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Glucose Oxidase in Conjunction with Catalase – An Effective System of Wine pH Management in Red Wine

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Abstract

Background and goals

High grape pH directly influences the quality of the subsequent wines. Acidulation of grape juice and grape must by tartaric acid is a common practice but can leave a wine's flavor unbalanced. Treatment with commercially available Catazyme 25 L, which contains the enzymes glucose oxidase (GOx) and catalase, was investigated as a valid way to lower high pH in red grape juice/must while simultaneously increasing acidity and lowering glucose and potential alcohol.

Methods and key findings

Tempranillo must and juice were treated with Catazyme 25 L at two concentrations (0.5 g/L and 1 g/L) over a 24-hr period under continuous aeration. Chemical and sensory analyses were performed on the resulting wines. Results indicated that the pH of Tempranillo juice was decreased by 0.84 when using Catazyme 25 L at a rate of 1.0 g/L. Similarly, addition of Catazyme 25 L at 0.5 g/L decreased pH from 4.6 to 4.0 and 3.8 in the must and juice, respectively. Use of Catazyme 25 L led to production of lower alcohol wines due to conversion of glucose to gluconic acid. Sensory evaluation of the wines indicated a positive impact of the enzyme blend on color, aroma, and in-mouth flavor.

Conclusions and significance

GOx in conjunction with catalase is an effective pH management system, and of particular value for winemaking in hot climates, where it can also help lower alcohol concentration while positively impacting the sensory profiles of the wines.

Key words: catalase, glucose oxidase, hot climate, wine, wine acidity, wine pH

Introduction

Glucose oxidase (GOx) with catalase

GOx is an enzyme that is produced naturally by the fungus *Aspergillus niger* and catalyzes the oxidation of D-glucose into D-gluconolactone, during which hydrogen peroxide is produced (Wong et al. 2008). Catalase, a common enzyme found in nearly all living organisms that are exposed to oxygen, rapidly breaks down hydrogen peroxide (H_2O_2) into water and oxygen (Chelikani et al. 2004). D-gluconolactone and water then non-enzymatically react to form gluconic acid. GOx is Generally Recognized as Safe (Wong et al. 2008) and has been used in several commercial applications, including glucose removal from dried egg; improvement of color, flavor, and shelf life of food materials; oxygen removal from fruit juices, canned beverages, and mayonnaise to prevent rancidity; and as an ingredient in toothpaste (Bankar et al. 2009). GOx has also been researched as a prefermentative treatment in Riesling and Müller-Thurgau grape juice and must to produce a reduced-alcohol wine and lower wine pH in Riesling wines (Pickering et al. 1999).

pH in winemaking

pH is related to the concentration of hydrogen ions [H+] in solution and is crucial for microbial stability, color, preservation, oxidation, tartrate stability, protein stability, and wine taste and astringency (Boulton 1980). The proper pH range for red wine is between 3.4 and 3.7, but in Texas, wines with a pH of 3.8 and higher are common. In wine, a higher pH facilitates a more rapid rate of oxidation and is inductive of more microbial spoilage. The protection provided by the use of potassium metabisulfite (KMBS), which acts as a wine preservative, is much more difficult to achieve at a higher wine pH. The color of wine itself is a remarkable phenomenon that depends on pH and the stability of anthocyanins, which belong to the flavonoid class of chemical compounds (Margalit 2004, Tang et al. 2019). Anthocyanins are water soluble natural pigments responsible for a wide variety of colors such as red, purple, and blue (Tang et al. 2019). Anthocyanin color is related to its structural formation, which is transformable and reversible depending on the pH value (Tang et al. 2019).

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GOx as an oxygen scavenger

The presence of O_2 is a problem in many food products (Wong et al. 2008), including wine. O_2 promotes bacterial growth and browning; therefore, it is desirable to remove O_2 from wine and wine headspace to maintain an anaerobic environment (Wong et al. 2008). The GOx reaction consumes O_2, which allows GOx to be used as an active O_2 scavenger (Ough 1975, Wong et al. 2008) in the GOx/catalase enzyme system for O_2 removal in wines with residual sugar before bottling.

Using GOx to produce reduced-alcohol wines

Another application of the GOx/catalase enzyme system is to produce low - or reduced-alcohol wines. The process reduces potential alcohol by converting glucose into gluconic acid (Bredie et al. 2018) during a prefermentative treatment.

The current study aims to investigate the potential of treatment with GOx plus catalase to decrease pH and increase acidity in red wines. The target of the treatments would be acidity related, rather than glucose/alcohol related. We hypothesize that by focusing on pH, the treatment will avoid previously reported potential shortcomings, such as the excessive production of gluconic acid, undesirable sensory characteristics, or color changes, while increasing acidity and decreasing pH to desirable levels. Herein, we use 'GOx' to indicate the level of Catazyme 25 L added (Catazyme 25 L is a proprietary combination of Glucose Oxidase and Catalase).

Materials and Methods

Prefermentation

Two different experimental trials were undertaken to observe the effects of Catazyme 25 L on the winemaking process with two laboratory trials (Batch 1 - must and Batch 2 - juice).

Batch 1 - Tempranillo must

Batch 1 was a laboratory experiment that used Tempranillo must (from previously frozen grapes) with a pH of 4.6 and a titratable acidity (TA) of 4.4 g/L, which was then separated into four different treatments. This experimental design was chosen to observe the effects of Catazyme 25 L in grape must. Dosing Catazyme 25 L directly into grape must is the most straightforward way of using GOx with catalase.

Each treatment in Batch 1 was carried out in 18.9-L food grade buckets with 12 L of must per bucket, and each treatment was duplicated for a total of eight buckets. Batch 1 treatments included control, aeration, 0.5 (g/L) GOx, and 1.0 (g/L) GOx. Control treatment consisted of 2 × 12-L buckets of must and received no chemical additions or mechanical treatments. Aeration treatment contained 2 × 12-L buckets of must fitted with one fish pump (Imagitarium, 3.6 L/min output) and two sparging stones (Aquaculture) per bucket. The 0.5 g/L GOx treatment consisted of 2 × 12-L buckets of must fitted with one fish pump, two sparging stones, and 6 g of Catazyme 25 L per bucket. The 1.0 g/L GOx treatment contained 2 × 12-L buckets of must with one fish pump, two sparging stones, and 12 g of Catazyme 25 L enzyme per bucket. The experiment was conducted for 24 consecutive hours while measuring pH, TA, glucose, and gluconic acid every 4 hrs.

