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LABORATORY

This section shows what we analyze and measure in our laboratory and how.

Overview Laboratory

This section covers our laboratory for measuring the chemical characteristics of grape juice, must in fermentation and maturing wine. This page explains why we need a laboratory, what we analyze and measure, and how we calibrate the instruments. The following pages describe each measurement and the associated laboratory process.

We know little, but we measure a lot

When I compare the art of viticulture and winemaking to my professional background in engineering and finance, I sense we are still in the dark ages. We know good wine when we drink it, but we have little scientific understanding of the chemical processes that lead to good wine. We have learned the importance of measuring specific physical and chemical characteristics of grape juice and wine mostly because we know what ranges they should be in to make drinkable wine and what to do if they are not. We do not understand most chemical processes leading to good wine and what and how to measure to optimize these processes. We know famous vignerons who have developed successful processes for their grapes, leading to high-priced wines. Still, they don't understand the underlying science – they are artists who have refined their craft based on recipes handed down over generations or based on processes they chanced upon.

The following questions and answers highlight how limited our current understanding is:

1. Exactly which chemical compounds in what combination in mature wine (phenolics and flavor compounds) create that wonderful combination of sensory experience in smelling, tasting, and drinking great wine? We don't know. We know which chemicals create bad tastes and smells, and we know the composition of some of the substances which are associated with specific flavors

2. Exactly how do specific cellaring processes (aging in barrels, adding wood chips, racking, etc.) change the chemical composition of wine? We don't know because we don't know the chemical composition of wine in the first place, let alone the change therein. But we know that specific cellaring techniques have led to wine selling for a higher price.

3. How do the steps in winemaking (enzyme addition, cold soak, fermentation, extended maceration, pressing) extract the "good" phenolics and flavor compounds from the grapes? What should we measure to guide us better on what to do, when, and how much? We still rely primarily on the smell and palate of experienced winemakers and their past successes to guide us through the art and craft.

4. What happens in the vineyard concerning phenolics and flavor compounds in the grape? How can we make the vines produce the suitable phenolic composition in the grapes, which then can be extracted in winemaking? We don't know, except that sunlight and irrigation affect the buildup of phenolics more than rootstock and temperature. But we know some viticultural practices that led to grapes for which some winemakers have tended to pay high prices.

So, my argument is: we still know (academically) very little about what exactly makes superb wines and why. We know a fair amount about the acceptable ranges for chemical properties we can easily measure. We also know how to intervene in winemaking to get these measures back into acceptable ranges. So science has helped us reduce the amount of lousy wine made, but we still need to understand more of the chemical processes for science to help us make outstanding wines.

Gifted and experienced winemakers can smell and taste the slightest aberration in wine – thus, the laboratory is not very important in small boutique wineries. In large wineries with industrialized processes, laboratories are essential to ensure consistency. We are a small boutique at best and lack the gifted nose and palate, so the laboratory is vital for us.

Measurement tools

Laboratory tools have become very sophisticated and expensive as technology has progressed, and industrial scalability has become essential. Consequently, many wineries have outsourced this function to specialized providers (e.g., ETS, Enartis Vinquiry, Signature Labs, etc.). Given our goal to keep the whole vineyard and winery process in-house, we decided to maintain a laboratory – it defies economic logic for the sake of understanding the entire picture.

While our laboratory is well equipped, it is no match to the sophisticated equipment currently employed at top commercial wineries and leading universities (primarily for various types of chromatography, spectrometry, nuclear magnetic resonance spectrometry, etc.).

The "wet" lab

In the old-school-"wet"-lab, we measure chemical properties by changing the chemical characteristic or composition of the sample to be measured: we measure boiling points, we add known quantities of a given reagent until a property changes, etc. In "wet" labs, you see a lot of glassware and bottles with reagents. Time-consuming processes are needed to measure every property of every sample.

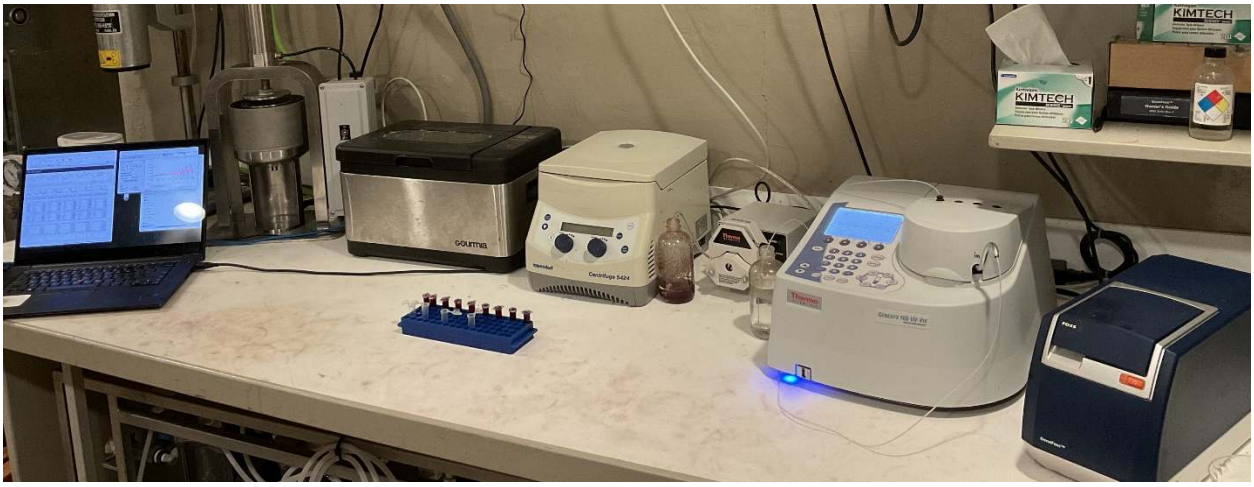
The main benefit of "wet"-labs is cost: fixed costs are $2,000 – 5,000 for glassware and tools. The variable cost averages $3 – 5 for reagents per sample. The main disadvantage is labor: A single measurement takes 5 to 45 minutes.

The new approach: spectrometry

The new approach has three steps: a) centrifuge samples to separate sediments, b) measure the spectral density over a wide range of frequencies, and c) derive, with specialized software, specific chemical properties of the sample from its spectral density curve.

We currently use two types of spectrometer setups:

- An OenoFoss Analyzer (www.foss.us/industry-solution/products/oenofoss/) for the basic chemical properties: Brix, Alcohol, Glucose, Fructose, Density, pH, Total Acidity, Volatile Acidity, Malic Acids, and Lactic Acids,
- A Genesys 10S UV-Vis spectrometer from Fisher Scientific with a 0.2mm quartz flow cell from Starna Cell fed into cloud software from WineXRay to measure phenolics (www.winexray.com).



The main benefits of spectrometry are speed and zero variable costs: less than 3 minutes per sample with 6-12 simultaneous measurements. The main disadvantage is high fixed costs: $5,000 – 40,000 per setup. Thus spectrometry can only be justified when hundreds of samples are to be analyzed yearly.

We introduced spectrometry in 2013 when we started measuring phenolics. We stopped using most of our "wet"-lab tools in 2017 when we bought an OenoFoss Analyzer to handle the increasing number of basic measurements.

What do we measure, and how?

Our approach to measurements has changed significantly since 2009. We started with a well-equipped "wet" lab to take basic measurements. We soon realized that measuring samples in a "wet" lab every time we interacted with the grapes, must, or wine became impractical – far too time-consuming. We started using spectrometry in 2013 to measure phenolics, and we bought an OenoFoss Analyzer in 2017 to measure the basic chemical properties. In 2019 we started experimenting with measuring dissolved CO2, dissolved oxygen, and, in 2021, Oxygen Reduction Potential ("ORP").

