

The HRATA aromatic attributes were analyzed by combining the data pretreatment method proposed by Koenig et al. [17] with variance analysis techniques recommended by Meyners et al. and Vidal et al. for RATA data [24,25]. First, a coding step was used to aggregate information from lower hierarchical levels to higher ones for each wine and taster when the superior levels were not used [17]. Then, two-way ANOVAs (wines + panelists) were conducted to identify attributes with significant differences among wines. Data were aggregated for each significant attribute across all subjects who rated this attribute at least once. The Dravnieks score was used to account for both the frequency of attribute selection and the assigned intensity values [25,26]. A standardized PCA was performed on the Dravnieks score matrix, including the family, categories, and terms. All ANOVAs were fitted using the lmer function from the lme4 package, and *p*-values were calculated with the step function from the lmerTest package [23]. PCAs were performed using the FactoMineR package [20].

3. Results

3.1. Chemical Analyses

3.1.1. Pre-Fermentation Acid Profiles

The acid profiles of the juice post adjustment, pre fermentation can be seen in Table 6. Only results for tartaric acid, GOx, and verjus treatments are presented here since ion exchange was performed post fermentation. Titratable acidity and malic, lactic, and tartaric acids were all significantly different. The GOx treatment had the highest titratable acidity and lactic acid. The lactic acid increase can be explained by the increased oxygen present during the GOx treatment. While not fully understood, oxygen accelerates the conversion of malic acid into lactic acid [27,28]. The verjus treatment had the highest malic acid content due to the naturally high malic acid concentrations found in verjus. It also showed higher tartaric acid levels compared to the GOx treatment, as verjus also contains tartaric acid. Meanwhile, the tartaric acid treatment recorded the highest tartaric acid levels.

Table 6. Acid profiles of adjusted juice.

	pH	Titratable Acidity [1]	Malic Acid [1]	Lactic Acid [2]	Tartaric Acid [1]
Tartaric acid	3.61 [a] (±0.0)	4.5 [c] (±0.5)	0.8 [b] (±0.1)	732 [b] (±38.0)	2.1 [a] (±0.1)
GOx	3.54 [b] (±0.1)	6.7 [a] (±0.6)	0.14 [c] (±0.0)	1061 [a] (±23.0)	1.1 [c] (±0.1)
Verjus	3.57 [a,b] (±0.0)	5.7 [b] (±0.3)	3.24 [a] (±0.2)	644 [c] (±60.0)	1.4 [b] (±0.2)

Concentrations are mean values (n = 3) expressed in g/L [1] and mg/L [2]. Titratable Acidity expressed as tartaric acid. pH expressed as pH units. Different letters indicate significantly different values based on Tukey's HSD test, $p = 0.05$.

3.1.2. Post-Fermentation Acid Profiles

The acid profiles of all the finished wines can be seen in Table 7. There is some variability between treatments on final pH; however they remained close to the goal of 3.6. Without any acidification treatment, the final pH of the control treatment was 3.91, which can have implications including microbial instability, color instability, poor taste, and much more. Titratable acidity for the GOx treatment was significantly different from all other treatments, having the highest value at 6.77, which can be explained by the Catalase 25 L converting glucose into gluconic acid (not measured) as shown in previous studies [12,29].

Table 7. Acid profiles of finished wine.

	pH	Titratable Acidity [1]	Malic Acid [1]	Lactic Acid [2]	Tartaric Acid [1]
Tartaric	3.63 [c] (±0.0)	5.6 [b] (±0.2)	0.1 [a] (±0.0)	1194 [d] (±33.0)	2.3 [a] (±0.2)
GOx	3.63 [c] (±0.1)	6.8 [a] (±0.5)	0.05 [b] (±0.0)	1248 [c] (±21.2)	1.9 [c] (±0.0)
Verjus	3.68 [b] (±0.0)	5.6 [b] (±0.2)	0.05 [b] (±0.0)	2199 [a] (±34.0)	2.1 [a,b] (±0.2)
Ion exchange	3.59 [d] (±0.0)	5.5 [b] (±0.2)	0.05 [b] (±0.0)	1283 [c] (±15.0)	2.0 [b,c] (±0.2)
Control	3.91 [a] (±0.0)	4.7 [c] (±0.2)	0.05 [b] (±0.0)	1339 [b] (±37.3)	2.1 [a,b] (±0.2)

Concentrations are mean values (n = 3) expressed in g/L [1] and mg/L [2]. Titratable Acidity expressed as tartaric acid. pH expressed as pH units. Different letters indicate significantly different values based on Tukey's HSD test, $p = 0.05$.

