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Research article

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

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

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. Commercially available Catazyme® 25L, 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 lowering glucose, lowering potential alcohol, and increasing acidity.

Methods and key findings: Tempranillo must and juice were treated with Catazyme® 25L at two levels of dosage (0.5 g/L and 1g/L) over a 24 hrs period under continuous aeration. Chemical and sensory

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analyses were performed on the resulting wines. Results indicated that the pH of Tempranillo juice was lowered by 0.84 units when using Catazyme® 25L at a rate of 1.0g/L. Similarly, addition of Catazyme® 25L at 0.5g/L decreased pH from must and juice, from 4.6 to 4.0 and 3.8, respectively. Lower alcohol wines were produced when using Catazyme® 25 L due to glucose being converted into gluconic acid. Sensory evaluation of the wines indicated a positive impact of the enzyme 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 in hot climate winemaking, where it can also help lower alcohol concentration, while positively impacting the sensory profiles of the wines.

Introduction

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

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taste and astringency (Boulton, 1980). The proper pH range for red wine is between 3.4 – 3.7, but in Texas it is common to see wines with a pH of 3.8 and higher. 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 upon pH and the stability of anthocyanins, which belong to the flavonoid class of chemical compounds (Tang et al., 2019, Margalit, 2004). 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 upon the pH value (Tang et al., 2019).

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

Using GOx to produce reduced-alcohol wines. Another application for 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 pre-fermentative treatment.

The current study aims to investigate the potential of GOX plus Catalase treatment as a means to decrease pH and increase acidity in red wines. As such, 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.

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Materials and Methods

Pre-fermentation. Two different experimental trials were undertaken to observe the effects of Catazyme® 25L 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 TA of 4.4g/L, the must was then separated into four different treatments. This experimental design was chosen to observe the effects of Catazyme® 25L in grape must. Dosing Catazyme® 25L directly into grape must is the most straightforward way of using GOx with Catalase.

Each treatment in Batch 1 was carried out in 5-gallon food grade buckets with 12 liters 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 x 12-liter buckets of must and received no chemical additions or mechanical treatments. Aeration treatment contained 2 x 12-liter buckets of must fitted with one fish pump (Imagitarium™, China, 0.95 gal/min output) with 2 sparging stones (Aquaculture, China) per bucket. 0.5 g/L GOx treatment consisted of 2 x 12-liter buckets of must fitted with one fish pump, 2 sparging stones, and 6 grams of Catazyme® 25L per bucket. 1.0 g/L GOx treatment contained 2 x 12-liter buckets of must and received one fish pump with 2 sparging stones along with 12 grams of Catazyme® 25L enzyme per bucket. The experiment was conducted for 24 consecutive hours while measuring pH, TA, glucose, and gluconic acid every 4 hours.

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

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

Vinification. After the 24-hour pre-fermentation research was concluded, the laboratory experiments of Batch 1 and Batch 2 method were then inoculated with Viti Levure MT *Saccharomyces cerevisae* yeast a rate of 1.67g/L for each bucket and given a 1.2g/L addition of Go-Ferm. Each bucket was fermented to dryness and then pressed into glass carboys using a bladder press. A 60 ppm addition of potassium metabisulfite (KMBS) was added to each carboy at this time for Batch 1. A 70 ppm addition of KMBS was added to each carboy for Batch 2 method. The wines were then sparged with Argon gas and sealed. Wines were racked twice and a one-time 70 ppm addition of KMBS was added to Batch 1, while a one-time 40 ppm KMBS addition was given to Batch 2 method. Wines were then bottled and stored in a 55° (10°C) chiller. Batch 1 wines were stored for three months before being chemically analyzed, while Batch 2 method wines were stored for eight months before being chemically analyzed.

Sensory evaluation. The Flash Profiling method (Kitzberger et al., 2016, Liu et al., 2016, Liu et al., 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 in glasses labeled with three-digit codes. The

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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 each treatment having two replicates. The first session was conducted in an air-controlled room (24°C) for attribute development and sample analysis. The total session time was 2 h. All eight samples were served all at once and panelists were given 1 h to generate attributes. A 30-min break was given to panelists, while 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 then 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 (XLSTAT help guide, Addinsoft, Paris, France).