As of 2023, the primary laboratory instruments we use are:

- **OenoFoss** Analyzer for measuring the basics: sugars (Brix, Glucose, and Fructose), alcohol (% Alcohol, Density), acids (pH, Total Acidity, Volatile Acidity, Malic Acids, Lactic Acids, Tartaric Acids, Gluconic Acids), nutrients (Yeast Available Nitrogen: Alpha Amino Acids, Ammonia)

- **WineXRay** for measuring phenolics: Anthocyanins (bound, free, and total), Tannins, and Total Iron-Reactive Phenols ("TIRPs"). The setup includes a centrifuge, a sipper pump, a spectrometer, and a laptop to connect to the WineXRay cloud for analysis.
- **Enotrex** for measuring essential characteristics of maturing berries (with OenoFoss), Potential Anthocyanins (with WineXray). The setup includes a precision press, a temperature-controlled bath, and WineXRay.
- **@Accuro** for measuring Oxidation Reduction Potential ("ORP") to monitor oxygenation during fermentation
- **Hanna HI84500 Titrator** for measuring SO2
- **DOTek** from Flotek and **Hanna HI9148** for measuring dissolved oxygen
- **Carbodoseur** from Laboratoires Dujardin-Sailleron for measuring dissolved CO2

Each of the following seven pages describes these instruments. The five pages which follow illustrate the now outdated "wet" lab measurement methods:

- **Brix** measured with Optical Refractometers and Hydrometers
- **Alcohol** measured with Eboulliometers
- **Acidity** (pH and Total Acidity) measured with a pH meter and titration
- **Volatile Acidity** measured with a Cash Still
- **Malic & Lactic Acids** measured with Paper Chromatography

Instrument calibration

Instruments used in wet labs should be calibrated every time before use. This calibration is time-consuming and requires calibrated reagent solutions with a relatively short shelf-life (a few months at best). This makes wet labs cumbersome.

Spectrometer-based instruments, which do not depend on chemical reactions, are usually calibrated by the manufacturer against typical must and wine samples from the winery using sophisticated wet-lab assays. This is expensive, but their calibrations are good for extended periods (years).

Measuring the chemical properties during fermentation is challenging because the must's chemical composition continually changes as it ferments (hour by hour). This makes many wet-lab measurements impractical – they take too much time. For the same reason, spectrometer-based instruments cannot be calibrated for measurements during fermentation. However, the

calibration adjustments can be interpolated from the results measured at the beginning and end of the fermentation when the samples are stable enough for wet-lab calibration. Details on how we calculate these adjustments can be found in the Data Management Section.

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Last updated: March 3, 2023

OenoFoss for basics

Initially, we built a conventional wet laboratory with lots of glassware and reagents to measure basic chemical properties. As a consequence, we learned a lot about introductory chemistry. However, measuring multiple samples every few days proved very time-consuming. So, in 2017, we purchased an **OenoFoss** Analyzer to replace the bulk of the glassware and reagents.

OenoFoss Analyzer

The Analyzer uses Near Infrared ("NIR") Spectrometry defined by wavelengths between 700 and 2500 nanometers. FOSS is a Danish company with a dominant food and agricultural analytics position. The key advantages of using OenoFoss are that it works with tiny samples (1 mL), returns results within only 2 minutes, and requires only infrequent calibration. The main disadvantage is cost. The instrument measures the following properties simultaneously:

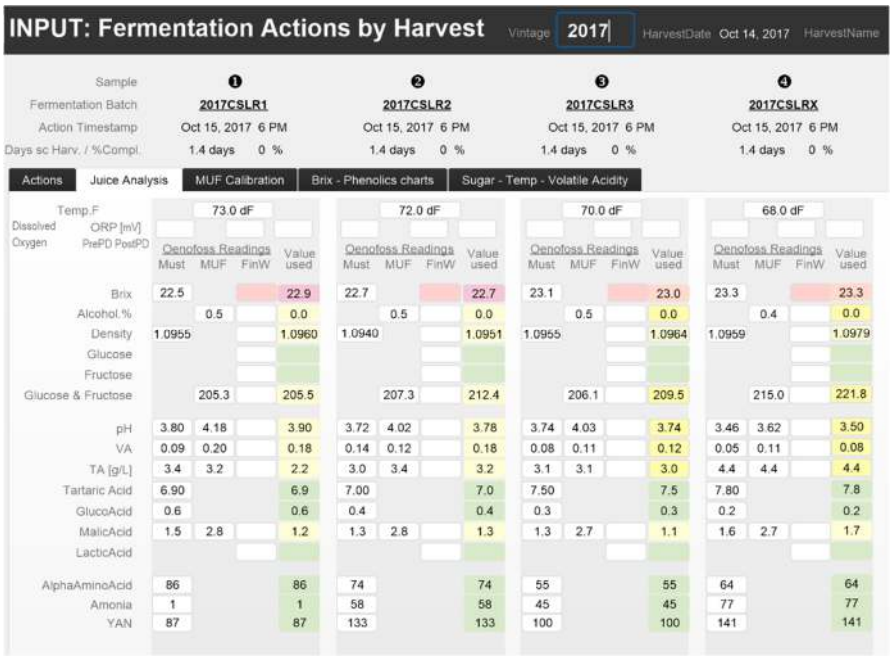
- sugars (Brix, Glucose, and Fructose),
- alcohol (% Alcohol, Density),
- acids (pH, Total Acidity, Volatile Acidity, Malic Acids, Lactic Acids, Tartaric Acids, Gluconic Acids), and
- nutrients (Yeast Available Nitrogen: Alpha Amino Acids, Ammonia

For more details, see https://www.fossanalytics.com/en/products/oenofoss

We use an Eppendorf 5424 centrifuge (https://www.eppendorf.com/product-media/doc/en/330723/Centrifugation_Operating-manual_Centrifuge-5424-R.pdf) for clarifying samples that contain suspended particles larger than 1 micrometer. (for more information, see the following page)