All wines spontaneously went through malolactic fermentation, leaving all wines with low levels of malic acid and varying levels of lactic acid. The verjus treatment had the highest amounts of lactic acid present, which can be explained by the presence of malic acid in the verjus that was added and was subsequently metabolized into lactic acid. The verjus treatment also had higher amounts of tartaric acid than the GOx and ion exchange treatments, but not as much as the tartaric acid treatment. This can be attributed to the presence of tartaric acid in the verjus that was added.

All treatments and control saw an increase in titratable acidity. While expected in the post-fermentation ion exchange treatment, it is hypothesized that in the other treatments and control the increase in titratable acidity can be explained by the potential presence of succinic acid (not measured). It has been shown by Coulter that post-fermentation increases in titratable acidity can be linked to succinic acid, which is a byproduct of alcoholic fermentation [30].

3.1.3. Other Chemical Parameters of Finished Wines

Figure 2 shows the levels of potassium and polyphenols in the finished wines. The GOx treatment wine had significantly higher levels of potassium compared to any other

treatment. We attributed this increase to the oxygen pumping involved in the treatment, which caused the pomace to break down more extensively, facilitating greater potassium extraction from the skins. After fermentation, the pomace from the GOx treatment was noticeably more degraded compared to the other treatments. Verjus, tartaric acid, and GOx treatments had similar levels of polyphenols. The ion exchange treatment had an increased amount of polyphenols when compared to the other treatments, but our findings concluded similarly to Lasanta's that the ion exchange treatment had a lower concentration of polyphenols when compared to the control, although not significantly different [7].

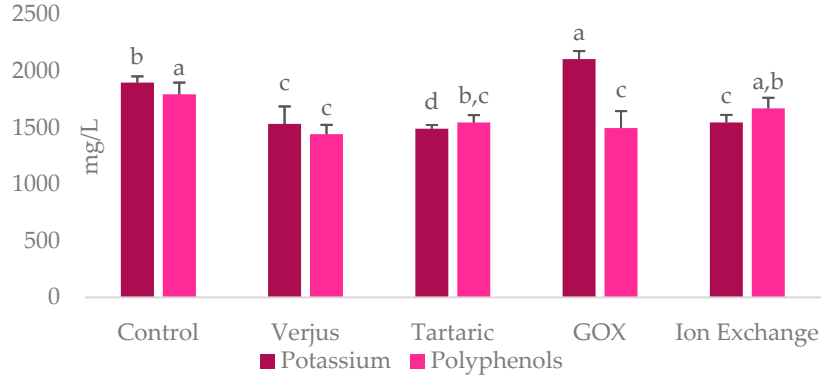


Figure 2. Potassium and polyphenols of finished wine. Concentrations are mean values (n = 3) expressed in mg/L. Different letters indicate significantly different values based on Tukey's HSD test $p = 0.05$.

Figure 3 shows the alcohol levels of finished wines. The verjus treatment had the lowest alcohol at 10.14%, which can be explained by the addition of low-sugar juice resulting in an overall decrease in fermentable sugar concentration and decreasing potential alcohol. The GOx treatment had the second lowest alcohol at 10.6%, which can be explained by the conversion of glucose in the must to gluconic acid during treatment, lowering the concentration of available sugars for fermentation and therefore the final alcohol concentration.