Chemistry. Initially, three-factor general linear model analysis of variance (ANOVA) with all two-way interactions were conducted by batch for each pH, TA, glucose, and gluconic acid. The main effects of wine treatment, replication, and time, as well as treatment*replication, replication*time, and

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157 treatment*time interactions were included. Further ANOVAs were undertaken for each chemical
158 monitored in the wines and included only those main effects and interactions found (significant p-value <
159 0.05) in the initial 3-factor 2-way ANOVA. Bonferroni correction with initial $p < 0.05$ and 8 ANOVA
160 tests were conducted, resulting in an adjusted p-value for significance $p < 0.00625$ (i.e., $p < 0.01$). Finally,
161 following statistical significance with ANOVA, Tukey's HSD was used for means comparisons. Using
162 the Bonferroni correction replications and their interactions were not found to significantly contribute;
163 thus, they are not included in the final ANOVA results table (Table 11).

164 **Sensory data analysis.** Generalized Procrustes analysis (GPA) using the Commandeur method in
165 XLSTAT (XLSTAT help guide, 2022; Gower, 1975) was used to explore aroma, flavor, and color data
166 from FP of the four treatment conditions. Each modality (i.e., aroma (by sniffing headspace), flavor (in-
167 mouth aroma, taste, and mouthfeel), and color were considered separately in GPA. To obtain the full
168 PANOVA Table, the constraint $2(n–1)*p>2+p*(p–1)$, where n is the number of products and p the
169 maximum number of dimensions per configuration, was considered[Varela and Ares, 2012].

170 Prior to GPA, FP sensory data were treated as previously described for white wine[5], and will be
171 briefly described here. For each panelist, any attribute included in their vocabulary that did not
172 discriminate between samples (i.e., same score given to all four samples on two or more of the four
173 judgements (2 production replications * 2 evaluation replications) was removed. Following removal of
174 non-discriminating attributes, Pearson's correlation (r) and associated p-value were determined for each
175 panelist's remaining attributes; pairwise across products, attributes significantly correlated with $r \geq +0.8$
176 ($p < 0.05$) were deemed redundant[5]. The attribute in the correlated pair with the greatest variation (i.e.,
177 maximum rating minus minimum rating across all samples and replications) between products was
178 retained for further analysis while the other attribute was removed. If the two correlated attributes were
179 found to have the same variation, their ratings were averaged for analysis. Finally, within each of the two
180 testing sessions, the two treatment replications were averaged, yielding two scores per attribute per

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sample for inclusion in GPA (Skrobot et al., 2020). Each modality (i.e., aroma (by sniffing headspace), flavor (in-mouth aroma, taste, and mouthfeel), and color were considered separately in GPA.

Results and Discussion

Chemical data. *Effects of GOx on pH and titratable acidity (TA).* Significantly larger decreases in pH over the 24 hours were observed for both Tempranillo must (Batch 1, Table 1) and Tempranillo juice (Batch 2, Table 2) treated with Catazyme® 25L at both 0.5 g/L and 1.0 g/L than either control (no treatment) or aerated wines. On average, must pH decreased from 4.6 [±0.07] to 3.9 [±0.01] and juice pH decreased from 4.6 [± 0.03] to 3.9 [±0.03] with addition of Catazyme® 25L at 1.0g/L. Similarly, addition of Catazyme® 25L at 0.5g/L decreased pH from must and juice, to 4.0 [±0.01] and 3.8 [±0.01], respectively.

Titratable Acidity (TA) also increased, going up 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 is responsible for the increase in acidity.

Effects of GOx on glucose and gluconic acid. Both Tempranillo juice and Tempranillo must laboratory trials in this study showed a decrease in glucose with a simultaneous increase in acidity in treatments using Catazyme® 25L, albeit not all changes were found to be statistically significant.

