

Final Report - Submitted June 2020

Project Title: Sensory Characteristics of Washington State Wines

Principal Investigator: Carolyn F. Ross

Summary:

Over the past decades, a trend in the wine industry has been higher alcohol wine. To address the associated challenges with high alcohol wines, microbiological strategies are helpful. The use of wine yeast to produce wine with reduced alcohol remains one of the simplest strategies for winemakers to implement. As determined using instrumental analyses, previous studies have demonstrated that the use of non-*Saccharomyces* yeasts modifies the volatile profile of the wine. However, mouthfeel may also be affected due to the phenomenon of pectin breakdown induced by these yeasts due to their production of pectinase. However, the full influence of these non-*Saccharomyces* yeast strains on the final sensory quality remains unknown. Therefore, the overall objective of this study was to examine the influence of different non-*Saccharomyces* yeasts on wine sensory and chemical attributes.

To investigate this objective, two studies were completed. In the first study, Chardonnay and Merlot were produced with either *Saccharomyces cerevisiae* (control) or a non-*Saccharomyces* yeast cocktail (*Cr. adeliensis*, *I. orientalis*, and *P. kluyveri*), with the addition *S. cerevisiae* after three days of fermentation. Pectin was added at 0.5 g/L to ensure sufficient substrate for the pectinase. In Chardonnay, pectin addition produced higher intensities of butter aroma and citrus flavors, increased floral and grassy aromas, along with increased sharpness and solvent flavor. Differences were also seen in the chemical analysis for Chardonnay, specifically in glycerol and galacturonic acid levels, which were highest in the non-*Saccharomyces* treatment with pectin and lowest in the *Saccharomyces* wine without pectin. In Merlot, the use of non-*Saccharomyces* yeasts produced wines with higher intensities of berry and dried fruit aromas, increased sharpness, as well as more intense berry, cherry, and geranium flavors. The addition of pectin also resulted in significant differences in viscosity and weight which related to the chemical components of galacturonic acid and glycerol levels. In addition, alcohol content was also significantly different, with pectin addition reducing alcohol.

In the second study, different pectin concentrations added to the wines during fermentation (Merlot:0.25 - 1.25 g/L. Chardonnay 0.25-1 g/L) were investigated. For Merlot, the addition of non-*Saccharomyces* yeasts was associated with notes of chocolate, berry, viscosity, drying and mouthcoating, with all treatments being similar except for the wine made with 1 g/L pectin. Merlot produced using non-*Saccharomyces* yeast with added 1 g/L pectin presented more animal, herbaceous and solvent aromas. The *S. cerevisiae* treatments were similar and described by their buttery and spicy flavor notes. For Chardonnay, the *Saccharomyces* treatments were related to attributes such

as honey, melon and green apple. The Chardonnay treatments made with non-*Saccharomyces* treatments with higher pectin were associated with pear, woody and animal notes. The Chardonnay wines produced with lower concentrations of pectin with non-*Saccharomyces* were described by their green, and ethanol notes, with viscosity, and drying mouthfeel. These findings suggest that during fermentation, the use of non-*Saccharomyces* yeasts that produce pectinase, when sufficient substrate is present, may be useful in modifying wine quality.

Final Report

Project Title: Sensory Characteristics of Washington State Wines

Project Duration: 3 years

WRAC Project Number: 2018-3.EN.RC1

Principal Investigator:

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Collaborator(s):

Charles Edwards, School of Food Science, Washington State University, Pullman WA. Participation: wine microbiology and wine chemistry

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Objectives and Experiments Conducted to Meet Stated Objectives:

The overall objective of this research program is to study the sensory and consumer acceptance of Washington State Wines. To address this overall objective, we aim to:

1. Evaluate the influence of selected non-*Saccharomyces* yeasts on the sensory profile of wines.
2. Determine the chemical changes associated with the application of non-*Saccharomyces* yeasts in Washington State red and white wines.

Methods:

Wine Production

Yeasts utilized:

The non-*Saccharomyces* yeasts (*Issatchenkia orientalis* J5-6-5, *Pichia kluyveri* P01C002, and *Cryptococcus adeliensis* P44A007) were previously isolated from vineyards at the Irrigated Agriculture Research and Extension Center (Prosser, WA., U.S.A.) (Bourret et al., 2013). Yeasts were maintained and starter culture was prepared as described in Aplin et al. (2019). *Saccharomyces cerevisiae* Lavin Clos and *S. cerevisiae* Enoferm Syrah were obtained from Lallemand Inc. (Montréal, Quebec, Canada).

Production of red and white wines in 2017:

In Fall 2017, wines were made using a two-way treatment structure of yeast and pectin addition (**Figure 1**). The first treatment (yeast) was either a cocktail of non-*Saccharomyces* strains (*Cryptococcus adeliensis*, *Issatchenkia orientalis*, and *Pichia kluyveri*) or *Saccharomyces cerevisiae*. The second treatment was the addition of pectin at 0.5 g/L or 0g/L.

Chardonnay grapes were harvested, crushed, destemmed, at which point 60 ppm of SO₂ was added. The non-*Saccharomyces* treatments were inoculated with the non-*Saccharomyces* cocktail and allowed to ferment for three days prior to the inoculation

with *S. cerevisiae*. Once the wines completed fermentation, they were racked with an additional 20 ppm of SO₂ and stored in cold storage. Fermentation was monitored by daily brix measurements and temperature.