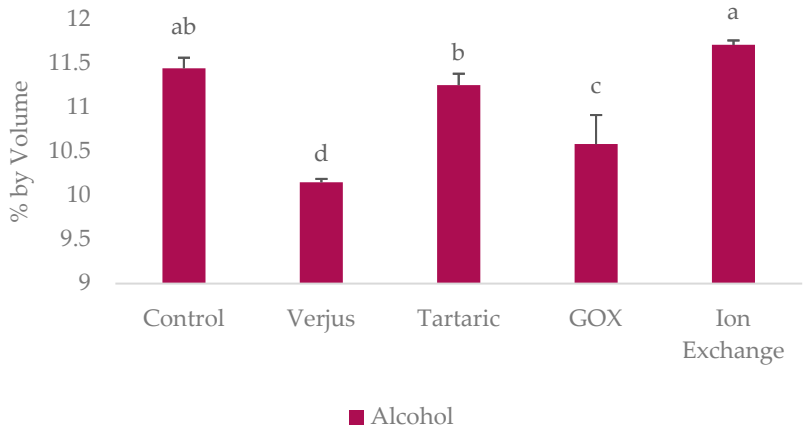


Figure 3. Alcohol of finished wine. Concentrations are mean values (n = 3) expressed in %. Different letters indicate significantly different values based on Tukey's HSD test $p = 0.01$.

3.2. GCMS Analysis of Finished Wines

Table 8 shows the aromatic compounds that had significant differences ($p = 0.01$) together with descriptions of the aromas found in the literature. Figure 4 shows the relevant PCA plots. Compounds with a star by them are displayed in Figure 4b, the PCA

plot of the compounds. The plots explain 70.4% of the variance. Aromatic compounds were separated according to acidification treatment.

Table 8. GCMS aromatic compounds, descriptions, and concentrations.