Data from the Batch 2 Tempranillo juice experiment shows that glucose levels dropped by an average of 14.1 g/L in the 1.0 GOx treatment over a 24-hour period followed closely by the 0.5 GOx treatment in which glucose levels dropped by an average of 13.1 g/L in the same time interval (Table 5). Batch 1 Tempranillo must data shows similar results, as glucose levels dropped an average of 11.9 g/L in the 1.0 GOx treatment over a 24-hour period and the 0.5 GOx treatment showed an average drop of 11.3 g/L, Table 6. There were no statistical differences for glucose levels between treatments for Batch 1, while in Batch 2 both treatments were significantly different from Control and Aeration. A slight decrease

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in glucose for the control and aeration treatments is shown, 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, this is likely due 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 both 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 al., 2017) in the skins, thus when juice is pressed-off from its skins shortly after harvest with no prolonged skin contact, there is a lack of both classes of compounds in the remaining juice. During the Tempranillo juice experiment, it was observed that 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 occurred.

Wines post-bottle. After the pre-fermentation experiment using Catazyme® 25L was concluded, both the Tempranillo juice and Tempranillo must were vinified and bottled aged. Batch 1, Tempranillo must, was bottled aged for 3 months before being tested for pH, titratable acidity (TA), and alcohol percentage. Batch 2 method, Tempranillo juice, was bottled aged for 8 months before being tested for pH, titratable acidity (TA), alcohol percentage, free SO_2, and volatile acidity (VA).

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For Batch 1 wines, Table 9 shows the average pH, TA, and alcohol percentage for all treatments analyzed 3 months after bottling. pH, TA, and alcohol percentage were found to be statistically different between treatments. Treatment 1.0 GOx had the lowest average pH (4.01), followed by 0.5 GOx (4.11). Control (no treatment) had the second highest average (4.66) and aeration treatment the highest (4.83). The TA for treatment 1.0 GOx was highest with an average of 8.1 g/L, followed by treatment 0.5 GOx 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 in the Control, with an average of 11.4%, followed closely by aeration treatment with an average of 11.3%. Treatment 0.5 GOx had an average alcohol concentration of 10.8%, while treatment 1.0 GOx had the lowest average alcohol with 10.6%.

Batch 2 method wines were analyzed 8 months after bottling and each parameter average for each treatment can be found in Table 10. pH, TA, alcohol percentage, and free SO_2 were all statistically significantly different by treatment; however, volatile acidity (VA) was not. Lowest average pH was observed in the 1.0 GOx (3.97), followed by treatment 0.5 GOx, which had an average pH of 4.08. Control and aeration treatments had the highest averages for pH 4.63 and 4.64, respectively. TA was highest in the 1.0 GOx treatment, with an average of 8.5 g/L, followed closely by treatment 0.5 GOx with the next highest average TA of 7.9 g/L. Aeration treatment had an average TA of 4.5 g/L, and control treatment had the lowest average TA of 3.6 g/L. Control treatment had an average alcohol concentration of 11.7%, followed by aeration treatment with an average alcohol of 11.5%. Treatments 0.5 GOx and 1.0 GOx had the lowest alcohol percentages with an average of 11.1% and 10.6%, respectively. Free SO_2 data revealed that GOx-treated wines held less free SO_2 when compared to Control or Aeration treatments. Treatment 0.5 GOx had an average free SO_2 level of 6 ppm, followed by treatment 1.0 GOx, which had an average free SO_2 level of 8 ppm. Control treatment held an average free SO_2 level of 18 ppm, while aeration treatment held the highest free SO_2 level with an average of 26 ppm. Although not statistically significant, VA was highest in aeration treatment with an average of 1.3 g/L of acetic acid.

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Treatment 1.0 GOx had the next highest VA with an average of 1.2 g/L of acetic acid. Control treatment had the next highest VA with an average of 1.1 g/L of acetic acid, and treatment 0.5 GOx had the lowest VA with an average of 1.0 g/L of acetic acid.

It is worth noting that for control wines, final parameters after bottling showed a pH of 4.66 and a 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 SO2 additions are often ineffective. It is of paramount importance for winemakers to manage pH and acidity early in the winemaking process and to monitor SO2 levels throughout the life of the wine pre-bottling. While these are extreme values, even for Texas, any pH over 3.7 should be seen as a cause 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 went through during storage and pre-processing.