The red wines were produced using Merlot grapes. The treatments were as described for the white wine (ie. the addition of pectin to either *Saccharomyces* or non-*Saccharomyces* yeast). To prevent browning after crushing and destemming, 25 ppm of SO₂ was added to the must, and daily punch downs were completed. The non-*Saccharomyces* treatments were fermented for three days prior by the non-*Saccharomyces* cocktail prior to the addition of *S. cerevisiae*. The musts were pressed when they reached 0°Brix. The wines were transferred into glass carboys. After dryness was reached, the red wines were placed in cold storage and racked. The red wines had a total of 60 ppm SO₂ added over two rackings. The final wine was then placed in cold storage. All red and white wine treatments were bottled in February of 2018. Sensory and chemical analysis were completed at least four months after bottling.

Production of red and white wines in 2018:

In Fall 2018, red and white wines were processed with non-*Saccharomyces* yeast strains and with varying pectin levels. Chardonnay grapes were harvested, crushed, destemmed, at which point 50 ppm of SO₂ was added and held at 15°C. Pectin was added at 0 g/l, 0.25 g/L, 0.5 g/L, 0.75 g/L, and 1 g/L, along with the addition of a cocktail of non-*Saccharomyces* strains (*Cr. adeliensis*, *I. orientalis*, and *P. kluyveri*). For comparison, two additional treatments were included, these being *S. cerevisiae* at 0 g pectin/L and 1 g pectin/l. After three days, *S. cerevisiae* was inoculated into the non-*Saccharomyces* treatments and allowed to finish fermentation. Fermentation was completed at 15°C, with brix monitored daily. Once the wines completed alcoholic fermentation, they had an additional 20 ppm of SO₂ added and placed in cold storage.

Red wines were produced with Merlot grapes with the addition of 25 ppm of SO₂. Pectin was added at 0 g/l, 0.25 g/L, 0.5 g/L, 0.75 g/L, 1 g/L and 1.25 g/L, along with the addition of a cocktail of non-*Saccharomyces* yeasts. For comparison, two additional treatments were included, *S. cerevisiae* at 0 g pectin/L and 0.75 g pectin/l. After inoculation of non-*Saccharomyces* yeasts or *S. cerevisiae*, all treatments were held at 15°C for three days to encourage non-*Saccharomyces* yeast growth. *S. cerevisiae* was added to the non-*Saccharomyces* treatments and all treatments were moved to 24°C to finish fermentation. Wines fermented until they reached between 0-5°Brix and were pressed. After dryness was reached, the red wines were placed in cold storage and racked.

Objective 1 Methods:

To complete the sensory analysis of the wines trained panels were conducted. Panelists (n=10 or 13) were selected and trained over 12 hours to recognize aroma, flavor, taste and mouthfeel attributes associated with Merlot or Chardonnay wines. A small expert panel evaluated the wines and created a list of attributes that were used for the trained panel. For each of the red wine attributes, standards were prepared to illustrate the particular parameter, with all attributes evaluated along a 15-cm line scale. Additional attributes such as estery and dried fruit were included based on published research that

showed association of these attributes with non-*Saccharomyces* yeast strains (Chambers et al., 2015; Sadoudi et al., 2012). The final list of attributes included aroma/flavor (estery, pungency, fruity, dried fruit, green, yeasty, ethanol, berry, earthy, barn, woody, sulfur, sweaty, chemical, and vegetal), taste (sweet, sour and bitter) and mouthfeel attributes (burning, astringency, roughness, viscosity, weight, drying, puckering and tingle). Following training, panelists evaluated the 2017 treatments in replicate and 2018 in triplicate.

Chardonnay were evaluated in a similar manner but for different sensory attributes. The attribute list included aroma and flavor (green apple, peach, citrus, tropical fruit, buttery, honey, ethanol, solvent, woody, grassy, melon, floral, pear, sulfur, and animal), taste (sweet, sour, and bitter) and mouthfeel attributes (burning, astringency, roughness, viscosity, weight, drying, tingle, puckering, and sharpness).

For all trained panels, during the first training session, panelists were instructed in the sampling protocol. Specifically, for aroma, panelists were instructed to remove the lid from the wine glass and perform three short, sharp sniffs, allowing 30 seconds to pass in between evaluations. For tasting, they were instructed to take the sample into the mouth, swish for 10-15 s, expectorate, wait for 30 seconds and then start evaluating, reporting the highest intensity for each attribute experienced. Throughout the training, responses were collected in the Compusense software, using a 15 cm unstructured line scale with anchor points at 'low' and 'high', corresponding to 10% and 90% of the scale respectively.

After the completion of training, final evaluations were conducted in the Washington State University Sensory Evaluation Facility in Pullman, WA. Samples (40 ml) were poured into ISO wine glasses 1 hour prior to evaluation and covered with a Petri glass, with each glass labeled with a random 3-digit code. The wine was served at room temperature, under white light, and in individual booths. At the six-month time point, treatments were presented in a randomized order in duplicate (Merlot) or triplicate (Chardonnay), over 2 or 3 sessions, respectively. At the 18-month time point, all treatments were presented in triplicate. To reduce fatigue, panelists were limited to six samples per evaluation and were required to take 5-minute breaks in between samples. Panelists were provided with a cuspidor, napkin, unsalted crackers, and water to rinse their palates.