Batch 2 - Tempranillo juice

Batch 2 was a laboratory experiment that used Tempranillo juice (from previously frozen grapes) with a pH of 4.6 and a TA of 3.2 g/L, which was separated into four different treatments. This experimental design was chosen to observe the effects of Catazyme 25 L in grape juice.

Each treatment was carried out in 18.9-L food grade buckets with 10 L of juice per bucket, and each treatment was duplicated for a total of eight buckets. Batch 2 treatments included control, aeration, 0.5 g/L GOx, and 1.0 g/L GOx. Control treatment contained 2 × 10-L buckets of juice and received no chemical or mechanical treatment. Aeration treatment consisted of 2 × 10-L buckets of juice and were fitted with one fish pump and two sparging stones per bucket. The 0.5 g/L GOx treatment consisted of 2 × 10-L buckets of juice that were fitted with one fish pump, two sparging stones, and 5 g of Catazyme 25 L per bucket. The 1.0 g/L GOx treatment consisted of 2 × 10-L buckets of juice fitted with one fish pump, two sparging stones, and 10 g of Catazyme 25 L per bucket. The experiment was conducted for 24 consecutive hours while measuring pH, TA, glucose, and gluconic acid every 4 hrs. After treatment, skins were added back to all buckets, and the wines were fermented with skin contact.

Vinification

After the 24-hr prefermentation research was concluded, the laboratory experiments of Batch 1 and Batch 2 were inoculated with Viti Levure MT *Saccharomyces cerevisae* yeast at a rate of 1.67 g/L for each bucket and given a 1.2 g/L addition of Go-Ferm. Each bucket was fermented to dryness and then pressed into glass carboys using a bladder press. A 60 mg/L addition of KMBS was added to each carboy at this time for Batch 1. A 70 mg/L addition of KMBS was added to each carboy for Batch 2. The wines were then sparged with argon gas and sealed. Wines were racked twice, and a one-time 70 mg/L addition of KMBS was added to Batch 1 and a one-time 40 mg/L KMBS addition was given to Batch 2. Wines were bottled and stored in a 10°C chiller. Batch 1 wines were stored for three months before chemical analysis, while Batch 2 wines were stored for eight months before chemical analysis.

Sensory evaluation

The Flash Profile (FP) method (Kitzberger et al. 2016, Liu et al. 2016, 2018) was used for sensory evaluation of the wines. Samples (30 mL) were taken out of a cooler, brought to room temperature for one hour, and served in 12-oz wine glasses labeled with three-digit codes. The presentation of the samples was randomized for each panelist. The first

session consisted of attribute generation, followed by three days of testing.

The panel was asked to identify aroma, flavor, and color attributes from control, aerated, and GOx-treated wines, with two replicates for each treatment. The first session was conducted in an air-controlled room (24°C) for attribute development and sample analysis. The total session time was two hours. All eight samples were served at once, and panelists were given one hour to generate attributes. A 30-min break was given to panelists, during which attributes were collated and written down on a whiteboard by the panel leader. Panelists were then asked to observe the total attributes accumulated and instructed to add or subtract attributes to their own list as they felt appropriate. Individual attributes were finalized and recorded for testing purposes.

The second, third, and fourth days consisted of testing sessions. Testing sheets were made for each panelist based on the final attribute list they had generated the previous day. Instructions were clearly stated on the testing sheets. Panelists were asked to rank attributes according to intensity on an ordinal scale anchored from 'low' to 'high'. In order, panelists evaluated aroma attributes, flavor attributes, and color attributes, taking a 30-min break between each section. All eight samples (30 mL) were placed in temperature controlled (24°C) individual booths and presented under red lights. Red lights were used for aroma and flavor evaluations. Upon completion of the aroma and flavor sections, lights were changed from red to white for color evaluation. As panelists evaluated each wine, they were asked to cleanse their palates using distilled water and unsalted crackers.

Statistical analyses

Statistical analysis for both Chemistry and Sensory used data was generated using XLSTAT (Addinsoft).

Chemistry

Initially, three-factor general linear model analysis of variance (ANOVA) with all two-way interactions were conducted by batch for pH, TA, glucose, and gluconic acid. The main effects of wine treatment, replication, and time, as well as treatment × replication, replication × time, and treatment × time interactions were included. Further ANOVA tests were undertaken for each chemical monitored in the wines and included only those main effects and interactions found to be significant ($p < 0.05$) in the initial three-factor two-way ANOVA. Bonferroni correction with initial $p < 0.05$ and eight ANOVA tests were conducted, resulting in an adjusted p value for significance of <0.00625 (i.e., $p < 0.01$). Following statistical significance with ANOVA, Tukey's honest significant difference was used for mean comparisons. Using the Bonferroni correction replications and their interactions were not found to significantly contribute; thus, they are not included in the final ANOVA results table.

Sensory data analysis

Generalized Procrustes analysis (GPA) using the Commandeur method in XLSTAT (as found on the website https://help.xlstat.com/6519-download-xlstat-help-documentation) (Gower 1975) was used to explore aroma, flavor, and color data from FP of the four treatment conditions. Aroma (by sniffing headspace), flavor (in-mouth aroma, taste, and mouthfeel), and color were considered separately in GPA. To obtain the full Procrustes ANOVA table, the constraint $2 (n - 1) \times p > 2 + p \times (p - 1)$, where n is the number of products and p the maximum number of dimensions per configuration, was considered (Varela and Ares 2012).

Prior to GPA, FP sensory data were treated as previously described for white wine (Botezatu et al. 2021), and is briefly described here. For each panelist, any attribute included in their vocabulary that did not discriminate between samples (i.e., same score given to all four samples on two or more of the four judgments [two production replications × two evaluation replications]) was removed. Following removal of non-discriminating attributes, Pearson's correlation (r) and associated p value were determined for each panelist's remaining attributes; pairwise across products, attributes significantly correlated with $r \geq +0.8$ ($p < 0.05$) were deemed redundant (Botezatu et al. 2021). The attribute in the correlated pair with the greatest variation (i.e., maximum rating minus minimum rating across all samples and replications) between products was retained for further analysis while the other attribute was removed. If the two correlated attributes were found to have the same variation, their ratings were averaged for analysis. Finally, within each of the two testing sessions, the two treatment replications were averaged, yielding two scores per attribute per sample for inclusion in GPA (Škrobot et al. 2020). Each modality (aroma [by sniffing headspace], flavor [in-mouth aroma, taste, and mouthfeel], and color) was considered separately in GPA.