Sensorial Properties. Flash profiling (FP) of the control and GOX-treated red Tempranillo wines here was conducted in a similar manner to that previously described for white wine (Pickering et al., 1999). While the rationale and utility of flash profiling are discussed in detail elsewhere (Kitzberger et al., 2016, Skrobot et al., 2020), the method used here was based on Bredie et al., (2018). The panel was composed of 9 assessors (aged 21–65 years) with over 200 hours of training and experience in descriptive analysis.

The number of attributes generated by each panelist ranged from 1–6 for color, 3–17 for aroma, and 5–19 for flavor. Following removal of redundant attributes within each panelist's lexicon, across all panelists 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 to assess the 3 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

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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 ($F = 5.03$, $p < .0001$; $F = 1.40$, $p < .05$; $F = 18.41$, $p < .0001$). Translation was significant for aroma ($F = 4.00$, $p < .0001$) and flavor ($F = 2.97$, $p < .0001$), while rotation was not significant for either. For aroma, scaling was also a significant ($F = 2.15$, $p < .04$) transformation.

Wines treated with low (0.5 GOx) and high (1.0 GOx) levels of GOx 0.5 GOx1.0 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 (Figures 1a and 1b). 0.5 GOx and 1.0 GOx treatments yielded wines described as 'ruby' and 'red', while Control and Aeration treated samples were described as 'brown' and 'tawny'.

Over the sample consensus space for both aroma (Figures 2a and 2b) and flavor (Figures 3a and 3b), Control samples 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 associated with 'sour', 'earthy', and 'fermented' aromas. A similar sample arrangement was observed in the GPA space for flavor, with 0.5 GOx and 1.0 GOx wines mapping together and Control and Aerated wines mapping together across dimension 1 (48%). In-mouth, 1.0 GOx and 0.5 GOx wines were described as 'cherry', 'fig/raisin', 'fruity', and 'sour', while Aerated and Control samples were described as 'musty', 'flat', 'sweet' and associated with 'vanilla', 'toffee', and 'caramel'.

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Conclusion

High pH grape juice and grape must is a ubiquitous problem in all hot grape-growing regions. The novelty of this work is that it provides an alternative for winemakers to quickly (2 - 24 hrs) and inexpensively lower the pH of high pH grape juice or must to optimal levels while at the same time 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 titratable acidity stays within reasonable limits, without need for post treatment de-acidification.

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	Batch 1 - Must pH Over Time in Hours						
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	4.6 ± 0.07 ab	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

Table 1 Statistical data for pH regarding batch1 (must) method experiments. Tukey's Honest Significant Difference (HSD) following a three-way analysis of variance (ANOVA) and Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L treatment), 1.0 (1.0 g/L treatment).

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	Batch 2 - Juice pH Over Time in Hours						
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	4.6 ± 0.03 ab	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

Table 2 Statistical data for pH regarding batch 2 (juice) method experiments. Tukey's Honest Significant Difference (HSD) following a three-way analysis of variance (ANOVA) and Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L treatment), 1.0 GOx (1.0 g/L treatment).

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	Batch 2 – Juice **Titratable acidity (g/L) Over Time in Hours**						
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	3.2 ± 0.11 ijk	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

Table 3 Statistical data for titratable acidity (g/L) regarding batch 2 (juice) method experiments. Tukey's Honest Significant Difference (HSD) following analysis of variance (ANOVA) and Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L treatment), 1.0 (1.0 g/L treatment).

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	Batch 1 – Must **Titratable acidity (g/L) Over Time in Hours**						
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	4.5 ± 0.1 hijk	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

Table 4 Statistical data for titratable acidity (g/L) regarding batch1 (must) method experiments. Tukey's Honest Significant Difference (HSD) following analysis of variance (ANOVA) and Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L treatment), 1.0 (1.0 g/L treatment).

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	Batch 2 – Juice **Glucose (g/L) Over Time in Hours**					
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**
Control	105.8 ± 0.2 abc	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

Table 5 Statistical data for glucose (g/L) regarding batch 2 (juice) method experiments. Tukey's Honest Significant Difference (HSD) following analysis of variance (ANOVA) and Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L treatment), 1.0 (1.0 g/L treatment).