Statistical Analysis

Data analysis for the trained panel data and chemical data was performed using XLSTAT 2017 (Addinsoft, Paris, France). Wine chemical analyses were evaluated using Least Significant Differences (LSD) *post-hoc* test. For the trained panel data, a three-way ANOVA was performed to determine if significant interactions may have affected the main effects, and mean separation was completed using Fisher's LSD. Principal Component Analysis (PCA) on the covariance matrix was performed on the trained panel data to produce biplots that aid in the visualization of each treatment sensory characterization.

Objective 2 Methods:

Juice chemistry was measured initially and daily during fermentation. The pH was measured using Fisher Scientific Accumet Basic AB15 Plus pH meter (Waltham, MA, U.S.A.) and °Brix with a hydrometer. Red wine cap and juice temperature was monitored with a thermometer.

After the completion of fermentation, wine chemistry measurements were completed to determine final wine composition. Titratable acidity was analyzed using titroLine Easy Autotitrator (Schott Instruments, Mainz, Germany) following standard methods (Ough and Amerine, 1988). Ethanol was measured with an ebulliometer (Alla France, France), volatile acidity with a cash still, reducing sugars with the Clinitest ® method, and free SO₂ using the aeration/ oxidation method (Ough and Amerine, 1988). Enzymatic kits based on spectrophotometric assays were used to measure concentrations of glycerol (Megazyme, K-GCROL; Ireland) and D-galacturonic acid (Megazyme, K-URONIC; Ireland).

Mannoprotein Concentration Analysis

Triplicate samples of the finished wine (3 ml) were filtered through a 30 x 10 mm Econo-Pac ® 10 DG disposable column (Bio-Rad Laboratories, Hercules, CA, U.S.A.) to isolate the mannoprotein fractions. A 250 µl aliquot of each mannoprotein-enriched wine fraction was supplemented with internal standard solution (20 µg ml⁻¹ myo-inositol in 50% (v:v) aqueous methanol) and the solution was evaporated to dryness. The residue was resuspended in methanolysis reagent (35 µl ml⁻¹ acetylchloride in anhydrous methanol) and the mixture was maintained at 80°C for 16 h. The sample was then dried under a gentle stream of nitrogen. The residue was taken up in a 1:1 (v:v) solution of N-methyl-N-(trimethylsilyl)trifluoroacetamide and pyridine, and maintained at 37°C for 30 min. These silylation reactions were performed just-in-time with a maximum delay of 2h before further processing.

The separation and analysis of derivatized mannose (hydrolyzed from wine mannoprotein samples) was achieved by gas chromatography–mass spectrometry (GC–MS). For absolute quantitation of mannose, a unique target ion was selected, with three qualifier ions being used for proper peak identification. Adjusted peak areas were converted to concentrations based on a calibration curve obtained with varying concentrations of a mannose authentic standard.

Summary of Major Research Accomplishments and Results by Objective:

Objective 1 Results:

Red wines: **Figure 2** shows the relationships among the analytical data with the sensory profiling of the Merlot produced in 2017. Both non-*Saccharomyces* treatments (with and without added pectin) were characterized by higher levels of D-galacturonic acid, and were associated with a sharp mouthfeel, along with dried fruit, cherry and berry notes. D-galacturonic are the fragments produced from the breakdown of pectin from enzymatic activity. The Merlot produced with *Saccharomyces* and added pectin was defined by its tingle mouthfeel, sweetness, and floral notes, as well as being closely related to the titratable acidity measurement. Merlot produced with *Saccharomyces* without added pectin was defined by a puckering mouthfeel, with woody and sulfur

notes. The *Saccharomyces* without added pectin was also related higher levels of glycerol and alcohol. Statistical analysis was also completed to determine the influence that the addition of pectin had alone as well as the influence of non-*Saccharomyces* yeast addition ($p < 0.1$). When pectin was added, there was a higher intensity of fruity aromas, while the treatments without pectin were higher in sulfur aroma, weight and viscosity. The addition of non-*Saccharomyces* yeast increased berry and dried fruit aroma, berry flavor and a sharp mouthfeel. The *S. cerevisiae* wine treatments were higher in viscosity and geranium flavor.

The Merlot produced in 2018 with varying pectin levels are shown in **Figure 3**. Separation was observed among the treatments with *S. cerevisiae* added at the start of fermentation and the treatments that were allowed to ferment for three days with the non-*Saccharomyces* yeasts. *S. cerevisiae* with no added pectin and 0.75 g pectin/L added were associated with buttery and spicy flavors. The non-*Saccharomyces* Merlot with 1 g/L added pectin was associated with animal, herbaceous and solvent notes. The rest of the non-*Saccharomyces* treatments were associated with chocolate, berry, and sulfur attributes, and a drying and viscous mouthfeel.