Calibration for measurements during fermentation

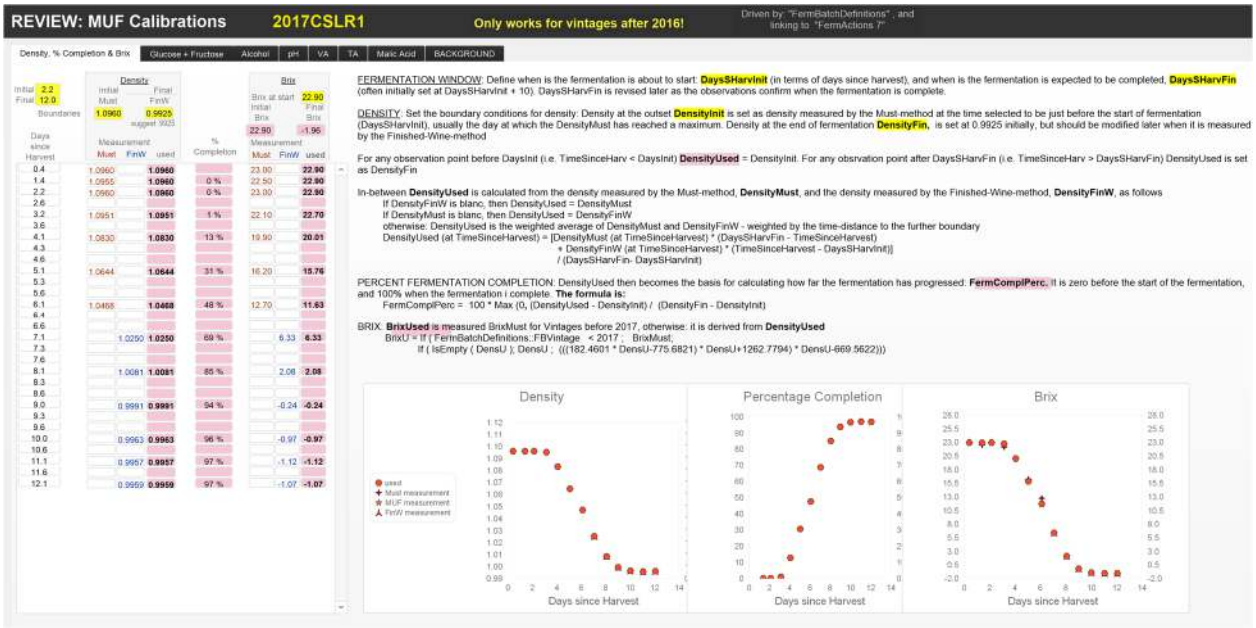
Foss calibrates the Analyzer for two measurements: for unfermented grape juice and must ("Must") or fully fermented must ("Finished Wine" or "FW"). Measures during fermentation ("Must under Fermentation" or "MUF") need to be adjusted by the boundary conditions at the beginning and end of the fermentation. We know that the "MUF"-measurements at the very beginning of the fermentation are still correct, i.e., calibrated, and we know that the "FW" measurements at the very end of the fermentation are also accurate, i.e., calibrated. The measurements during fermentation, which are not pre-calibrated, must be adjusted. In a nutshell, we adjust the MUF measurements by linearly interpolating the errors at the beginning and end of the fermentation. The adjustment to the "MUF" measurement at the beginning is the difference between the "Must" and "MUF" measurements. The adjustment at the end of the fermentation is the difference between the "MUF" and the "FW" measurements. In between, the adjustment is the linear interpolation between the initial and the final adjustment based on the percentage of completion of the fermentation. Consequently, the adjusted measures are only firm once the fermentation is complete.



We enter the Must, MUF, and FW measurements in the INPUT: Fermentation Actions by Harvest for all active fermentation batches. This screenshot illustrates how we entered the data for four fermentation batches on October 15, 2017, at 6 pm. The white boxes hold the Must, MUF, and FW data provided by the Analyzer, and the colored boxes under "Value Used" show the adjusted measures.

The adjusted measures will only be computed after the interpolation parameters have been established. The process of adjustments is documented in the layout REVIEW: MUF

Calibrations. The following eight screenshots show different tabs in that layout for the fermentation batch 2017CSLR1 at the end of the fermentation. Each of these tabs shows what boundary conditions need to be defined and how the adjustments are calculated. Each tab also contains a graphic of the raw and the adjusted measures. Note this MUF calibration needs to be updated at the beginning and end of each fermentation.



REVIEW: MUF Calibrations — 2017CSLR1 — Only works for vintages after 2016!

Driven by: "FermBatchDefinitions" , and linking to "FermActions 7"

Density, % Completion & Brix | Glucose + Fructose | Alcohol | pH | VA | TA | Malic Acid | BACKGROUND

Initial 2.2
Final 12.0

Glucose + Fructose [g/L]

Days since Harvest	% Complet	Initial Must	Initial MUF	Final MUF	Final FinW	Measurement MUF	Measurement FinW	Measurement used
		Boundaries						
		Delta						
			205.5	205.5	3.0	0.4		
			0.0		2.6			
0.4								205.5
1.4	0 %					205.3		205.5
2.2	0 %					205.5		205.5
2.6								
3.2	1 %					207.4		207.4
3.6								
4.1	13 %					178.9		178.9
4.3								
4.6								
5.1	31 %					136.9		136.9
5.3								
5.6								
6.1	48 %					97.7		97.7
6.4								
6.6								
7.1	69 %					53.3	10.1	51.5
7.3								
7.6								
8.1	85 %					21.9	18.9	19.7
8.3								
8.6								
9.0	94 %					5.2	4.1	2.8
9.3								
9.6								
10.0	96 %					2.6	0.4	0.1
10.6								
11.1	97 %					3.0	0.5	0.5
11.6								
12.1	97 %					3.0	0.4	0.4

1. Define the boundary conditions "before and at DaysInit" and at "DaysFin and after".The boundary conditions are:

Before and at DaysInit, i.e. when TimeSinceHarvest <= **DaysSHarvInit**: **G&FUsed**(TimeSinceHavest) = **G&FMust**(TimeSinceHarvest) = **G&FUsedInit**

At DaysFim and after, i.e. when TimeSinceHarvest => **DaysSHarvInit**: **G&FUsed**(TimeSinceHavest) = **G&FFinW**(TimeSinceHarvest) = **G&FUsedFin**

2. Calculate the errors at the boundary, i.e. the differences between the MUF measurements and the Must and FinW measurements respecively: G&FMUFInit, G&FMustInit, G&FMUFFin and G&FFinWFin have to be read from the listing of all obsrvations and entered by hand. For G&F there is no Must-based measurement, therefore we assume G&FMustInit = G&FMUFInit and G&FDeltaInit = 0.

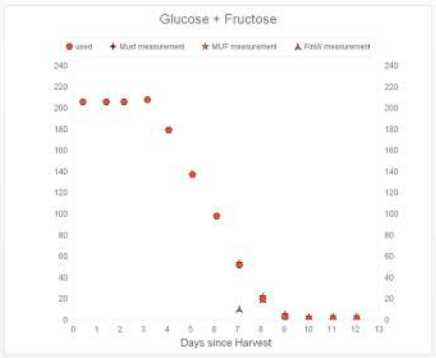
At TimeSinceHarvest = **DaysSHarvInit**: **G&FDeltaInit** = G&FMUF(DaysInit) - G&FMust(DaysInit) = **G&FMUFInit** - **G&FMustInit**

At TimeSinceHarvest = **DaysSHarvFin**: **G&FDeltaFin** = G&FMUF(DaysFin) - G&FFinW(DaysFin) = **G&FMUFFin** - **G&FFinWFin**

The third step is to calculate the adjustment to G&FMUF when TimeSinceHarvest is inbetween the boundary days, DaysInit and DaysFin. The adjustment depends on the percentage of completion of the fermentation (FermComplPerc) and the errors at the boundary

G&FUsed(TimeSinceHarvest) = G&FMUF(TimeSinceHarvest) + adjustment

= **G&FMUF**(TimeSinceHarvest)
- **FermComplPerc/100 * G&FDeltaFin**
- **(1 - FermComplPerc/100) * G&FDeltaInit**




```
If ( TimeSinceHarv < FermBatchDefinitions::DaysSHarvINIT;
FermBatchDefinitions::GandFMustInit;
  If ( TimeSinceHarv > FermBatchDefinitions::DaysSHarvFIN;
  GandFFinW ;
    If ( IsEmpty (GandFFinW) & IsEmpty (GandFMUF); GandFMUF;
      If ( IsEmpty ( GandFMUF) ; GandFFinW;
          GandFMUF
            - FermComplPerc FB1D / 100 * FermBatchDefinitions::GandFDeltaFin
            - (1 - FermComplPerc FB1D / 100) * FermBatchDefinitions::GandFDeltaInit

))))
```

REVIEW: MUF Calibrations — 2017CSLR1 — Only works for vintages after 2016!