Compounds	Descriptions	Control	GOx	Verjus	Tartaric	Ion Exchange	Source
Alcohols							
1-Butanol, 2-methyl-	Fermented, yeasty	497	3851	2119	2577	n.d. [1]	[31]
1-Hexanol *	ethereal, fruity, alcoholic	823	616	966	815	1003	[31]
Phenylethyl Alcohol *	Rose, honey	647	545	889	681	762	[32]
1-Butanol, 3-methyl-	Cheese	1186	177	n.d.	644	n.d.	[33]
1-Propanol, 2-methyl- *	Sweet, musty	767	111	88.8	75.7	88.5	[34]
3-Hexen-1-ol, (Z)- *	Refreshing, citrus	47.3	20.3	73.9	51.7	58.7	[35]
2,3-Butanediol, [R-(R *,R *)]-	Fruity, creamy, buttery	29.1	57	37.3	47.5	40.8	[31]
3-Hexen-1-ol *		47.8	16.4	38.8	42.2	44.5	
1-Heptanol *	Musty, leafy, vegetative	33.7	23.8	37.9	33.4	36.8	[31]
Benzyl alcohol *	Jasmine, hyacinth	30.3	16.8	30.7	23.8	33	[31]
(S)-(+)-3-Methyl-1-pentanol *	Unpleasent, chemical, fusel	22.1	19.8	28.2	22	24.3	[36]
1-Octanol *	Waxy, green, citrus, floral	24	11.7	32.3	23.7	24.2	[31]
2-Ethyl-1-hexanol	Citrus, fresh, floral, oily, sweet	16.4	16.3	16.8	20.6	22.4	[31]
1-Pentanol, 3-methyl-	Fusel, cognac, wine, fruity	18.9	22.5	14.7	18.8	n.d.	[31]
1-Pentanol	Fermented, yeasty, winey	6.8	3.1	4.8	418	5.1	[31]
3-Hexen-1-ol, (E)-	Green, leafy	12.8	11.6	14	11.8	15.5	[31]
1-Butanol	Fusel, oil, sweet, balsam, whiskey	10.3	9.2	9.6	12.8	12.3	[31]
Esters							
Octanoic acid, ethyl ester *	Fruity, sweet	631	257	586	551	579	[35]
Hexanoic acid, ethyl ester *	Fruity, sweet	404	242	337	336	391	[35]
Decanoic acid, ethyl ester *	Fruity, sweet	163	39	176	129	113	[35]
1-Butanol, 3-methyl-, acetate *	Banana, fruit, fresh	116	48.7	116	126	100	[35]
Formic acid, 2-propenyl ester	Pungent	n.d.	n.d.	674	n.d.	n.d.	[37]
Methyl formate *	Pleasant, ethereal	50	81	72	53.2	56.5	[38]
Butanedioic acid, diethyl ester *	Mild, fruity, cooked apple	47	42.3	67.6	50.2	60.2	[31]
Ethyl (S)-(-)Lactate	Butter, cream	31.8	51.7	67.7	44.1	66	[31]
Propanoic acid, 2-hydroxy-, ethyl ester	Etheral, buttery	32.7	60.5	n.d.	39.3	n.d.	[37]
Ethyl 9-decenoate *	Fruity, fatty	24.8	7.7	25	22.2	21.8	[31]
Isoamyl lactate *	Banana	12.4	18.9	27.6	16.3	20.4	[39]
Formic acid, heptyl ester	Fruity, floral, rose	36.1	n.d.	n.d.	n.d.	n.d.	[37]
Acetic acid, hexyl ester *	Green, fruity, sweet, fatty, fresh	9.4	n.d.	9.6	9.9	20.5	[31]
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester *	Cheese, rancid	9.2	9.5	13.2	9.9	10.4	[35]
Acids							
Hexanoic acid *	Cheese, fatty	167	134	242	173	202	[35]
Propanoic acid, anhydride *		499	1.5	677	595	302	
Octanoic acid *	Fatty, waxy, vegetable, cheesy	133	79.1	252	144	148	[31]
Acetic acid *	Vinegar	52.6	21	37.5	56.8	61	
Butanoic acid, 2-methyl-		29.4	54.6	31.1	45.9	45.8	
Propanoic acid, 2-methyl-, anhydride		n.d.	n.d.	131	n.d.	n.d.	
Propanoic acid	Soy	16.8	19.1	19.2	17.5	26.1	[32]
Butanoic acid	Cheese, stinky	12.9	11.9	14.8	14.5	15.6	[32]
n-Decanoic acid	Unpleasent, sour, fatty, citrus	11.7	7.2	25.8	13.9	12.9	[31]
Propanoic acid, 2-methyl-		8.2	12.4	8	9.9	9.5	
Other							
Azetidine		866	1156	1365	1060	1089	
Ethoxyacetylene *		946	1307	369	1399	593	
Cyclobutane, methyl-		n.d.	1015	1252	562	1307	
1,2,5-Oxadiazole		460	781	527	693	777	
Aziridine, 2-methyl-		n.d.	1202	n.d.	1018	n.d.	
Urea		42.8	70	71.2	n.d.	90	
1H-Tetrazole		651	9.2	464	0.2	0.2	
Propanediamide, 2-amino-		20.1	21.6	29.3	24.1	30.6	
Ammonium acetate *		21.7	17.4	27	23	25.4	
Formic acid hydrazide		19.1	19.5	17.4	26.6	21.8	
Oxiranemethanol, (R)-		n.d.	10.3	22.8	9.7	8.9	
Cyclobutanone		n.d.	n.d.	995	713	1561	
1,4-Dioxane-2,6-dione		n.d.	801	343	n.d.	n.d.	
3(2H)-Thiophenone, dihydro-2-methyl-		n.d.	n.d.	23.7	n.d.	n.d.	

Concentrations are mean values (n = 3) expressed in ug/L [1]. Compounds with * indicate they have a cos2 greater than 0.9 (25 compounds) and were plotted on Figure 4b below. Source indicates where the compound descriptors were found.

The tartaric acid and ion exchange treatments were most closely related to the control. The GOx and verjus treatments were most different from the control and each other.