White wines: The relationships among the analytical measures and the sensory profiling of the wine treatments produced in 2017 are shown in **Figure 4**. For the white wines, the Chardonnay without added pectin (both *Saccharomyces* and non-*Saccharomyces*) were clustered in the same quadrant. The Chardonnay made with *Saccharomyces* but without added pectin was described as being more drying, woody and rough while the non-*Saccharomyces* without added pectin was described as drying, with some weight and ethanol burn; both of these treatments were closely related to high levels of volatile acidity and pH. The Chardonnay made with non-*Saccharomyces* with added pectin was described as having more viscosity and tingle, along with notes of citrus and tropical fruit, in addition to being related to higher glycerol levels. Finally, the Chardonnay made with *Saccharomyces* with added pectin was described as having a sharp mouthfeel, along with green apple, grassy and floral notes, and a relationship with titratable acidity. Statistical analysis was also completed to determine the influence that the addition of pectin had alone as well as the influence of non-*Saccharomyces* yeast addition ($p < 0.1$). When pectin was added, there was a higher intensity of grassy and floral aroma, and solvent flavor. The addition of non-*Saccharomyces* yeast increased buttery aroma and citrus flavor in the Chardonnay.

The white wines produced in 2018 were profiled **Figure 5**. The analysis showed a similar trend to the red wines where there was a distinct separation between the non-*Saccharomyces* and *S. cerevisiae* treatments. The *Saccharomyces* treatments were related to attributes such as honey, melon and green apple. The non-*Saccharomyces* treatments with higher pectin were associated with pear, woody and animal. The rest of the non-*Saccharomyces* Chardonnay wines were related to green, and ethanol notes, with higher viscosity, and a drying mouthfeel

Objective 2 Results:

Red wines: The chemical analysis for the Merlot from 2017, showed some interesting differences (**Table 1**), with the most notable being the concentration of D-galacturonic

acid present in the wines. This is the most abundant compound in pectin and high D-galacturonic acid levels are indicative of pectin breakdown in the wine. The wine samples that were produced with the non-*Saccharomyces* yeast, regardless of pectin addition, contained higher concentrations of D-galacturonic acid than the wines produced with *Saccharomyces*, suggesting higher activity of pectinase activity by the non-*Saccharomyces* strains.

For the Merlot produced in 2018, the chemical analysis is shown in **Table 2**. There were no significant differences among alcohol content, glycerol, pH and mannoprotein concentrations. However, there were significant differences between the acetic acid levels and D-galacturonic acid levels among the different pectin levels. For acetic acid, the Merlot wines made with the highest level of pectin addition were significantly different from the Merlot with no pectin addition for the non-*Saccharomyces* yeasts treatments; a similar trend was observed in the *S. cerevisiae* treatments. All the wines made with non-*Saccharomyces* yeasts, with the exception of 0.25 and 0.75 g pectin/L, were higher in D-galacturonic acid levels than the *S. cerevisiae* treatments. This indicates that the non-*Saccharomyces* yeasts were able to utilize the pectin to produce the D-galacturonic acid fragments better than the *S. cerevisiae* treatments.

White wines: Chemical analysis of the 2017 Chardonnay are shown in **Table 3**. Unlike the Merlot, the D-galacturonic acid was highest in the treatments that had added pectin and was not influenced by the yeast. Glycerol levels were higher in the Chardonnay made with non-*Saccharomyces* yeast treatments, regardless of pectin addition. However, the *Saccharomyces* treatment with pectin added was significantly higher in glycerol levels than its non-pectin counterpart.

For the Chardonnay produced in 2018, the chemical analysis is shown in **Table 4**. No significant differences were noted among the glycerol, acetic acid, titratable acidity, and alcohol contents regardless of the amount of pectin added. The mannoprotein concentrations were higher in the treatments without any pectin added.

These results indicate that the use of non-*Saccharomyces* yeasts, along with the addition of pectin, exert a significant sensory impact, with chemical changes in glycerol, galacturonic acid and alcohol levels. These findings signify that the use of non-*Saccharomyces* yeasts that utilize pectin may be useful in modifying the sensory properties of wines made through traditional methods.

Outreach and Education Efforts - Presentations of Research:

Results of this research were communicated through the WSU extension publications, WSU Viticulture and Enology Extension News (VEEN; Paup and Ross, 2019), the annual meeting of the Institute of Food Technologists (2020), and the annual meeting of the Washington Wine Growers (2018, 2019, and 2020).

Research Success Statements:

This research has shown that the use of non-*Saccharomyces* yeasts that produce pectinase can modify the chemical and sensory profile. By utilizing these yeasts at the start of fermentation prior to the addition of *S. cerevisiae*, non-*Saccharomyces* yeasts

can modify the aromatics of the final wine, enhancing some attributes such as fruity and chocolate notes. Additionally, they can change the chemical composition, increasing compounds such as glycerol while not increasing compounds such as acetic acid that can detract from wine quality. An increase in D-galacturonic acid was also observed in the treatments with added pectin and non-*Saccharomyces* yeasts. Finally, through this project, a new methodology for quantification of mannoprotein was developed and tested.

Budget:

General summary of how the funds were used: these funds were used to fund the PhD (Food Science) graduate student, Victoria Paup, to complete this research. Funds were also used for consumables related to the cost of wine analysis and sensory profiling of the wines.