Results and Discussion

Chemical data

Effects of GOx on pH and TA

Significantly larger decreases in pH over 24 hrs were observed for Tempranillo must (Batch 1; Table 1) and Tempranillo juice (Batch 2; Table 2) treated with Catazyme 25 L at 0.5 g/L (0.5 GOx) and 1.0 g/L (1.0 GOx) compared with the control (no treatment) and aerated wines. Must pH decreased from 4.6 (±0.07 SD) to 3.9 (±0.01) and juice pH decreased from 4.6 (±0.03) to 3.9 (±0.03) with addition of Catazyme 25 L at 1.0 g/L. Similarly, addition of Catazyme 25 L at 0.5 g/L decreased pH of the must and juice to 4.0 (±0.01) and 3.8 (±0.01), respectively.

TA also increased from an average of 3.2 g/L (±0.11) to 7.9 g/L (±0.06) in the juice trial (Batch 2; Table 3), and from an average of 4.5 g/L (±0.1) to 7.6 g/L (±0.3) in the must trial (Batch 1; Table 4). The production of gluconic acid by the enzymes was responsible for the increase in acidity.

Effects of GOx on glucose and gluconic acid

A decrease in glucose with a simultaneous increase in acidity in treatments using Catazyme 25 L were observed with both Tempranillo juice and Tempranillo must laboratory trials, albeit not all changes were found to be statistically significant.

Data from the Batch 2 Tempranillo juice experiment show that glucose levels decreased by an average of 14.1 g/L with the 1.0 GOx treatment and 13.1 g/L with the 0.5 GOx treatment over a 24-hr period (Table 5). Batch 1 Tempranillo must data shows similar results, with glucose levels decreasing by an average of 11.9 g/L with the 1.0 GOx treatment and 11.3 g/L with the 0.5 GOx treatment over a 24-hr period (Table 6). Glucose levels were not statistically different among treatments in Batch 1, whereas the glucose levels in both GOx treatment groups were significantly different from control and aeration treatments in Batch 2. A slight decrease in glucose was observed with the control and aeration treatments, but this may be due to the onset of spontaneous fermentation.

Gluconic acid production was highest when using only grape juice. Batch 2 (Table 7) method treatment 1.0 GOx showed a final gluconic acid average of 12.6 g/L compared to only 0.12 g/L in the control treatment, and treatment 0.5 GOx showed a final gluconic acid average of 10.3 g/L compared to the 0.12 g/L in the control treatment. The Batch 1 (Table 8) Tempranillo must experiment showed similar results, although gluconic acid production was lower, which is likely related to treating must instead of juice. Treatment 1.0 GOx still had the highest average production of gluconic acid at 7.2 g/L, while treatment 0.5 GOx had a final average gluconic acid level of 5.6 g/L. Gluconic acid is not a natural acid found in grapes or wine but can be caused by the infection of certain fungi such as *Botrytis* or *Aspergillus*. Fungus-infected fruit may be the best explanation for the trace amounts of gluconic acid in the control and aeration treatments.

Color change in juice

The color of red wine is attributed to the presence of polyphenols, such as anthocyanins and tannins (Valencia et

Table 1 Means and standard deviations for pH of Batch 1 (must) method experiments.

	Batch 1 - Must pH over time						
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	4.6 ± 0.07 ab[b]	4.5 ± 0.04 ab	4.5 ± 0.00 abc	4.4 ± 0.00 cde	4.6 ± 0.01 a	4.5 ± 0.01 abc	4.4 ± 0.03 cd
Aeration	4.5 ± 0.03 ab	4.6 ± 0.00 bcd	4.4 ± 0.01 bcd	4.4 ± 0.01 cde	4.5 ± 0.01 abc	4.4 ± 0.01 cde	4.3 ± 0.01 efg
0.5 GOx	4.6 ± 0.01 a	4.4 ± 0.00 defg	4.3 ± 0.02 ghi	4.1 ± 0.01 jkl	4.2 ± 0.01 hij	4.0 ± 0.03 klm	4.0 ± 0.01 mn
1.0 GOx	4.6 ± 0.02 a	4.4 ± 0.01 def	4.3 ± 0.02 fgh	4.0 ± 0.01 lmn	4.2 ± 0.02 ijk	4.0 ± 0.01 lmn	3.9 ± 0.01 m

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

Table 2 Means and standard deviations for pH of Batch 2 (juice) experiments.

	Batch 2 - Juice pH over time						
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	4.6 ± 0.03 ab[b]	4.6 ± 0.05 ab	4.7 ± 0.01 a	4.7 ± 0.01 a	4.7 ± 0.01 a	4.7 ± 0.01 a	4.7 ± 0.01 a
Aeration	4.6 ± 0.01 b	4.6 ± 0.02 b	4.6 ± 0.01 ab	4.7 ± 0.01 a	4.6 ± 0.01 ab	4.6 ± 0.01 b	4.5 ± 0.02 c
0.5 GOx	4.6 ± 0.01 b	4.5 ± 0.01 c	4.3 ± 0.01 d	4.2 ± 0.04 e	4.1 ± 0.02 ef	4.0 ± 0.02 fg	3.8 ± 0.01 h
1.0 GOx	4.6 ± 0.01 b	4.5 ± 0.01 c	4.3 ± 0.01 d	4.2 ± 0.03 e	4.1 ± 0.03 fg	4.1 ± 0.04 gh	3.9 ± 0.03 i

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

Table 3 Means and standard deviations for titratable acidity (g/L) of Batch 2 (juice) experiments.