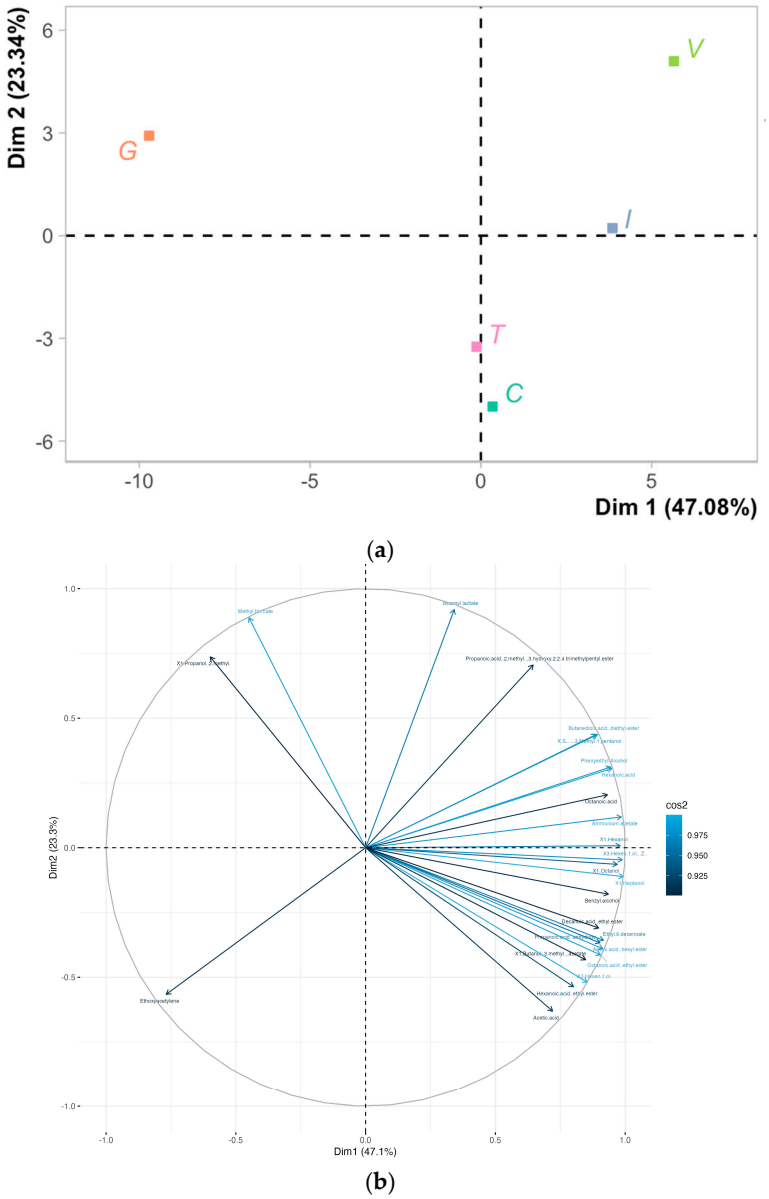


Figure 4. Normalized PCA plots based on mean values (n = 3) of significant GCMS aromatic compounds (p–value < 0.05): (**a**) Scores plot (C, control; T, tartaric; I, ion exchange; G, GOx; V, verjus) and (**b**) correlation loadings plot of GCMS aromatic compounds.

The verjus treatment was associated with the compounds isoamyl lactate and propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester. Isoamyl lactate is described as banana and propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester is described as having cheese and rancid characteristics.

The GOx treatment was associated with the compounds methyl formate and 1-Propanol, 2-methyl-. Methyl formate is described as pleasant and ethereal and 1-Propanol, 2-methyl- is described as ethereal and winey.

The ion exchange and tartaric acid treatments did not have as clear a separation of compounds in Figure 4b as the other treatments. Ion exchange had double the amount of acetic acid, hexyl ester which is described as green, fruity, and fresh, and only slightly higher amounts of benzyl alcohol which is described as jasmine and hyacinth. Tartaric acid had slightly higher amounts of 1-Butanol, 3-methyl-, acetate, which is described as banana and fruity, and ethoxyacetylene (no descriptors found).

3.3. Sensory Analyses

3.3.1. Affective Test

Figure 5 shows the response counts for the preference test. Table 9 shows the results of the Friedman's test which concluded there were no significant differences ($p = 0.438$) in consumer's preference for treatments. Samples that are preferred by some are not liked by others.