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	Batch 1 – Must **Glucose (g/L) Over Time in Hours**						
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	112.1 ± 0.9 a	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

Table 6 Statistical data for glucose (g/L) regarding batch1 (must) method experiments. Tukey's Honest Significant Difference (HSD) following analysis of variance (ANOVA) and Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L treatment), 1.0 (1.0 g/L treatment).

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	Batch 2 – Juice **Gluconic Acid (mg/L) Over Time in Hours**					
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**
Control	100.3 ± 6.7 a	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

Table 7 Statistical data for gluconic acid (mg/L) regarding batch 2 (juice) method experiments. Tukey's Honest Significant Difference (HSD) following analysis of variance (ANOVA) and Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L treatment), 1.0 (1.0 g/L treatment).

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	Batch 1 – Must **Gluconic Acid (mg/L) Over Time in Hours**						
Treatment	**0 hrs**	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**	**20 hrs**	**24 hrs**
Control	207 ± 11.5 g	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

403

404 **Table 8** Statistical data for gluconic acid (mg/L) regarding batch1 (must) method experiments.
405 Tukey's Honest Significant Difference (HSD) following analysis of variance (ANOVA) and
406 Bonferroni correction such that $p < 0.01$ was required to reach significance. 0.5 GOx (0.5 g/L
407 treatment), 1.0 (1.0 g/L treatment).

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Final Average Parameters - Batch 1 Wines			
Wine/Treatment	**pH**	**TA(g/L)**	**Alcohol %**
Control	4.66 a	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

410

411 Table 9 Final average parameters for batch 1 Tempranillo wines 3 months after bottling. pH,
412 titratable acidity(g/L), and alcohol percentage. Different letters following values indicate
413 different statistical groupings.

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Final Average Parameters – Batch 2 Wines					
Wine/Treatment	**pH**	**TA (g/L)**	**Alcohol %**	**Free SO_2 (mg/L)**	**VA (g/L)**
Control	4.63 a	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

419

420 **Table 10** Final average parameters for batch 2 Tempranillo wines 8 months after bottling. pH,
421 titratable acidity(g/L), alcohol percentage, free SO_2 (mg/L), and volatile acidity(g/L). Different
422 letters following values indicate different statistical groupings.

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	F-value, p-value							
	pH		**titratable acidity**		**gluconic acid**		**glucose**	
	batch 1	**batch 2**	**batch 1**	**batch 2**	**batch 1**	**batch 2**	**batch 1**	**batch 2**
Overall ANOVA	136.40, <0.0001	316.77, <0.0001	89.73, <0.0001	294.04, <0.0001	259.99, <0.0001	241.52, <0.0001	1.01, 0.49	111.27, <0.0001
Variables								
treatment	534.36, <0.0001	1896.82, <0.0001	235.59, <0.0001	445.1, <0.0001	1716.58, <0.0001	1197.83, <0.0001	0.93, 0.43	362.86, <0.0001
time	253.02, <0.0001	299.42, <0.0001	236.08, <0.0001	1508.96, <0.0001	214.46, <0.0001	267.26, <0.0001	2.72, 0.02	180.52, <0.0001
time* treatment	31.21, <0.0001	111.42, <0.0001	16.64, <0.0001	89.44, <0.0001	74.99, <0.0001	89.16, <0.0001	0.88, 0.60	37.87, <0.0001

Table 11 F-values and p-values for final ANOVA model results for each compound in each batch and for the variables 'treatment', 'time', and the 'time*treatment' interaction.