	Batch 2 – Juice titratable acidity (g/L) over time						
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	3.2 ± 0.11 ijk[b]	3.2 ± 0.2 ijk	3.2 ± 0.04 jk	3.1 ± 0.06 k	3.2 ± 0.05 ijk	3.3 ± 0.04 ijk	3.6 ± 0.05 hi
Aeration	3.2 ± 0.07 ijk	3.4 ± 0.07 ijk	3.4 ± 0.06 ijk	3.4 ± 0.06 ijk	3.6 ± 0.07 ij	4.0 ± 0.42 gh	4.2 ± 0.11 g
0.5 GOx	3.3 ± 0.11 ijk	4.3 ± 0.33 g	4.8 ± 0.15 f	5.4 ± 0.12 de	5.9 ± 0.22 d	6.4 ± 0.34 c	6.9 ± 0.34 b
1.0 GOx	3.1 ± 0.02 k	4.1 ± 0.11 g	5.0 ± 0.15 g	5.7 ± 0.08 d	6.4 ± 0.11 c	7.0 ± 0.11 b	7.9 ± 0.06 a

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

al. 2017), in the skins; thus, there is a lack of both classes of compounds in the remaining juice when juice is pressed off from its skins shortly after harvest with no prolonged skin contact. During the Tempranillo juice experiment, the aeration provided for GOx caused the juice to change color (brownish hue) during the treatment. The placement of skins back into the juice during fermentation allowed for red color to reappear, thereby eliminating the browning color that had been observed.

Wines postbottle

After the prefermentation experiment using Catazyme 25 L was concluded, the Tempranillo juice and must were vinified and bottle aged. Batch 1 (Tempranillo must) was bottled aged for three months before being tested for pH, TA, and alcohol percentage. Batch 2 (Tempranillo juice) was bottled aged for eight months before being tested for pH, TA, alcohol percentage, free SO_2, and volatile acidity (VA).

Table 4 Means and standard deviations for titratable acidity (g/L) for Batch 1 (must) experiments.

	Batch 1 – Must titratable acidity (g/L) over time						
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	4.5 ± 0.1 hijk[b]	4.6 ± 0.1 hijk	4.6 ± 0.1 hijk	4.9 ± 0.1 fghi	5.4 ± 0.1 def	5.8 ± 0.1 cd	6.5 ± 0.2 b
Aeration	4.4 ± 0.3 ijkl	3.8 ± 0.2 lmn	3.6 ± 0.1 n	3.8 ± 0.2 mn	4.1 ± 0.2 klm	4.9 ± 0.2 efghi	4.8 ± 0.2 ghij
0.5 GOx	4.3 ± 0.00 jklm	4.6 ± 0.2 hijk	5.0 ± 0.5 efgh	5.4 ± 0.2 defg	5.6 ± 0.4 cd	5.9 ± 0.1 cd	7.4 ± 0.1 a
1.0 GOx	4.4 ± 0.3 ijkl	4.6 ± 0.1 hijk	4.8 ± 0.2 ghij	5.5 ± 0.1 de	6.1 ± 0.1 bc	5.9 ± 0.3 bcd	4.6 ± 0.3 a

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

Table 5 Means and standard deviations for glucose (g/L) for Batch 2 (juice) experiments.

	Batch 2 – Juice glucose (g/L) over time					
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**
Control	105.8 ± 0.2 abc[b]	106.6 ± 0.4 a	104.5 ± 0.3 bcdeg	104.1 ± 0.2 def	104.5 ± 0.7 cdef	106.9 ± 1.9 a
Aeration	106.1 ± 0.8 ab	105.6 ± 0.6 abcde	104.6 ± 0.7 bcdef	104.3 ± 0.1 cdef	104.4 ± 0.1 cdef	103.7 ± 0.5 f
0.5 GOx	107.1 ± 0.5 a	103.3 ± 0.2 f	100.6 ± 0.4 gh	99.0 ± 0.5 hi	98.5 ± 0.7 ij	97.2 ± 0.9 jk
1.0 GOx	105.8 ± 0.5 abcd	104.1 ± 0.4 ef	101 ± 0.2 g	99.4 ± 0.6 ghi	98.4 ± 0.4 ij	95.7 ± 0.6 k

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

Table 6 Means and standard deviations for glucose (g/L) for Batch 1 (must) experiments.

	Batch 1 – Must glucose (g/L) over time						
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	112.1 ± 0.9 a[b]	109 ± 0.7 a	108 ± 0.9 a	93 ± 33.2 a	97 ± 21.4 a	108.4 ± 1.7 a	107.5 ± 1.5 a
Aeration	111.8 ± 0.1 a	110 ± 1.2 a	107.8 ± 0.3 a	108.1 ± 0.6 a	108.7 ± 0.4 a	100.1 ± 13.3 a	103 ± 0.9 a
0.5 GOx	110.5 ± 0.3 a	107.2 ± 0.3 a	104.3 ± 0.2 a	104.3 ± 0.3 a	94.8 ± 16.8 a	101.2 ± 0.8 a	99.5 ± 0.6 a
1.0 GOx	110.2 ± 0.3 a	106.1 ± 0.8 a	104.5 ± 0.3 a	106.2 ± 4.0 a	103.5 ± 0.8 a	102.3 ± 1.0 a	98.4 ± 0.2 a

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

Table 7 Means and standard deviations for gluconic acid (mg/L) for Batch 2 (juice) experiments.

	Batch 2 – Juice gluconic acid (mg/L) over time					
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**
Control	100.3 ± 6.7 a[b]	85.5 ± 17.8 b	220.3 ± 8.8 b	241.0 ± 11.4 c	84.0 ± 4.1 d	84.3 ± 20.5 c
Aeration	71.3 ± 22.2 a	203.8 ± 79.8 b	238.3 ± 15.6 b	263.3 ± 14.6 c	180.0 ± 17.2 c	197.0 ± 23.4 c
0.5 GOx	123.0 ± 96.7 a	2826.0 ± 74.6 a	4612.5 ± 241.8 a	6569.5 ± 549.2 b	7811.3 ± 819.4 b	8913.0 ± 1042.2 b
1.0 GOx	90.5 ± 11.7a	3245.0 ± 276.9 a	5311 ± 345.4 a	8269.3 ± 1566.2 a	9175.8 ± 533.7 a	11018.0 ± 705.5 a

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

For Batch 1 wines, Table 9 shows the average pH, TA, and alcohol percentage for all treatments analyzed three months after bottling. pH, TA, and alcohol percentage were found to be statistically different between treatments. The average pH was lowest with the 1.0 GOx treatment (4.01), followed by the 0.5 GOx treatment (4.11). Control (no treatment) had the second highest average (4.66), and aeration treatment had the highest pH value (4.83). The TA for the 1.0 GOx treatment was highest with an average of 8.1 g/L, followed by the 0.5 GOx treatment (7.1 g/L). Control treatment had an average TA of 4.8 g/L, and aeration treatment had the lowest average TA at 3.8 g/L. Alcohol percentage was highest with the control treatment (average of 11.4%), followed closely by aeration treatment (average of 11.3%). The 0.5 GOx treatment had an average alcohol concentration of 10.8%, while the 1.0 GOx treatment had the lowest average alcohol concentration (10.6%).