Driven by: "FermBatchDefinitions" , and linking to "FermActions 7"

Density, % Completion & Brix | Glucose + Fructose | Alcohol | pH | VA | TA | Malic Acid | BACKGROUND

Initial 2.2
Final 12.0

Alcohol [%]

Days since Harvest	% Complet	Initial Must	Initial MUF	Final MUF	Final FinW	Measurement MUF	Measurement FinW	Measurement used
		Boundaries						
		Delta						
			0.0	0.6	12.3	13.0		
			0.6		-0.7			
0.4								0.0
1.4	0 %					0.5		0.0
2.2	0 %					0.6		0.0
2.6								
3.2	1 %					0.8		0.2
3.6								
4.1	13 %					2.5		2.1
4.3								
4.6								
5.1	31 %					3.9		3.7
5.3								
5.6								
6.1	48 %					7.0		7.0
6.4								
6.6								
7.1	69 %					10.1	9.8	10.4
7.3								
7.6								
8.1	85 %					12.1	11.7	12.6
8.3								
8.6								
9.0	94 %					12.9	11.8	13.5
9.3								
9.6								
10.0	96 %					12.5	13.1	13.2
10.6								
11.1	97 %					12.6	13.1	13.3
11.6								
12.1	97 %					12.3	13.0	13.0

Note: the calculation process is the same as for Glucose + Fructose, except that The initial Alcohol-Must is set to zero.

1. Define the boundary conditions "before and at DaysInit" and at "DaysFin and after".The boundary conditions are:

Before and at DaysInit, i.e. when TimeSinceHarvest <= **DaysSHarvInit**: **AlcUsed**(TimeSinceHavest) = **AlcMust**(TimeSinceHarvest) = **AlcUsedInit**

At DaysFim and after, i.e. when TimeSinceHarvest => **DaysSHarvInit**: **AlcUsed**(TimeSinceHavest) = **AlcFinW**(TimeSinceHarvest) = **AlcUsedFin**

2. Calculate the errors at the boundary, i.e. the differences between the MUF measurements and the Must and FinW measurements respecively: AlcMUFInit, AlcMustInit, AlcMUFFin and AlcFFinWFin have to be read from the listing of all observations and entered by hand. For Alcohol there is no Must-based measurement, therefore we assume AlcMustInit = 0.

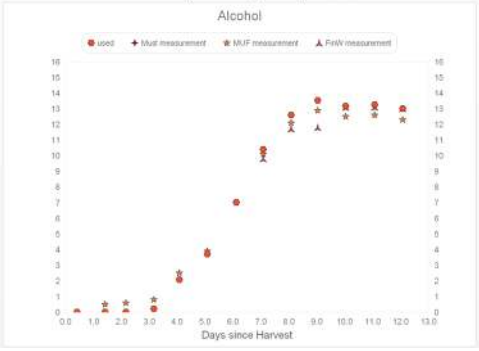
At TimeSinceHarvest = **DaysSHarvInit**: **AlcDeltaInit** = AlcMUF(DaysSHarvInit) - AlcMust(DaysSHarvInit) = **AlcMUFInit** - **AlcMustInit**

At TimeSinceHarvest = **DaysSHarvFin**: **AlcDeltaFin** = AlcMUF(DaysSHarvFin) - AlcFinW(DaysSHarvFin) = **AlcMUFFin** - **AlcFinWFin**

The third step is to calculate the adjustment to AlcMUF when TimeSinceHarvest is inbetween the boundary days, DaysInit and DaysFin. The adjustment depends on the percentage of completion of the fermentation (FermComplPerc) and the errors at the boundary

AlcUsed(TimeSinceHarvest) = AlcMUF(TimeSinceHarvest) + adjustment

= **AlcMUF**(TimeSinceHarvest)
- **FermComplPerc/100 * AlcDeltaFin**
- **(1 - FermComplPerc/100) * AlcDeltaInit**




```
If ( TimeSinceHarv < FermBatchDefinitions::DaysSHarvINIT;
FermBatchDefinitions::AlcMustInit;
  If ( TimeSinceHarv > FermBatchDefinitions::DaysSHarvFIN;
  AlcFinW ;
    If ( IsEmpty (AlcFinW) and IsEmpty (AlcMUF); AlcMUF;
      If ( IsEmpty ( AlcMUF) ; AlcFinW;
          AlcMUF -
              (1- FermComplPerc FB1D / 100) * FermBatchDefinitions::AlcDeltaInit
              - FermComplPerc FB1D / 100 * FermBatchDefinitions::AlcDeltaFin

))))
```

REVIEW: MUF Calibrations — 2017CSLR1 — Only works for vintages after 2016!

Driven by: "FermBatchDefinitions" and linking to: "FermActions 7"

Density, % Completion & Brix | Glucose + Fructose | Alcohol | pH | VA | TA | Malic Acid | BACKGROUND

Initial: 2.2 Final: 12.0

pH

Days since Harvest	% Complet	Initial Must	Initial MUF	Final MUF	Final FinW	Measurement Must	Measurement MUF	Measurement FinW	used
Boundaries		3.90	4.27	3.51	3.47				
Delta		0.37		0.04					
0.4						3.77			3.90
1.4	0 %					3.80	4.18		3.90
2.2	0 %					3.90	4.27		3.90
2.6									
3.2	1 %					3.88	4.33		3.96
3.6									
4.1	13 %					3.70	3.94		3.61
4.3									
4.6									
5.1	31 %					3.89	3.93		3.66
5.3									
5.6									
6.1	48 %					3.94	3.66		3.45
6.4									
6.6									
7.1	69 %						3.67	3.65	3.53
7.3									
7.6									
8.1	85 %						3.70	3.61	3.61
8.3									
8.6									
9.0	94 %						3.70	3.62	3.64
9.3									
9.6									
10.0	96 %						3.46	3.41	3.41
10.6									
11.1	97 %						3.49	3.46	3.44
11.6									
12.1	97 %						3.51	3.47	3.47

Note: the calculation process is the same as for Glucose + Fructose.

1. Define the boundary conditions "before and at DaysInit" and at "DaysFin and after",The boundary conditions are:

Before and at DaysInit, i.e. when TimeSinceHarvest <= **DaysSHarvInit:** **pHUsed**(TimeSinceHavest) = **pHMust**(DaysSHarvInit) = **pHUsedInit**

At DaysFim and after, i.e. when TimeSinceHarvest => **DaysSHarvFin:** **pHUsed**(TimeSinceHavest) = **pHFinW**(DaysSHarvFin) = **pHUsedFin**

2. Calculate the errors at the boundary, i.e. the differences between the MUF measurements and the Must and FinW measurements respecively: pHMUFInit, pHMustInit, pHMUFFin and pHFinWFin have to be read from the listing of all observations and entered by hand.