Entire project budget: \$87,000 (Awarded \$29,000 in Year 1, Year 2 and Year 3)
Remaining balance: \$0

References

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Figures and Tables

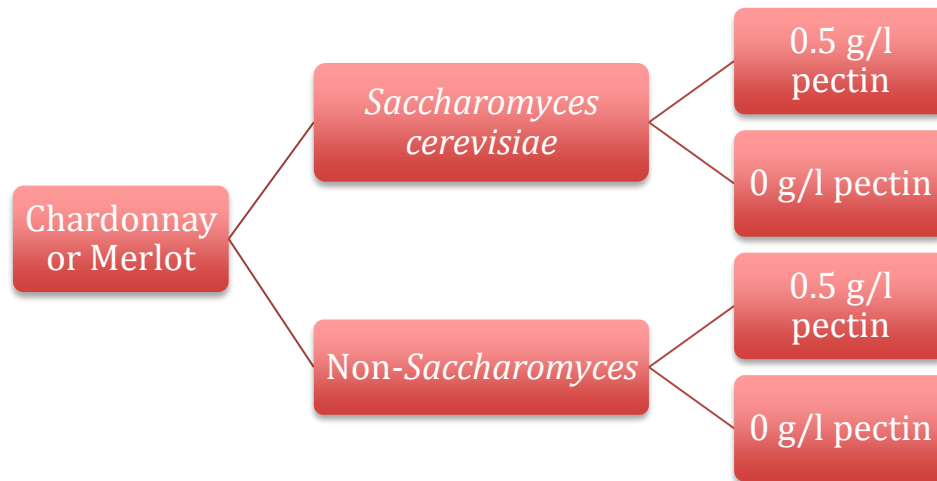


Figure 1. Schematic representation of the treatments implemented in the Chardonnay and Merlot wine fermentation. *Saccharomyces cerevisiae* was added to the non-*Saccharomyces* treatments after three days. Pectin was added at the start of fermentation.

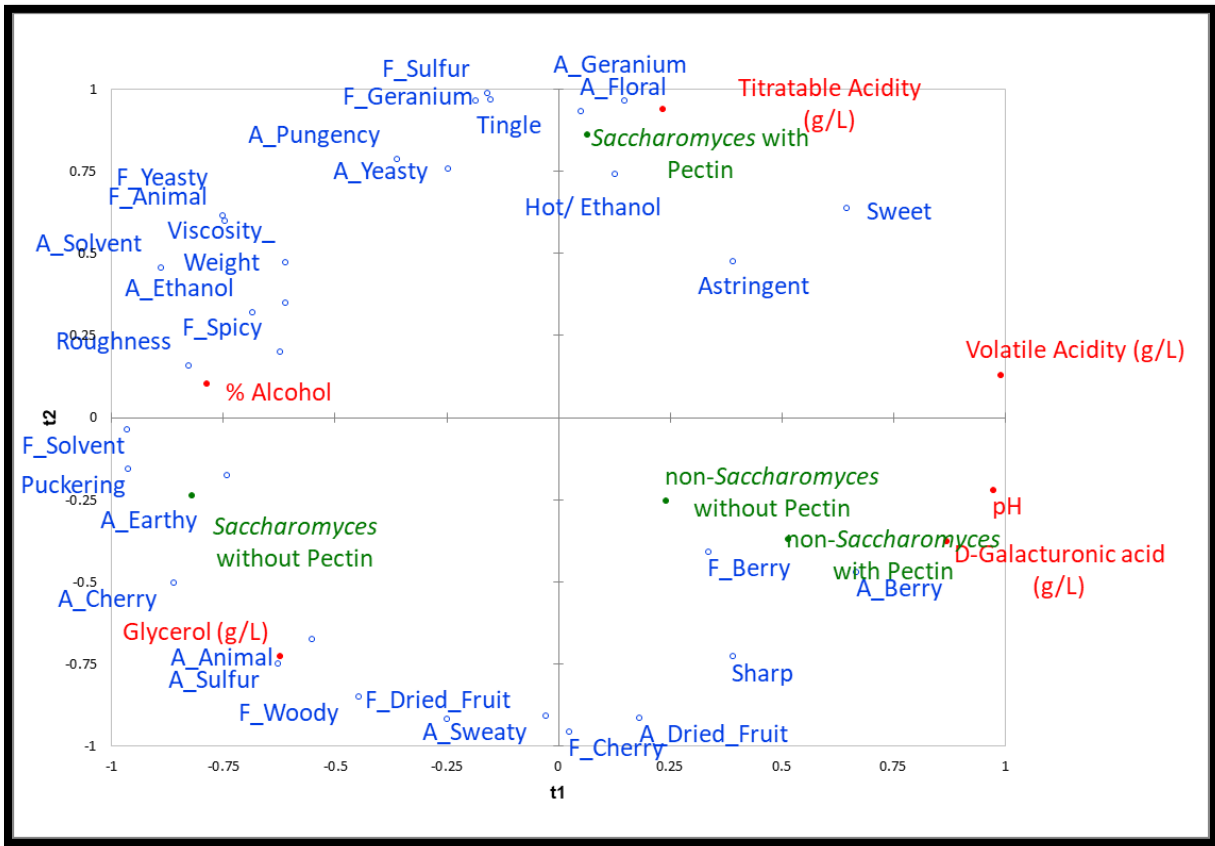


Figure 2. Merlot (2017) profiling using both chemical and sensory data. The four wine samples are shown in green and include Saccharomyces (with and without added pectin) and non-Saccharomyces (with and without added pectin).