Batch 2 wines were analyzed eight months after bottling. Each parameter average for each treatment can be found in Table 10. pH, TA, alcohol percentage, and free SO_2 were all significantly different by treatment; however, VA was not. Lowest average pH was observed with the 1.0 GOx treatment (3.97), followed by the 0.5 GOx treatment (4.08). Control and aeration treatments had the highest pH averages of 4.63 and 4.64, respectively. Average TA was highest with the 1.0 GOx treatment (8.5 g/L), followed closely by 0.5 GOx treatment (7.9 g/L). Average TA was 4.5 g/L with aeration treatment and 3.6 g/L with control treatment. Average alcohol concentration was 11.7% with control treatment and 11.5% with aeration treatment. The 0.5 GOx and 1.0 GOx treatments had the lowest alcohol concentrations, with averages of 11.1% and 10.6%, respectively. Free SO_2 data revealed that GOx-treated wines held less free SO_2 compared to control or aeration treatments. The average free SO_2 levels with the 0.5 and 1.0 GOx treatments were 6 and 8 mg/L, respectively, and were 18 mg/L with control treatment and 26 mg/L with aeration treatment. Although not statistically significant, average VA was numerically highest with

Table 8 Means and standard deviations for gluconic acid (mg/L) of Batch 1 (must) experiments.

	Batch 1 – Must gluconic acid (mg/L) over time						
Treatment[a]	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	207 ± 11.5 g[b]	260.8 ± 49.2 g	191.5 ± 23.4 g	183.5 ± 17.9 g	200.8 ± 20.3 g	195.8 ± 46.8 g	186.8 ± 16 g
Aeration	200 ± 11.5 g	208.3 ± 14.3 g	210.3 ± 21.7 g	215.0 ± 23.3 g	226.3 ± 17.4 g	172.5 ± 91.2 g	231.8 ± 36 g
0.5 GOx	198 ± 5.8 g	2474.3 ± 335.6 f	3078 ± 307.2 ef	4067.3 ± 258.7 d	5317.5 ± 579.4 c	5138.8 ± 212.7 c	5616.8 ± 469.3 bc
1.0 GOx	188 ± 9.2 g	3440 ± 159.8 de	3508.5 ± 141.9 de	5678.3 ± 1276.3 bc	5712 ± 288.7 bc	6398 ± 93.5 ab	7177.5 ± 234.5 a

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

Table 9 Final average pH, titratable acidity (TA, g/L), and alcohol percentage for Batch 1 Tempranillo wines three months after bottling. Different letters following values indicate different statistical groupings.

	Final average parameters - Batch 1 wines		
Treatment[a]	**pH**	**TA (g/L)**	**Alcohol (%)**
Control	4.66 a[b]	4.8 a	11.4 a
Aeration	4.83 b	3.8 b	11.3 a
0.5 GOx	4.11 c	7.1 c	10.8 b
1.0 GOx	4.01 c	8.1 d	10.6 b

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

Table 10 Final average pH, titratable acidity (TA, g/L), alcohol percentage, free SO_2 (mg/L), and volatile acidity (VA, g/L) for Batch 2 Tempranillo wines eight months after bottling.

	Final average parameters – Batch 2 wines				
Treatment[a]	**pH**	**TA (g/L)**	**Alcohol (%)**	**Free SO_2 (mg/L)**	**VA (g/L)**
Control	4.63 a[b]	3.6 a	11.7 a	18 a/b	1.1 a
Aeration	4.64 a	4.5 b	11.5 a	26 a	1.3 a
0.5 GOx	4.08 b	7.9 c	11.1 b	6 c	1.0 a
1.0 GOx	3.97 c	8.5 c	10.6 c	8 b/c	1.2 a

[a]0.5 GOx, 0.5 g/L glucose oxidase treatment; 1.0 GOx, 1.0 g/L glucose oxidase treatment.
[b]Means followed by different letters are significantly different at $p < 0.01$.

the aeration treatment (1.3 g/L of acetic acid), followed by the 1.0 GOx treatment (1.2 g/L of acetic acid), control treatment (1.1 g/L of acetic acid), and the 0.5 GOx treatment (1.0 g/L of acetic acid).

It is worth noting that after bottling, the control wines had a final pH of 4.66 and TA of 4.8 g/L (Batch 1) and a pH of 4.63 with a TA of 3.6 g/L (Batch 2). At these pH and TA values, microbiological instability is highly likely, and SO_2 additions are often ineffective. It is of paramount importance for winemakers to manage pH and acidity early in the winemaking process and to monitor SO_2 levels throughout the life of the wine before bottling. While these are extreme values, even for Texas, any pH over 3.7 should be seen as a reason for increased caution and attention. In this particular case, we attribute the overly high pH to extra extraction of potassium from grape skins during the freezing/thawing process that the grapes experienced during storage and pre-processing.

Sensorial properties

FP of the control and GOx-treated red Tempranillo wines was conducted in a similar manner as that previously described for white wine (Pickering et al. 1999). While the rationale and utility of FP are discussed in detail elsewhere (Kitzberger et al. 2016, Škrobot et al. 2020), the method used here was based on Bredie et al. (2018). The panel was composed of nine assessors (age 21 to 65 years) with over 200 hrs of training and experience in descriptive analysis.

The number of attributes generated by each panelist ranged from one to six for color, three to 17 for aroma, and five to 19 for flavor. Following removal of redundant attributes within each panelist's lexicon, a final total of 15 color (i.e., visual evaluation), 34 aroma (i.e., headspace sniff evaluation), and 33 flavor (i.e., in-mouth evaluation) attributes were gathered across all panelists to assess the three treated samples and the untreated control by FP.