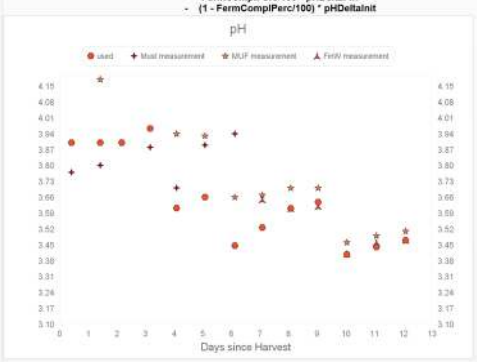
At TimeSinceHarvest = **DaysSHarvInit** **pHDeltaInit** = pHMUF(DaysSHarvInit) - pHMust(DaysSHarvInit) = **pHMUFInit** - **pHMustInit**

At TimeSinceHarvest = **DaysSHarvFin** **pHDeltaFin** = pHMUF(DaysSHarvFin) - pHFinW(DaysSHarvFin) = **pHMUFFin** - **pHFinWFin**

The third step is to calculate the adjustment to pHMUF when TimeSinceHarvest is inbetween the boundary days, DaysInit and DaysFin. The adjustment depends on the percentage of completion of the fermentation (FermComplPerc) and the errors at the boundary

pHUsed(TimeSinceHarvest) = pHMUF(TimeSinceHarvest) + adjustment

= **pHMUF**(TimeSinceHarvest)
- **FermComplPerc/100 * pHDeltaFin**
- **(1 - FermComplPerc/100) * pHDeltaInit**




```
If ( TimeSinceHarv < FermBatchDefinitions::DaysSHarvINIT;
FermBatchDefinitions::pHMustInit;
  If ( TimeSinceHarv > FermBatchDefinitions::DaysSHarvFIN;
  pHFinW ;
    If ( IsEmpty (pHFinW) and IsEmpty (pHMUF); pHMUF;
      If ( IsEmpty ( pHMUF) ; pHFinW;
          pHMUF -
              (1- FermComplPerc FB1D / 100) * FermBatchDefinitions::pHDeltaInit
              - FermComplPerc FB1D / 100 * FermBatchDefinitions::pHDeltaFin

))))
```

REVIEW: MUF Calibrations — 2017CSLR1 — Only works for vintages after 2016!

Driven by: "FermBatchDefinitions" and linking to: "FermActions 7"

Density, % Completion & Brix | Glucose + Fructose | Alcohol | pH | VA | TA | Malic Acid | BACKGROUND

Initial: 2.2 Final: 12.0

VA [g/L]

Days since Harvest	% Complet	Initial Must	Initial MUF	Final MUF	Final FinW	Measurement Must	Measurement MUF	Measurement FinW	used
Boundaries		0.18	0.14	0.11	0.34				
Delta		-0.04		-0.23					
0.4						0.12			0.18
1.4	0 %					0.09	0.20		0.18
2.2	0 %					0.18	0.14		0.18
2.6									
3.2	1 %					0.15	0.11		0.15
3.6									
4.1	13 %					0.24	0.15		0.21
4.3									
4.6									
5.1	31 %					0.36	0.33		0.43
5.3									
5.6									
6.1	48 %					0.33	0.09		0.22
6.4									
6.6									
7.1	69 %						0.06	0.26	0.23
7.3									
7.6									
8.1	85 %						0.07	0.29	0.27
8.3									
8.6									
9.0	94 %						0.07	0.24	0.29
9.3									
9.6									
10.0	96 %						0.10	0.29	0.32
10.6									
11.1	97 %						0.08	0.31	0.30
11.6									
12.1	97 %						0.11	0.34	0.34

Note: the calculation process is the same as for Glucose + Fructose.

1. Define the boundary conditions "before and at DaysInit" and at "DaysFin and after",The boundary conditions are:

Before and at DaysInit, i.e. when TimeSinceHarvest <= **DaysSHarvInit:** **VAUsed**(TimeSinceHavest) = **VAMust**(DaysSHarvInit) = **VAUsedInit**

At DaysFim and after, i.e. when TimeSinceHarvest => **DaysSHarvInit:** **VAUsed**(TimeSinceHavest) = **VAFinW**(DaysSHarvFin) = **VAUsedFin**

2. Calculate the errors at the boundary, i.e. the differences between the MUF measurements and the Must and FinW measurements respecively: VAMUFInit, VAMustInit, VAMUFFin and VAFinWFin have to be read from the listing of all obsevrations and entered by hand .

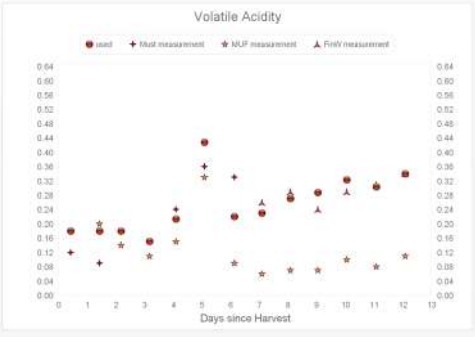
At TimeSinceHarvest = **DaysSHarvInit** **VADeltaInit** = VAMUF(DaysSHarvInit) - VAMust(DaysSHarvInit) = **VAMUFInit** - **VAMustInit**

At TimeSinceHarvest = **DaysSHarvFin** **VADeltaFin** = VAMUF(DaysSHarvFin) - VAFinW(DaysSHarvFin) = **VAMUFFin** - **VAFinWFin**

The third step is to calculate the adjustment to VAMUF when TimeSinceHarvest is inbetween the boundary days, DaysSHarvInit and DaysSHarvFin. The adjustment depends on the percentage of completion of the fermentation (FermComplPerc) and the errors at the boundary

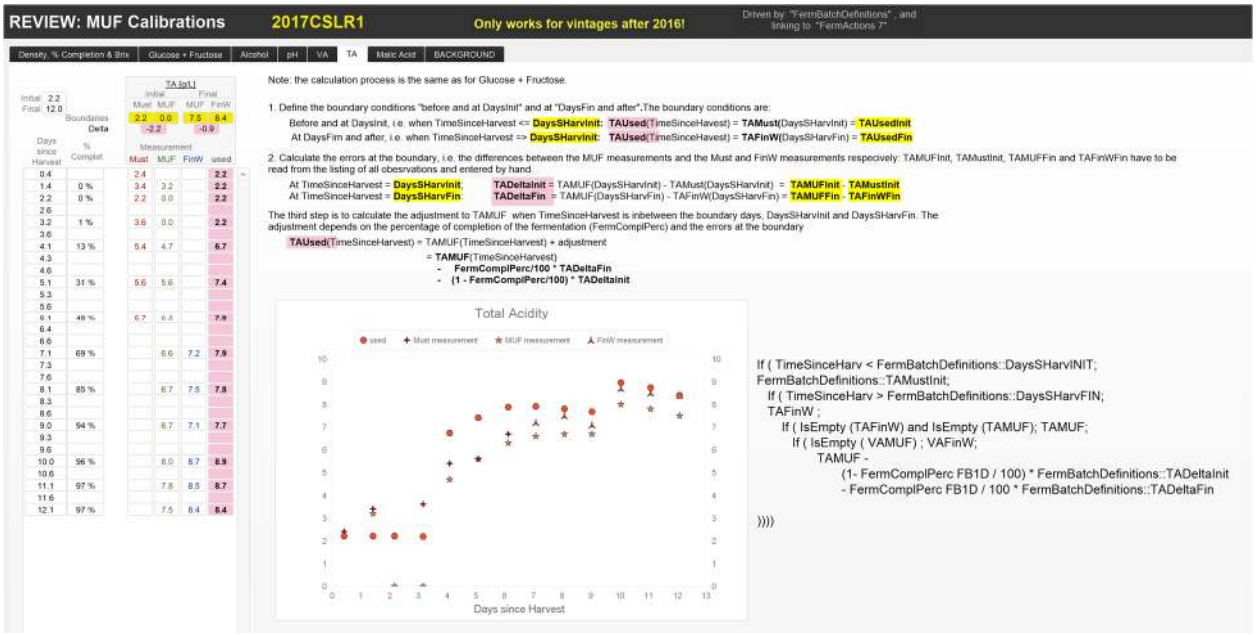
VAUsed(TimeSinceHarvest) = VAMUF(TimeSinceHarvest) + adjustment