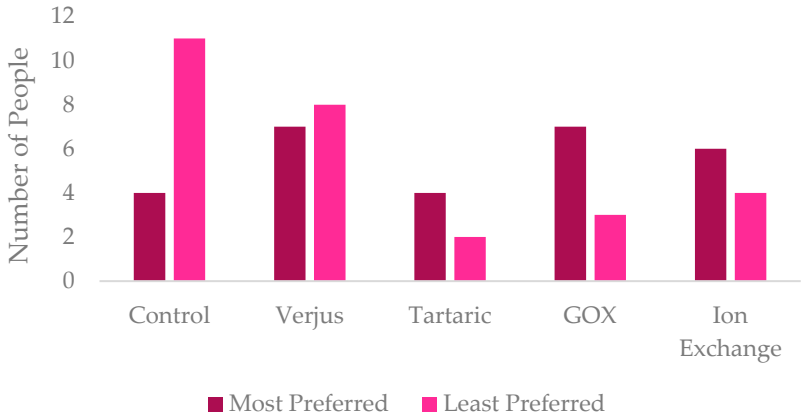


Figure 5. Preference test counts.

Table 9. Preference ranking per treatment.

	Rank Sum	Rank Mean	Group
Tartaric	78,000	2786	A
GOx	79,000	2821	A
Ion exchange	79,000	2821	A
Verjus	87,000	3107	A
Control	97,000	3464	A

Sum scores and means per treatment. N = 28 $p = 0.438$.

3.3.2. HRATA Test

Figure 6 shows the relevant PCA plots. The plots explain 78.69% of the variance.

Based on the examined parameters, the samples were separated according to the acidification treatments. The tartaric acid and ion exchange treatments were most closely related to the control, while the verjus and GOx treatments were the most different.

The verjus treatment was associated with the following descriptors: hay/straw, dried, sweaty, lactic, and wet wool/wet dog. The "lactic" descriptor is explained by the verjus treatment having the highest amounts of lactic acid present.

The GOx treatment was associated with the following descriptors: citrus, caramel, honey, hazelnut, lemon, fruity, strawberry, and berry. The descriptors for the GOx treatment are similar to the findings of Botezatu's previous study using GOx to acidify [29].

Of the four mouthfeel descriptors scored by panelists, "drying" was the only significant descriptor ($p = 0.05$). Tartaric acid was ranked the highest for drying, while GOx and verjus were ranked the lowest.