GPA analysis of variance (PANOVA) indicated that the greatest transformation effect for color, aroma, and flavor was translation (correction for variation associated with attribute intensities [Kitzberger et al. 2016]). Scale transformation (correction for variations associated with the use of different scale amplitudes by panelists), rotation transformation (correction for different interpretations of the terms and indicates the panelists' agreement or disagreement with respect to the sample) (Kitzberger et al. 2016), and translation were all significant for color ($F = 5.03$, $p < 0.0001$; $F = 1.40$, $p < 0.05$; $F = 18.41$, $p < 0.0001$). Translation was significant for aroma ($F = 4.00$, $p < 0.0001$) and flavor ($F = 2.97$, $p < 0.0001$), whereas rotation was not significant for either. For aroma, scaling was also a significant transformation ($F = 2.15$, $p < 0.04$).

Wines treated with low (0.5 GOx) and high (1.0 GOx) levels of GOx were clearly distinguished from the control (untreated) and aerated samples by color. The first two dimensions explained 82% of the total variance, with the first dimension accounting for 61% of variation (Figure 1A and 1B). The 0.5 GOx and 1.0 GOx treatments yielded wines that were described as 'ruby' and 'red', while control- and aeration-treated samples were described as 'brown' and 'tawny'.

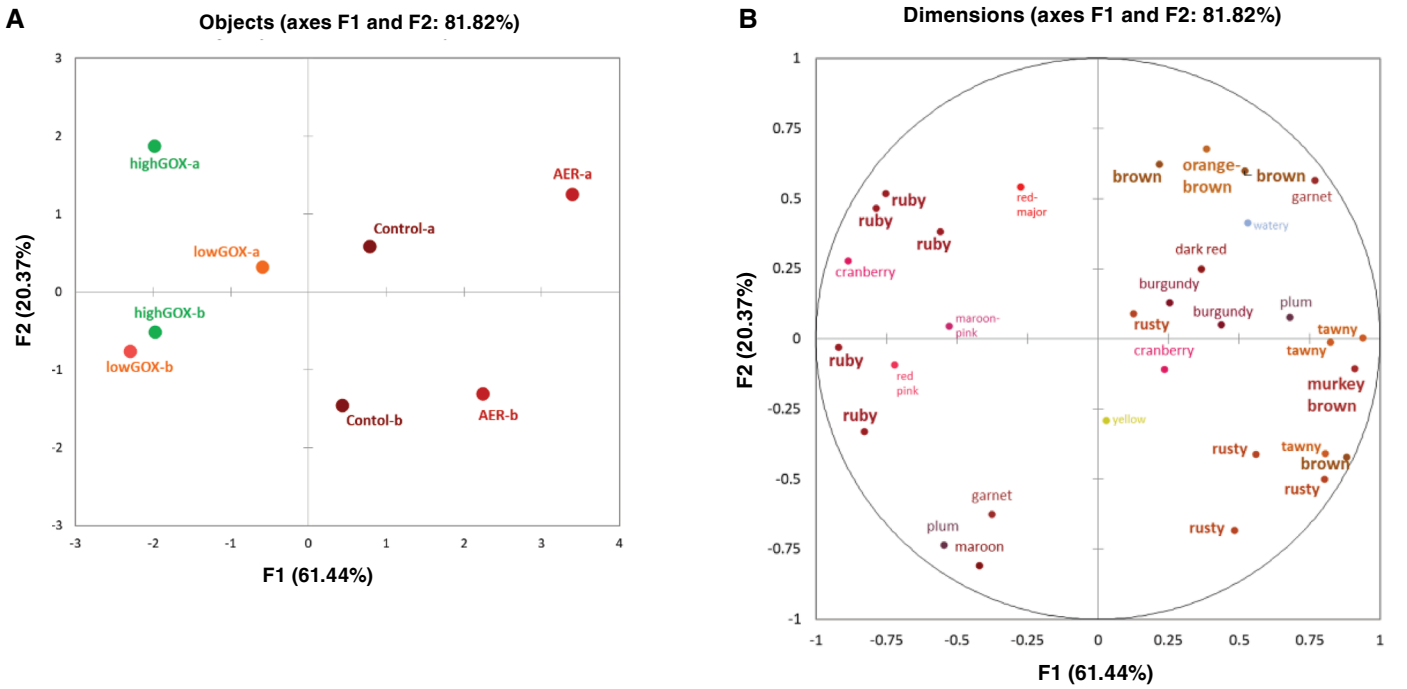

Figure 1 Plots of (**A**) sample consensus space by (**B**) color attributes. (**A**) Sample consensus space of untreated (Control) samples and Aeration (AER) and glucose oxidase (GOx)-treated samples by level (0.5 GOx, lowGOX; 1.0 GOx, highGOX) and evaluation replicate (a, b) resulting from (**B**) color characterization by panelists using Flash Profile via generalized Procrustes analysis. Attribute labels are colored to match attribute color descriptors. In alphabetical order, the final attributes for color are: brown, burgundy, cranberry, dark red, garnet, maroon, maroon-pink, orange-brown, plum, red pink, red-major, ruby, rusty, tawny, watery, yellow.