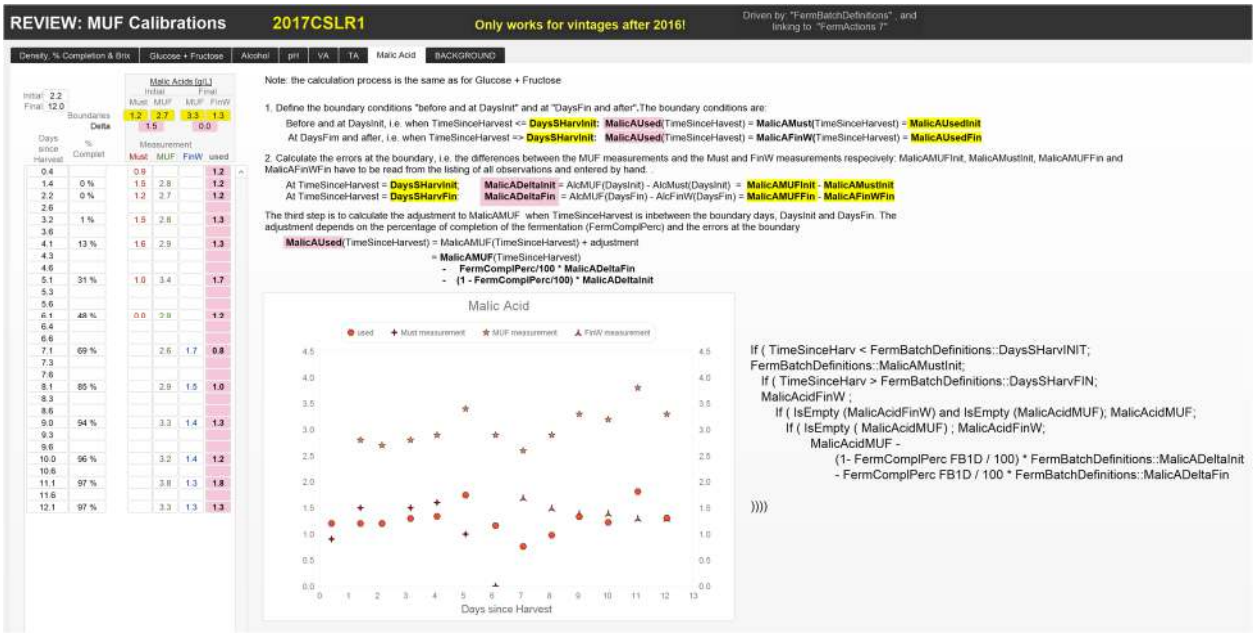
= **VAMUF**(TimeSinceHarvest)
- **FermComplPerc/100 * VADeltaFin**
- **(1 - FermComplPerc/100) * VADeltaInit**




```
If ( TimeSinceHarv < FermBatchDefinitions::DaysSHarvINIT;
FermBatchDefinitions::VAMustInit;
  If ( TimeSinceHarv > FermBatchDefinitions::DaysSHarvFIN;
  VAFinW ;
    If ( IsEmpty (VAFinW) and IsEmpty (VAMUF); VAMUF;
      If ( IsEmpty ( VAMUF) ; VAFinW;
          VAMUF -
              (1- FermComplPerc FB1D / 100) * FermBatchDefinitions::VADeltaInit
              - FermComplPerc FB1D / 100 * FermBatchDefinitions::VADeltaFin

))))
```





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Next Page: WineXRay for measuring phenolics

Last updated: March 3, 2023

WineXRay for measuring Phenolics

Phenolics are organic chemicals in grapes and wines responsible for their taste, mouthfeel, and color. There are thousands of different phenolic molecules, some small, others huge, some have simple organic structures, and others are very complex. They constantly interact with other chemicals and with each other as grapes grow, during fermentation into wine, and cellaring. These chemical processes are very complex and not yet fully understood.

The most important classes of phenolics in red wine are tannins and anthocyanins.

- Anthocyanins are water-soluble pigments extracted from grape skins. They interact with themselves and provide color. Anthocyanins represent a family of different compounds. We measure their concentration in ppm of Malvidin equivalents (Malvadin is a simple type of Anthocyanin).
- Tannins are also water-soluble but are extracted more slowly from the skin, seeds, and pulp. They impart an astringent character to the wine. We measure Tannin concentration in ppm of Catechin equivalents (Catechin is a simple type of Tannin).
- Bound Anthocyanins result from the combination of Anthocyanins and Tannins. They are stable polymeric pigments and thereby provide color stability to the wine. The concentration of Bound Anthocyanins can be measured by absorbance at 520 nM.

Mature cabernet sauvignon grapes contain around 3.5 mg per grape of tannins – of these, approximately 58% are in the seeds, 38% in the grape skins, and 7 % in the flesh – all very different in structure and size. During fermentation, different proportions of these seed, skin, and flesh tannins get extracted and combined with anthocyanins (also present in the grapes). Later, these chemicals continue interacting with each other and the tannins and other chemicals in wine barrels. Typical Cabernet Sauvignon wine has around 700-1000 mg/L in tannins and 800-1100 mg/L in anthocyanins (concentrations are higher for Petit Syrah and lower for Merlot, Zinfandel and much lower for Pinot.

While we know very little about chemical processes, there is progress. In 2006 scientists at UC Davis, in cooperation with the Australian Wine Research Institute,, published papers describing how to measure, almost in real-time, the effect of different winemaking techniques on the extraction of tannins and anthocyanins. They measured the wine's absorption of ultraviolet light, correlatingd the results with phenolic compositions of similar wines analyzed using wet lab chemistry (the Harbertson-Adams Assay).Th. By 2012 the methodology evolved into two commercial applications: One is the Tannin Portal created by the Australian Wine Research

Institute, which sells representative spectral data to analytic laboratories and large wineries so they can compare the spectra they measure locally (see https://www.awri.com.au/wp-content/uploads/tannin_portal_faq.pdf). The other is a platform created by a commercial startup, WineXRay (see https://www.winexray.com). Since then, many other commercial implementations have come to market.

WineXRay

Since 2013, we use the WineXRay platform developed by Gianni Colantuoni & Scott McLeod to estimate:

Free and Total Anthocyanins (basis of color and textural qualities)

Anthocyanins bound to tannins (basis of stable color and add textural qualities)

Protein-Precipitable Tannins (important for wine style and age-ability)

Total Iron-Reactive Phenolics: the amount of all phenolic compounds

This table shows typical levels for 2012 vintages of different grape varieties (extracted from a WineXRay presentation). Clearly, the typical phenolic and tannin levels vary significantly across grape varietals.