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A) **B)**

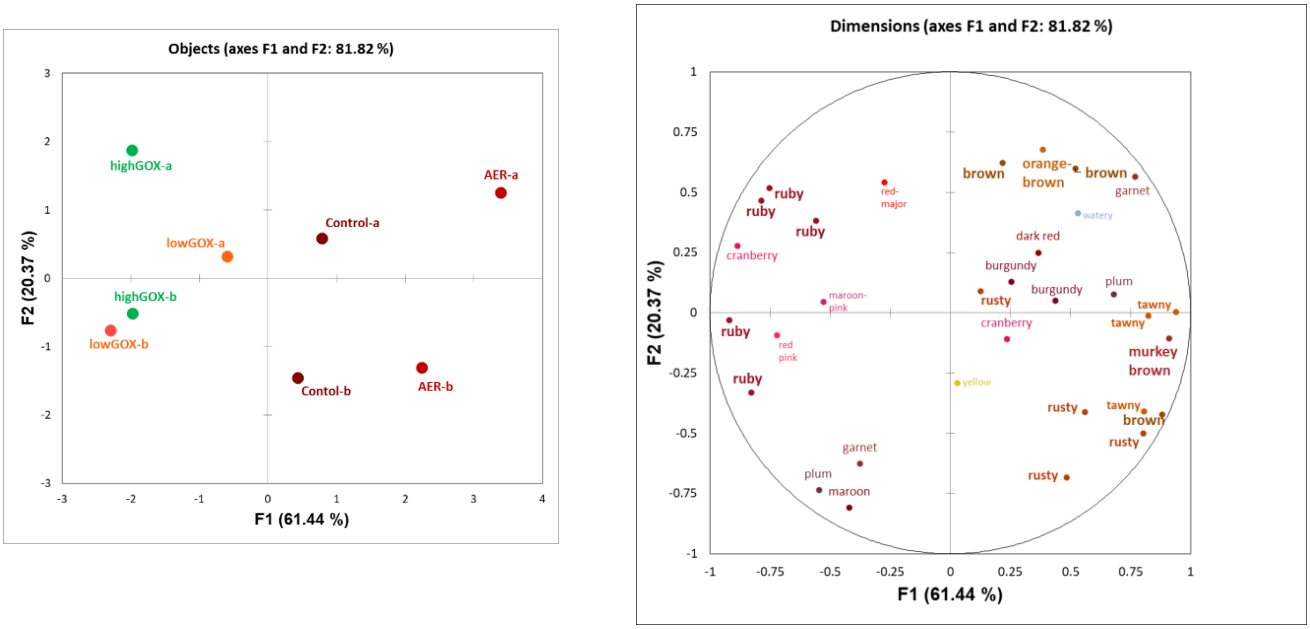

Figure 1 Plots of (A) sample consensus space by (B) color attributes.

(A) Sample consensus space of untreated (Control) samples and Aeration (AER) and 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.