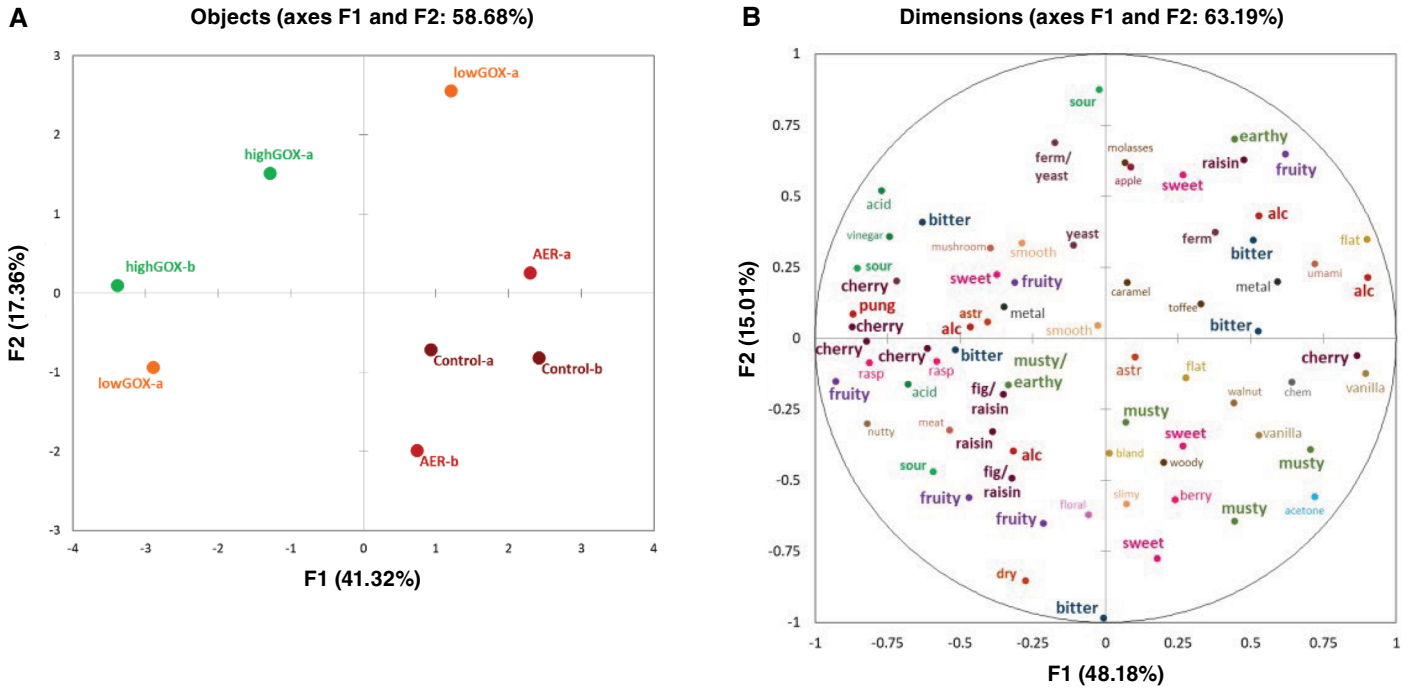

Figure 2 Plots of (**A**) sample consensus space by (**B**) aroma (via sniff) attributes. (**A**) Sample consensus space of the untreated (Control) samples and Aeration (AER) and glucose oxidase (GOx)-treated samples by level (0.5 GOx, lowGOX; 1.0 GOx, highGOX) and evaluation replicate (a, b) resulting from (**B**) aroma characterization by panelists using Flash Profile via generalized Procrustes analysis. Similar colors of attribute words indicate similar aroma notes. Attribute words of similar color indicate similar aroma notes. In alphabetical order and grouped, the final attributes (with abbreviations) for aroma were: acetone, acidic/vinegar, alcohol (Alc)/acrid/pungent (Pung), almond, apple, bitter, bland/flat, caramel, cedar, chemical (Chem)/medicinal (Med)/smokey, cherry/dark fruits/figs/raisins/sherry, citrus, earthy, ethyl acetate, fermented (Ferm), floral, fruit, herbal, honey, jam, molasses, moldy, musty, nuts, perfume, sherry, smooth, sour, sweet, vanilla, vomit/black tea/umami/meat/mushroom.

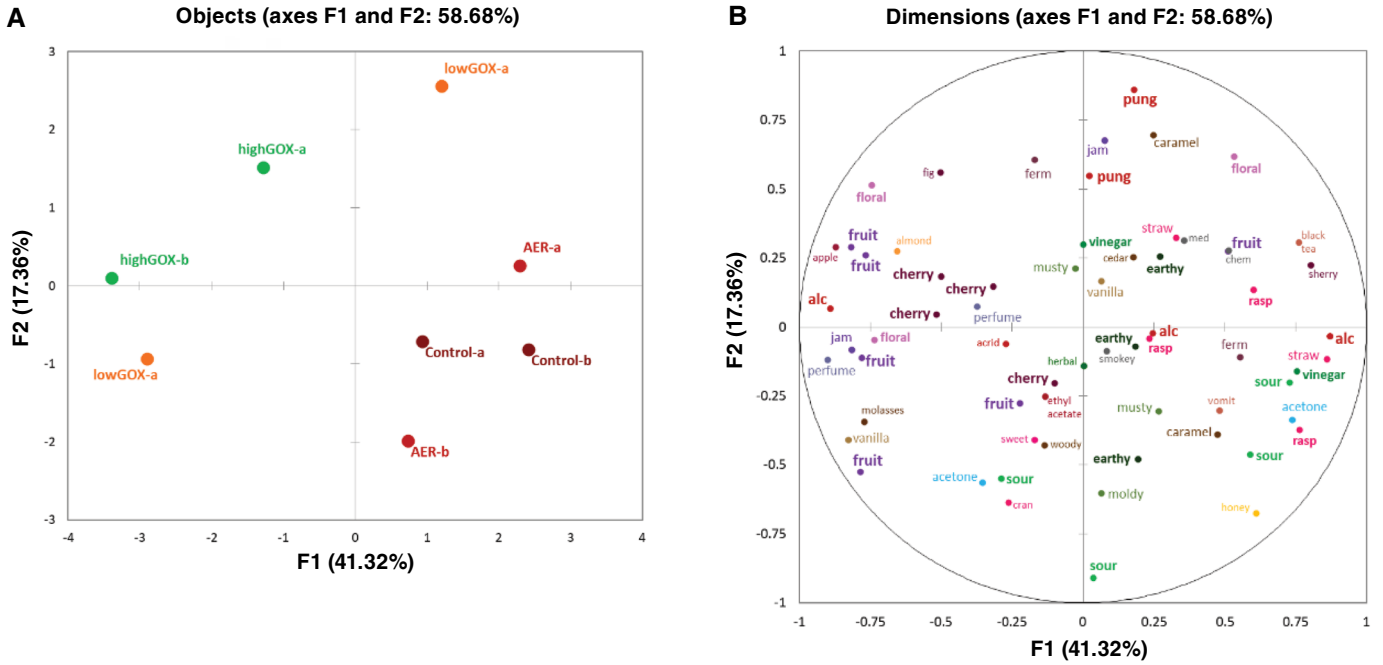

Figure 3 Plots of (**A**) sample consensus space by (**B**) flavor (in-mouth) attributes. (**A**) Sample consensus space of the untreated (control) samples and aeration (AER) and glucose oxidase (GOx)-treated samples by level (0.5 GOx, lowGOX; 1.0 GOx, highGOX) and evaluation replicate (a, b) resulting from (**B**) flavor characterization by panelists using Flash Profile via generalized Procrustes analysis. Attribute words of similar color indicate similar flavor notes. In alphabetical order and grouped, the final attributes (with abbreviations) for flavor (in-mouth) are: acetone, acid, alcohol (Alc)/pungent (Pung), apple, astringent (Astr)/dry, berry, bitter, bland, caramel, chemical (Chem), cherry, fermented (Ferm)/ yeast, flat, floral, fruity, meat/umami/mushroom, metallic (Metal), musty/earthy, nutty, fig/raisin, raspberry (Rasp), slimy, smooth, sour, sweet, toffee, vanilla, vinegar, walnut, woody.