	Total Anthos	Free Anthos	Bound Anthos	Tannins	Total IRPs
12CS	959	700	231	1026	2386
12ME	634	479	138	806	1968
12PS	1338	1018	290	1716	3524
12ZI	316	254	53	528	1529
12PN	406	334	50	266	1366

Laboratory Process

The measurement process we established requires the following equipment:

An Eppendorf 5424 Centrifuge to settle solids in the samples

A supply of 2mL test tubes for the centrifuge

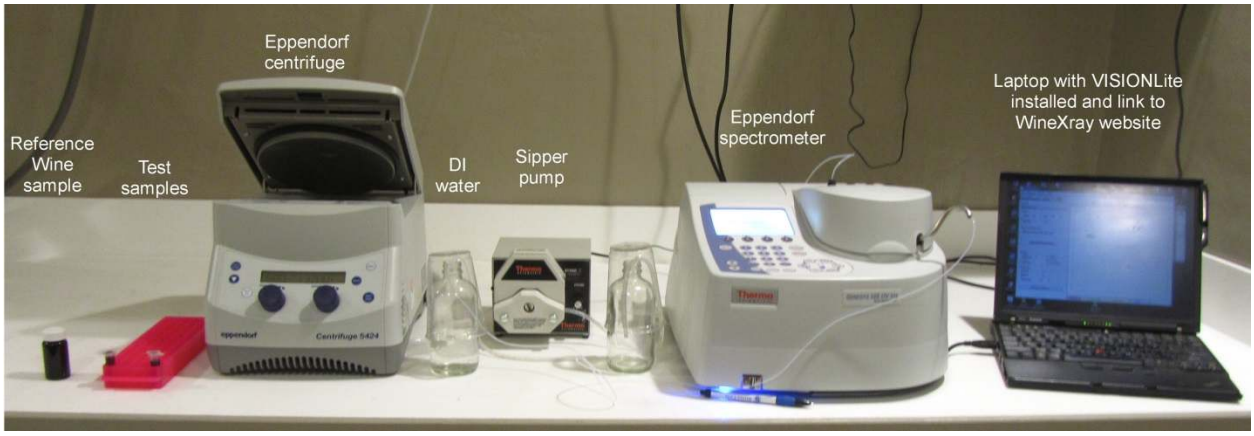
A sipper system from Fisher Scientific (Model 72-310-080)

A Quartz Flow Cell 0.2mm from Starna Cell (Cat # 584.4-Q-0.2)

A Genesys 10S UV-Vis spectrometer from Fisher Scientific and

A laptop computer with VISIONLite software installed and a live link to the WineXray website

Here is an annotated picture:



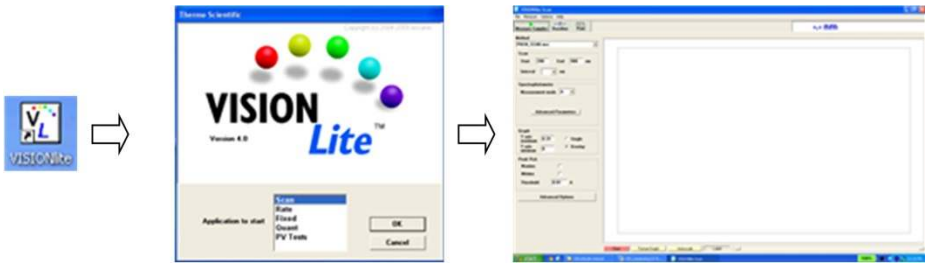
The process itself consists of 4 steps

Step 1: Prepare samples.

- Pour 2 mL of reference wine 2009 CSV CH into a test tube and label it R
- Take the sample to be measured, strain it, pour 2 mL into a test tube, and label it S
- Centrifuge both samples at 13,500 rpm for 5 minutes with the Eppendorf 5424 centrifuge
- Decant both test tubes into new test tubes labeled the same: R and S

Step 2: Initialize the spectrometer.

- Connect the laptop to the spectrophotometer, turn the spectrometer on, and launch the VISIONlite software in "Scan" mode using its associated shortcut.

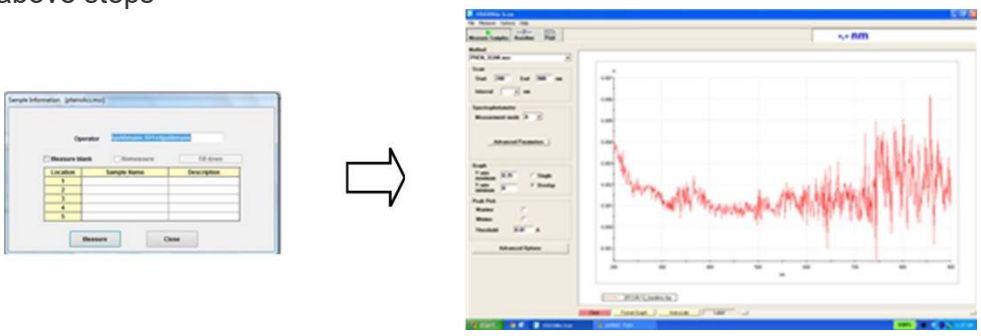


- Place the flow-through cell in the "B" position (Starna Quartz Flow Cell 0.2mm; cat. # 584.4-Q-0.2). Using the sipper Fisher Scientific Model 72-310-080, flush the flow-through cell with DI water for two to three minutes.

- Click the "Baseline" button to zero the spectrophotometer readings.

- Place the flow-through cell in the "1" position, and click the "Measure Sample" button. A Sample Information window opens. Type in "yyyy-mm-dd baseline" and click "Measure." The Sample Information will open again on top of the VisionLite window; click Close.

- Click Autoscale under the spectrum graph. Assure that all the absorbance readings are less than 0.005 AU. The image shows a typical baseline spectrum. In case the absorbance readings are greater than 0.005 AU, flush the flow-through cell in the reverse direction using the cell-cleaning solution followed by DI water, and repeat the above steps

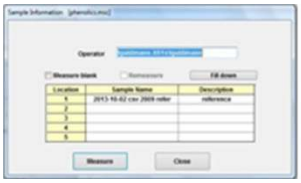


The system is now ready to measure the spectra of the wine samples.

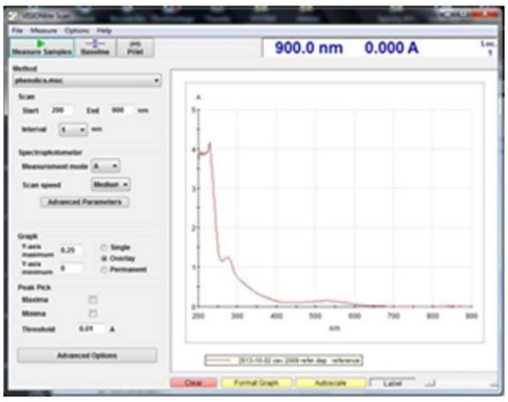
Step 3: Measure the spectra. Do the following for each sample:

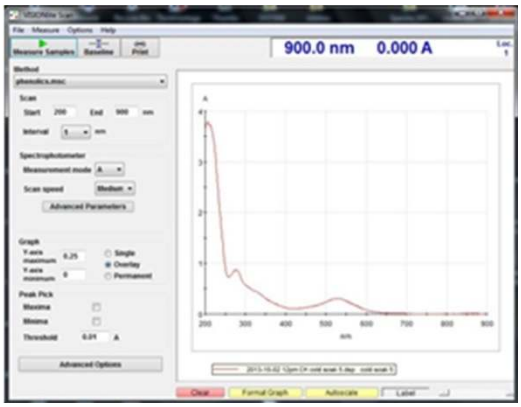
- Use the sipper unit to prime the flow-through cell in position "1" in the spectrophotometer with the wine sample.

- Click the "Measure Sample" button to run the spectrophotometer in scanning mode. A sample information screen comes up to fill in the sample name
  Fill in "yyyy-mm-dd csv 2009 refer" if the sample is the reference wine; "yyy-mm-dd samplename" if the sample is the test sample. Then click the "Measure" button.




- The spectrophotometer will now measure the sample's absorbances at wavelengths from 200nm to 900nm. When complete, the Sample Information window will open on top of the VisionLite window; click "Close" and the "Autoscale" button to see the entire spectrum. Examine reasonableness.