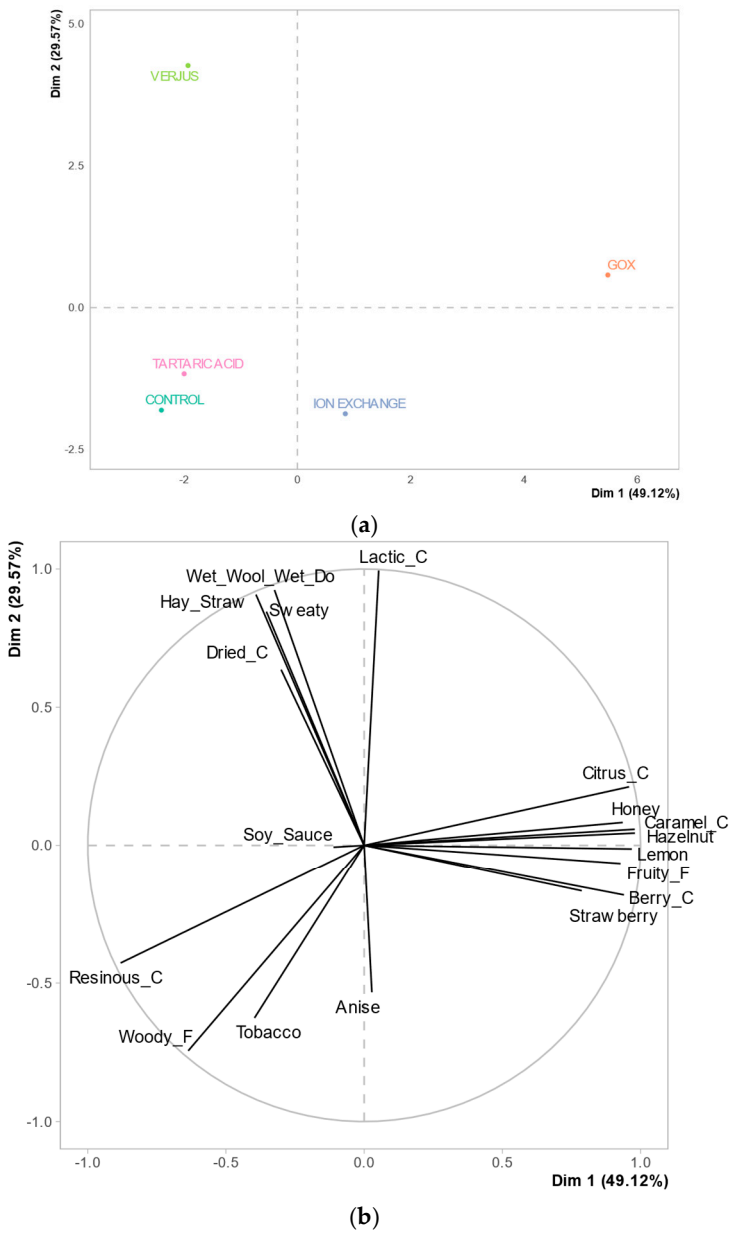


Figure 6. Normalized PCA plots based on Dravnieks' scores for significant families, categories, and terms from HRATA data (p–value < 0.05): (**a**) Scores plot and (**b**) correlation loadings plot of sensory attributes.

4. Discussion

All acidification treatments were effective in lowering the pH to more suitable levels for red wine production. There were notable chemical and sensorial differences between treatments. These differences can be utilized by winemakers to impart different flavors and create different styles of wine.

The verjus and GOx treatments were the most sensorily different compared to the control and other treatments. The verjus addition led to an increase in malic acid in the must and consequently increased the amount of lactic acid in the finished wine since the wines went through malolactic fermentation spontaneously. The verjus treatment produced undesirable aromatic compounds like 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester, which has been described as cheesy and rancid and which consumers described as "sweaty, lactic, and wet wool/wet dog".

We believe the descriptors may stem more from the spontaneous malolactic fermentation occurring at high pH prior to acidification, rather than solely from the addition of verjus. The extra malic acid in the must likely provided additional nutrients for the already established lactic acid bacteria (LAB). Previous studies have shown that similar rancid and sweaty descriptors can develop in wines undergoing malolactic fermentation at high pH [40,41].

We hypothesize that inoculating early or co-inoculating with a controlled and desirable LAB strain could potentially out-compete a native strain and have a more positive outcome. Verjus chemistry will vary depending on variety, harvest date, etc., so bench trials must be conducted before use.

Wines produced with GOx had the highest TA, highest amount of potassium, and the most desirable flavor attributes, including "citrus, caramel, honey, hazelnut, lemon, fruity, strawberry, and berry." The most significant aromatic compounds for this treatment include methyl formate and 1-Propanol, 2-methyl-, which are described as ethereal, pleasant and sweet, musty, respectively. In the preference test, this treatment had the highest number of most preferred with the lowest number of least preferred. While this treatment does require some additional equipment for enzyme activation, we do not believe this to be a large barrier for winemakers to implement. We recommend GOx as an acidification technique for red wine production, as it was not only effective but also provided the wine with positive sensory descriptors that made clear differences in the wine.

Due to rising temperatures that result in increased Brix levels in grapes, around the world, wines have trended toward having higher amounts of alcohol. Both the verjus and GOx treatments produced wines lower in alcohol, providing winemakers with options to both remediate high pH and decrease potential alcohol before fermentation.

The tartaric acid treatment was most closely related, both chemically and sensorially, to the control, as shown in both the HRATA and GCMS PCA loading plots. Of all the mouthfeel attributes evaluated in the HRATA study, tartaric acid was significantly different ($p = 0.05$) for drying. If winemakers desire to make a wine that has more of a drying effect on the mouth, or is closely related to the control wine, then tartaric acid could be a good option.

As more acidification options become available and approved for wine production, they should be further investigated in order to understand their complete impact on wine. Future studies should also evaluate gluconic and succinic acids. More research utilizing other varieties, including white varietals, should be performed in order to provide winemakers clear recommendations.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to no appropriate data repository available for datasets.

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