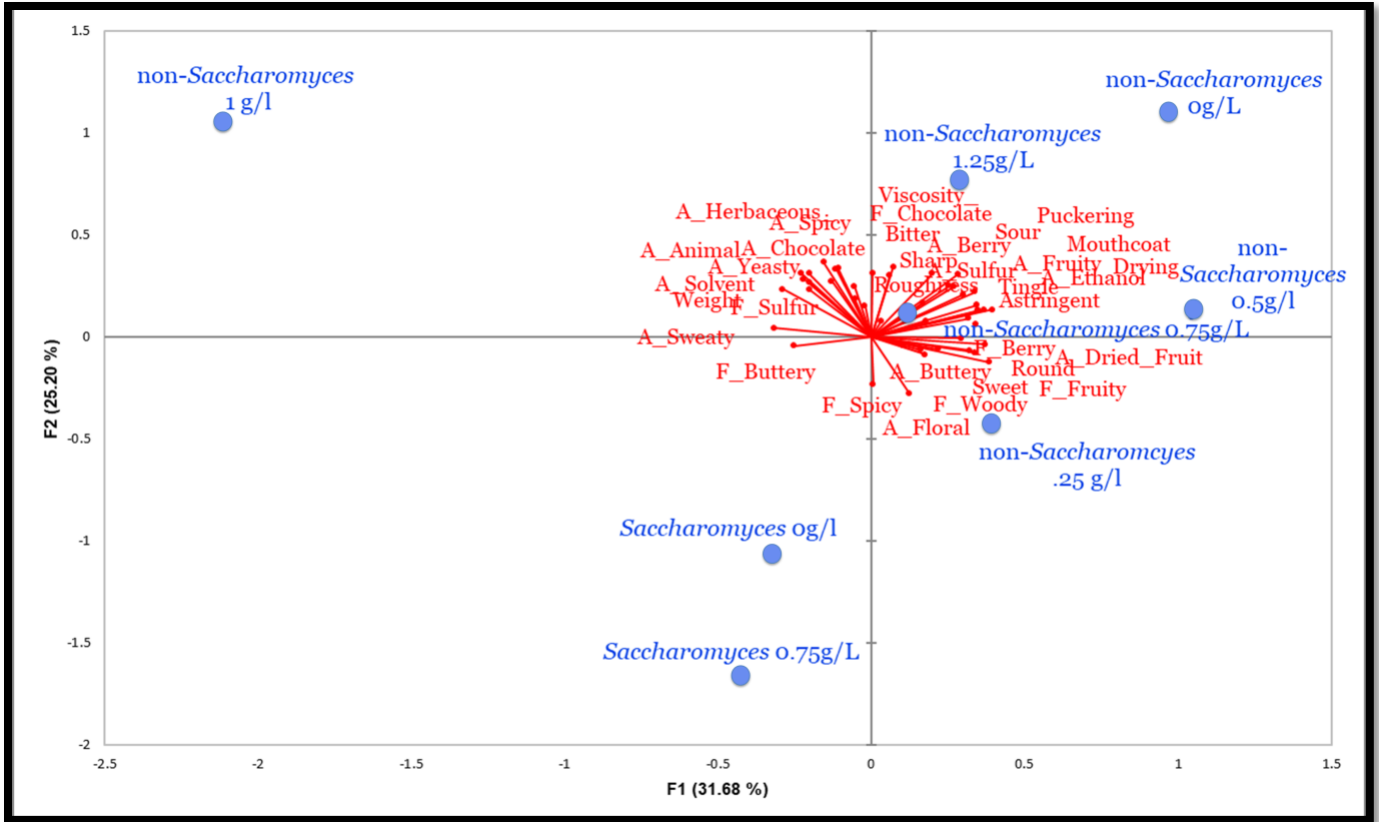


Figure 3. Principal component analysis of the red wines made in 2018 with *Saccharomyces* or *non-Saccharomyces* yeasts, with different concentrations of pectin added. The A represents aroma attributes and the F indicates flavor attributes.