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A) **B)**

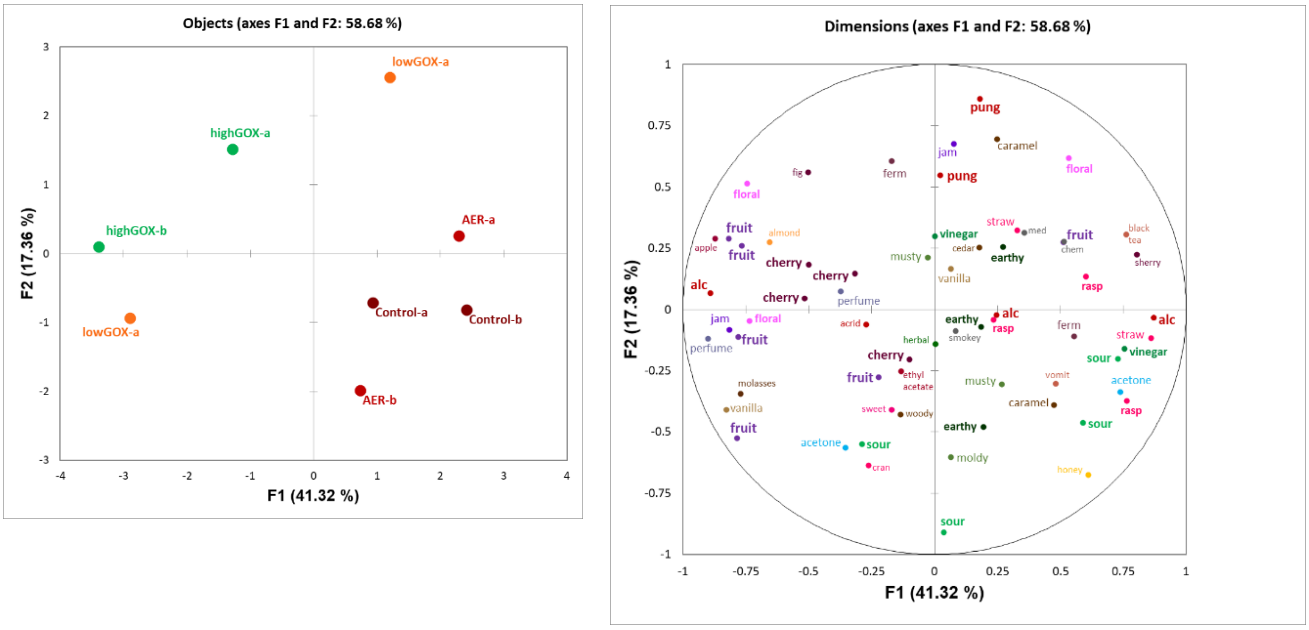

Figure 2 Plots of (A) sample consensus space by (B) aroma (via sniff) attributes. A) Sample consensus space of the untreated (Control) samples and Aeration (AER) and GOX-treated samples by level (0.5 GOx, lowGOX; 1.0 GOx, highGOX) and evaluation replicate (a, b) resulting from (B) aroma characterization 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, Carmel, 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.

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A) **B)**

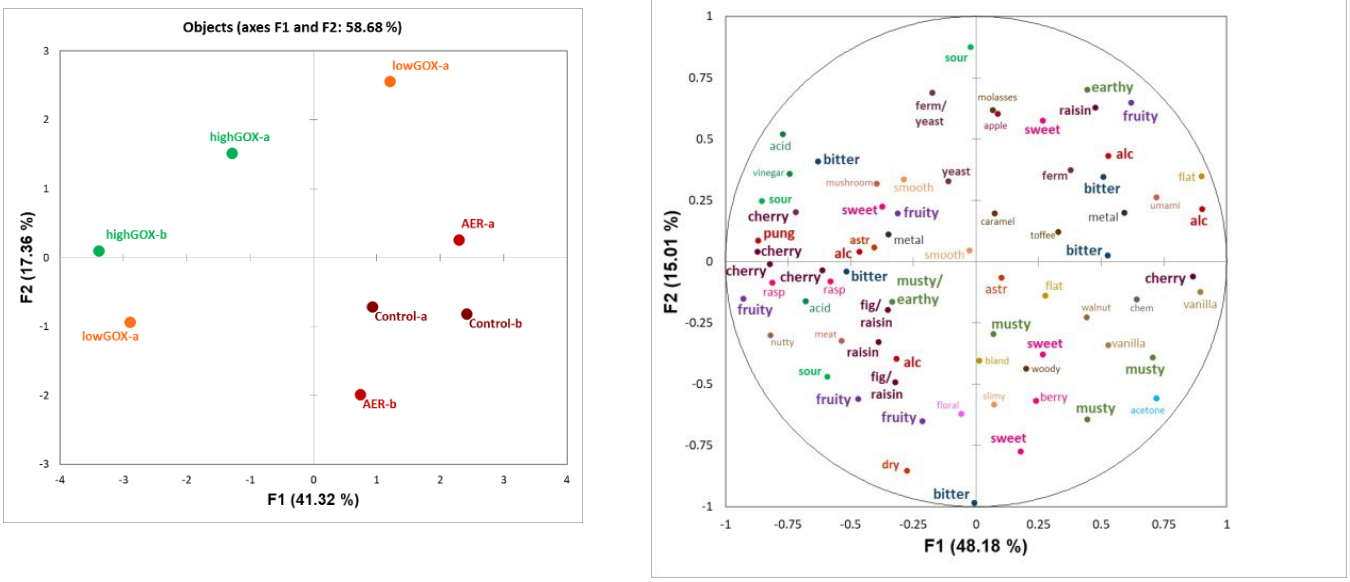

Figure 3 Plots of (A) sample consensus space by (B) flavor (in-mouth) attributes. A) Sample consensus space of the untreated (control) samples and aeration (AER) and GOX-treated samples by level (0.5 GOx, lowGOX; 1.0 GOx, highGOX) and evaluation replicate (a, b) resulting from (B) flavor characterization 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.