Over the sample consensus space for aroma (Figure 2A and 2B) and flavor (Figure 3A and 3B), control and aerated samples were closely associated, as were 0.5 GOx and 1.0 GOx samples. The first two dimensions explained 57% of the total variance for aroma and 63% for flavor. For both aroma and flavor, dimension 1 explained the greatest variation and provided the clearest differentiation of samples.

For aroma, dimension 1 (41%) separated control and aerated samples from 0.5 GOx and 1.0 GOx samples. GOx-treated wines were associated with 'cherry,' 'fruit,' 'alcohol,' and 'floral' attributes, and the aerated and control wines were associated with 'sour,' 'earthy,' and 'fermented' aromas. A similar sample arrangement was observed in the GPA space for flavor, with 0.5 and 1.0 GOx wines mapping together and control and aerated wines mapping together across dimension 1 (48%). For in-mouth characteristics, 1.0 and 0.5 GOx wines were described as 'cherry,' 'fig/raisin,' 'fruity,' and 'sour,' whereas aerated and control samples were described as 'musty,' 'flat,' and 'sweet,' and associated with 'vanilla,' 'toffee,' and 'caramel.'

Conclusion

High pH grape juice and grape must are a ubiquitous problem in all hot grapegrowing regions. The novelty of this work is that it provides an alternative for winemakers to quickly (2 to 24 hrs) and inexpensively lower the pH of high pH grape juice or must to optimal levels while simultaneously increasing TA, reducing alcohol levels, and protecting against oxidation in residual sugar wines. By focusing on acidity rather than sugars/alcohol, the treatment proves to be applicable, as the levels of gluconic acid produced are not detrimental to the sensorial profile of the wines, and the TA stays within reasonable limits without a need for posttreatment de-acidification.

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References

Bankar S, Bule M, Singhal R and Ananthanarayan L. 2009. Glucose oxidase – an overview. Biotechnol Adv 27:489-501. DOI: 10.1016/j.biotechadv.2009.04.003

Botezatu A, Elizondo C, Bajec M and Miller R. 2021. Enzymatic management of pH in white wines. Molecules 26:2730. DOI: 10.3390/molecules26092730

Boulton R. 1980. The general relationship between potassium, sodium, and pH in grape juice and wine. Am J Enol Vitic 31:182-186. DOI: 10.5344/ajev.1980.31.2.182

Bredie WLP, Liu J, Dehlholm C and Heymann H. 2018. Flash Profile Method. *In* Descriptive Analysis in Sensory Evaluation. Kemp SE et al. (Eds), pp. 513–533. Wiley & Sons. DOI: 10.1002/9781118991657.ch14

Chelikani P, Fita I and Loewen PC. 2004. Diversity of structures and properties among catalases. Cell Mol Life Sci 61:192-208. DOI: 10.1007/s00018-003-3206-5

Gower JC. 1975. Generalized Procrustes Analysis. Psychometrika 40:33–51 DOI: 10.1007/bf02291478

Kitzberger CSG, Scholz MBS and da Silva JBGD. 2016. Free choice profiling sensory analysis to discriminate coffees. AIMS Agric Food 1:455-469. DOI: 10.3934/agrfood.2016.4.455

Liu J, Grønbeck MS, di Monaco R, Giacalone D and Bredie WLP. 2016. Performance of Flash Profile and Napping with and without training for describing small sensory differences in a model wine. Food Qual Prefer 48:41-49. DOI: 10.1016/j.foodqual.2015.08.008

Liu J, Bredie WL, Sherman E, Harbertson JF and Heymann H. 2018. Comparison of rapid descriptive sensory methodologies: free-choice profiling, flash profile and modified flash profile. Food Res Int 106:892-900. DOI: 10.1016/j.foodres.2018.01.062

Margalit Y. 2004. Concepts in Wine Chemistry. 2d Ed. Wine Appreciation Guild, San Francisco.

Ough CS. 1975. Further investigations with glucose oxidase-catalase enzyme systems for use with wine. Am J Enol Vitic 26:30-36. DOI: 10.5344/ajev.1975.26.1.30

Pickering GJ, Heatherbell DA and Barnes MF. 1999. The production of reduced-alcohol wine using glucose oxidase treated juice. Part I. Composition. Am J Enol Vitic 50:291-298. DOI: 10.5344/ajev.1999.50.3.291

Škrobot DJ, Tomić JM, Dapčević-Hadnadev TR, Novaković AR, Hadnadev MS, Delić JD et al. 2020. Flash profile as a rapid descriptive analysis in sensory characterization of traditional dry fermented sausages. Food Feed Res 47:55-63. DOI: 10.5937/FFR2001055S

Tang B, He Y, Liu J, Zhang J, Li J, Zhou J et al. 2019. Kinetic investigation into pH-dependent color of anthocyanin and its sensing performance. Dyes Pigm 170. DOI: 10.1016/j.dyepig.2019.107643

Valencia P, Espinoza K, Ramirez C, Franco W and Urtubia A. 2017. Technical feasibility of glucose oxidase as a prefermentative treatment for lowering the alcoholic degree of red wine. Am J Enol Vitic 68:386-389. DOI: 10.5344/ajev.2017.16005

Varela P and Ares G 2012. Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization. Food Res Int 48:893-908. DOI: 10.1016/j.foodres.2012.06.037

Wong C, Wong K and Chen X. 2008. Glucose oxidase: natural occurrence, function, properties and industrial applications. Appl Microbiol Biotechnol 78:927-938. DOI: 10.1007/s00253-008-1407-4