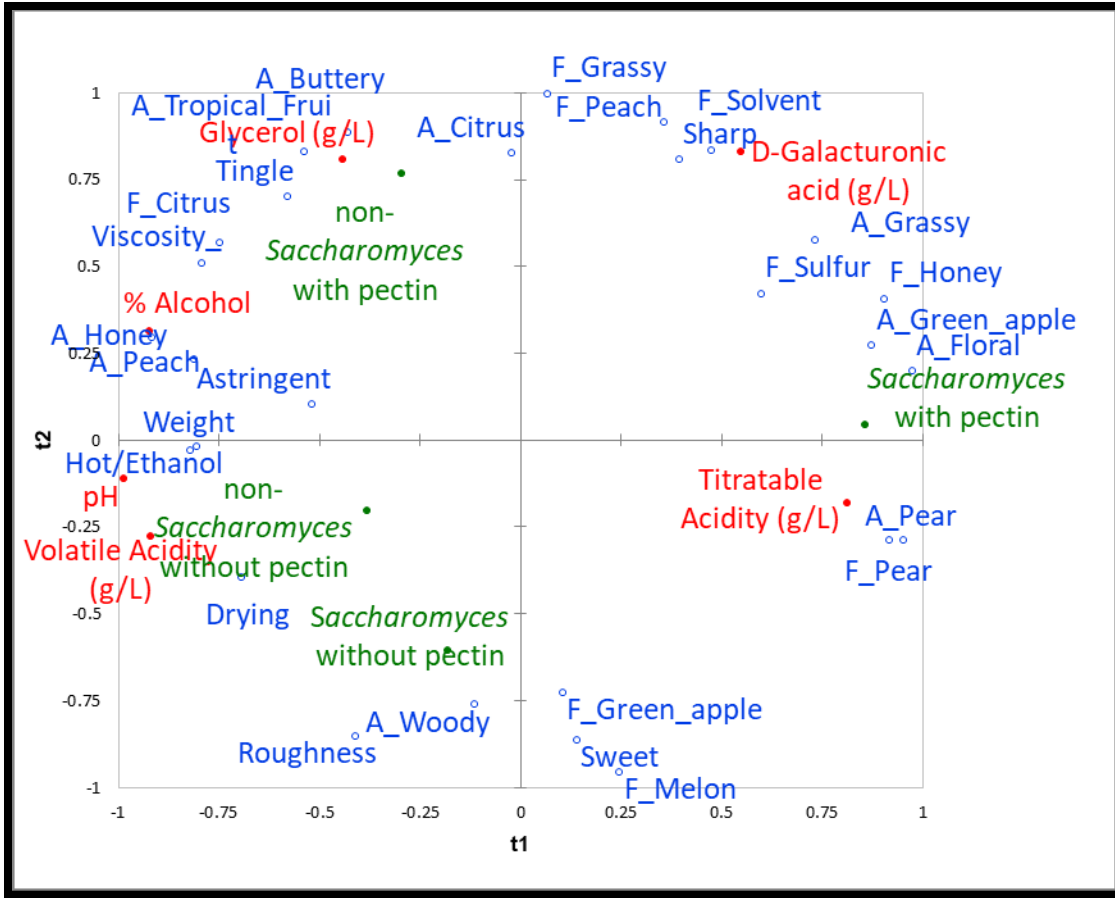


Figure 4. Chardonnay (2017) profiling using both chemical and sensory data. The four wine samples are shown in green and include *Saccharomyces* (with and without added pectin) and non-*Saccharomyces* (with and without added pectin).

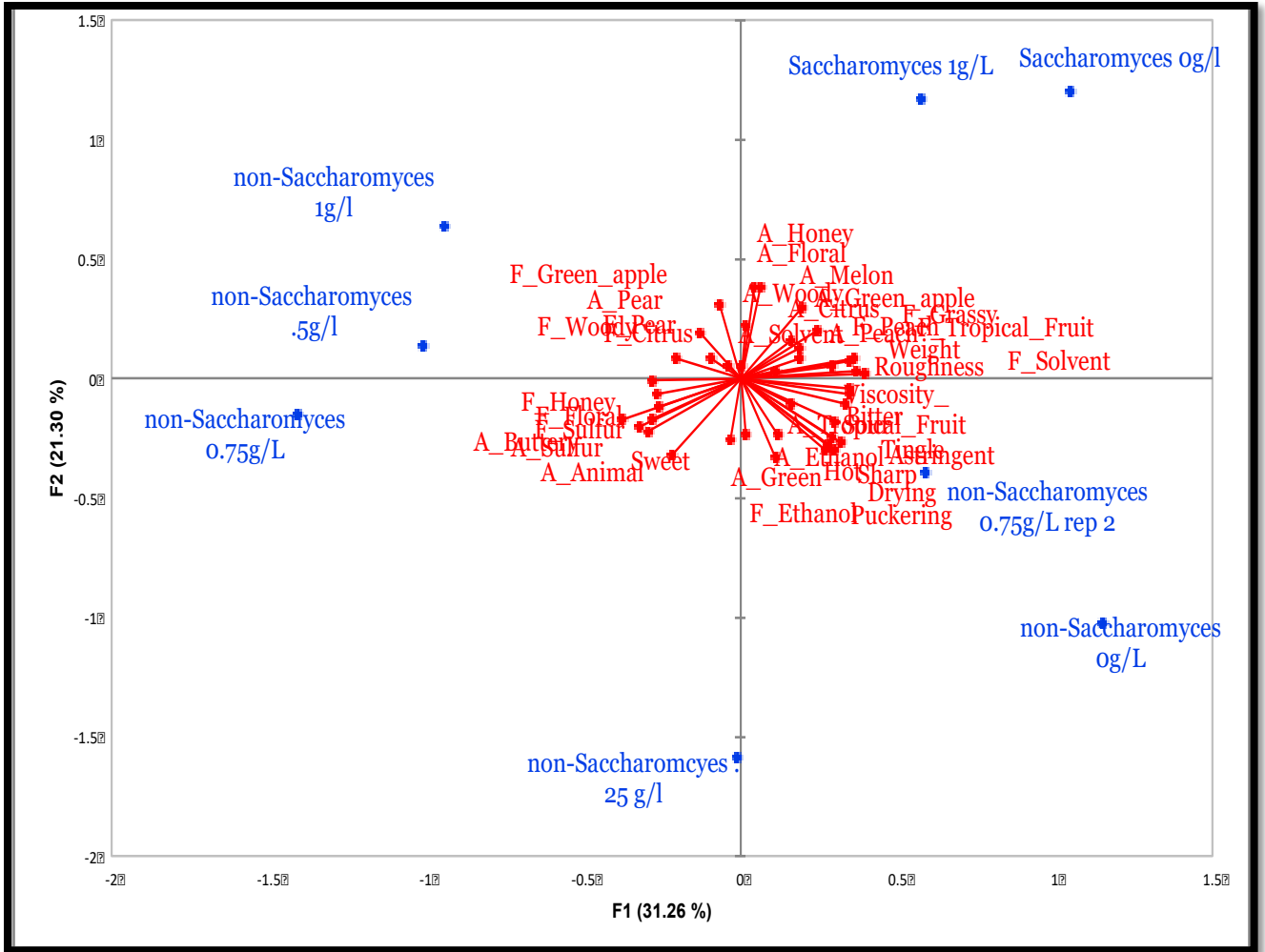


Figure 5. Principal component analysis of the Chardonnay made in 2018 with *Saccharomyces* or non-*Saccharomyces* yeasts, with different concentrations of pectin added. The A represents aroma attributes and the F indicates flavor attributes.

Table 1. Chemical profile, including mannoprotein concentrations, in the Merlot produced in 2017.

Sample	Mannoprotein concentration (mg/L)	pH	TA (g/L)	VA (g/L)	% Alcohol	D-Galacturonic acid (g/L)	Glycerol (g/L)
Non- <i>Saccharomyces</i> with pectin	109.19	3.43a	0.75ab	0.654a	13.6b	0.509b	10.28b
Non- <i>Saccharomyces</i> without pectin	119.55	3.4a	0.74b	0.646a	14a	0.545a	10.05b
<i>Saccharomyces</i> with pectin	105.01	3.36a	0.78a	0.643a	13.9ab	0.359c	9.73c
<i>Saccharomyces</i> without pectin	125/21	3.3a	0.74b	0.6a	14.1a	0.276d	10.71a

Table 2. Chemical profile of the Merlot produced in 2018.

Yeast	Pectin Level (g/L)	pH	Titratable Acidity (g/L)	Acetic Acid (g/L)	D-Galacturonic acid (g/L)	% Alcohol	Glycerol (g/L)	Mannoprotein (mg/L)
<i>Saccharomyces</i>	0	3.86	5.8a	0.36b	0.121c	13.13a	10.3a	163.61a
<i>Saccharomyces</i>	0.75	4.03	4.99c	0.475ab	0.139bc	11.99a	10.33a	133.5a
non- <i>Saccharomyces</i>	0	4.02	5.39b	0.412b	0.181a	11.53a	9.747a	151.36a
non- <i>Saccharomyces</i>	0.25	3.94	5.21bc	0.456ab	0.15abc	13.52a	11.207a	169.64a
non- <i>Saccharomyces</i>	0.5	3.87	5.5ab	0.447ab	0.177a	13.46a	11.10a	171.93a
non- <i>Saccharomyces</i>	0.75	3.98	4.178e	0.537ab	0.152abc	12.76a	11.27a	201.98a
non- <i>Saccharomyces</i>	1.0	3.94	4.59d	0.505ab	0.172ab	11.19a	9.61a	127.93a
non- <i>Saccharomyces</i>	1.25	4.02	5.0c	0.6a	0.183a	12.46a	10.84a	142.22a

Table 3. Chemical profile, including mannoprotein concentrations, in the Chardonnay produced in 2017.

Sample	Mannoprotein concentration (mg/L)	pH	TA	VA	% Alcohol	D Galacturonic acid (g/L)	Glycerol (g/L)
Non- <i>Saccharomyces</i> with pectin	128.73	3.26a	0.83a	0.324a	11.75a	0.353a	7.04a
Non- <i>Saccharomyces</i> without pectin	117.31	3.28a	0.87a	0.357a	11.7a	0.19b	6.53b
<i>Saccharomyces</i> with pectin	111.53	3.2a	0.906a	0.285a	11.6a	0.351a	5.66c
<i>Saccharomyces</i> without pectin	95.98	3.26a	0.84a	0.334a	11.7a	0.177c	5.16d

Table 4. Chemical profile of the Chardonnay produced in 2018.

Yeast	Pectin addition (g/L)	pH	Titratable acidity (g/L)	Acetic acid (g/L)	% Alcohol	Glycerol (g/L)	Mannoprotein (mg/L)
<i>Saccharomyces</i>	0	3.87	4.53	0.19a	13.8a	6.82a	178.94
<i>Saccharomyces</i>	1	3.89	4.36	0.06a	13.74a	6.358a	130.1
Non- <i>Saccharomyces</i>	0	3.86	4.59	0.22a	14.6a	6.94a	195.23
Non- <i>Saccharomyces</i>	0.25	3.84	4.48	0.19a	14.01a	6.74a	147.79
Non- <i>Saccharomyces</i>	0.5	3.9	4.44	0.14a	14.2a	6.69a	157.61
Non- <i>Saccharomyces</i>	0.75	3.82	4.61	0.12a	14.28a	6.59a	130.07
Non- <i>Saccharomyces</i>	1	3.81	4.13	0.12a	13.74a	6.325a	101.05