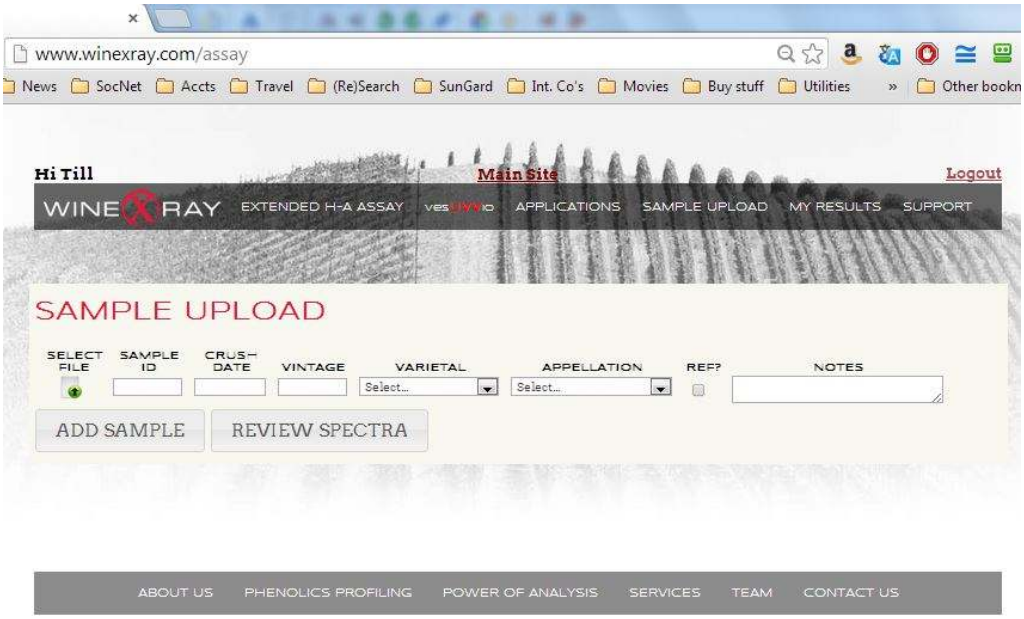


- Click File, then Save spectrum to save the spectrum data twice, first in dsp format, then in csv format in a folder on the laptop labeled with the date of the test sample.

- Rinse the tubes and the Flow Cell using DI water for 30 seconds with the sipper.

Step 4: Upload the spectra and get phenolic results:

- Open the wineXray website (http://www.winexray.com/Account/Login?ReturnUrl=%2fassay) and log in with your username and password. On the next screen, click on "SAMPLE UPLOAD."



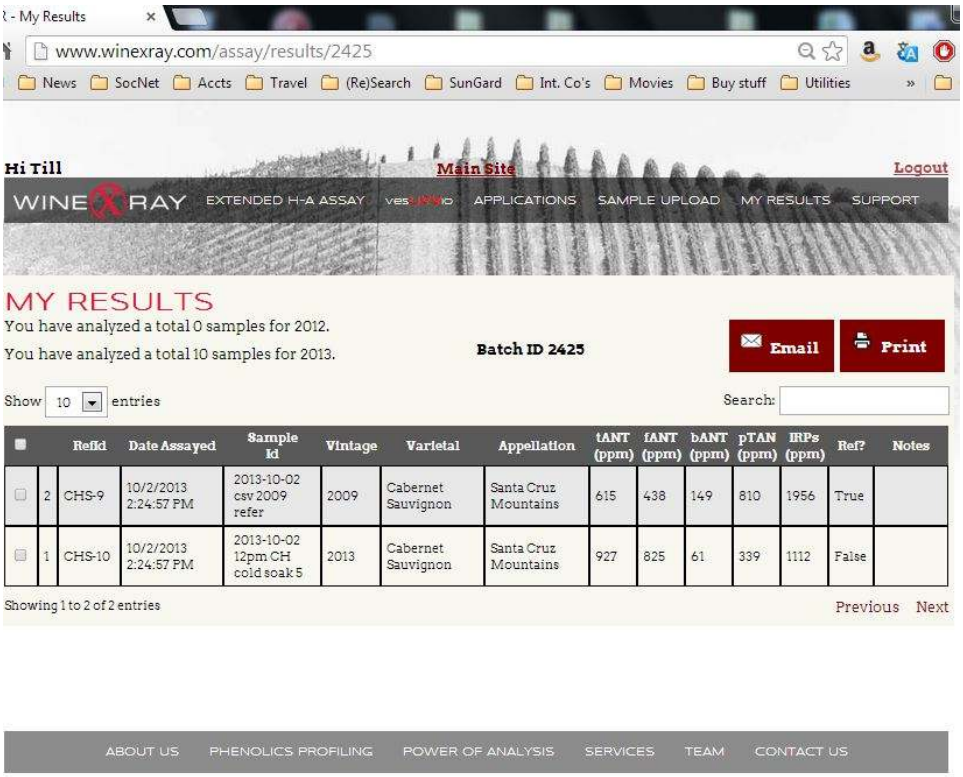
- Click "SELECT FILE" and point to the dsp spectrum file of the reference sample; then fill in the fields Crush Date, Vintage, Varietal, and Appellation and click the "REF?" button to get the checkmark.

  Click another line of "SELECT FILE" and point to the dsp spectrum file of the test sample; then fill in the fields Crush Date, Vintage, Varietal, and Appellation.

  You may add more spectral files at this point

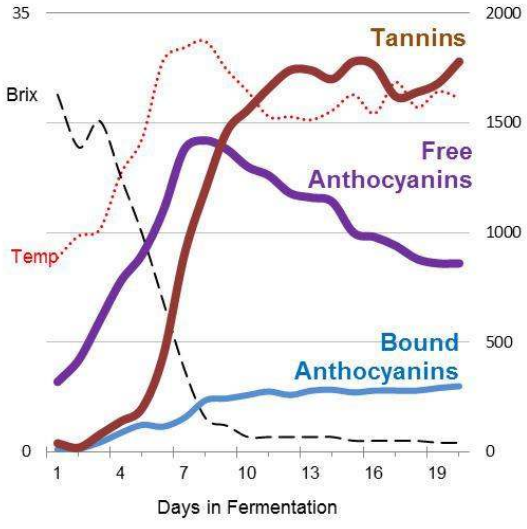
- Click the "REVIEW SPECTRA" button and inspect the graph for reasonableness

- Click "Submit Samples," and you will get the phenolic profiles back within a minute. Save the results for future analysis.

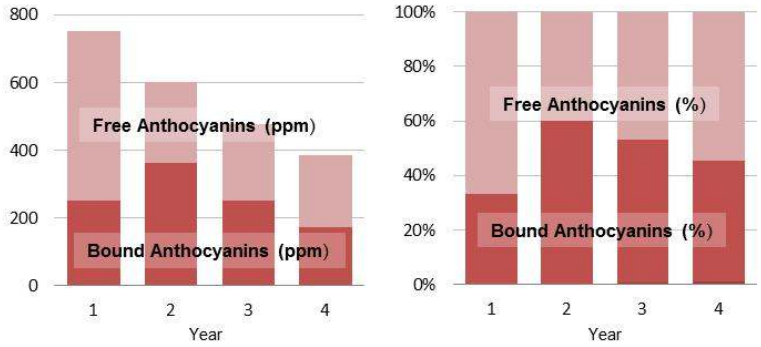


Use Cases

One typical use of these measures is to monitor fermentations with daily sampling. The chart on the right (adapted from a WineXRay presentation) shows the extraction of tannins and anthocyanins into the juice. Because of the quick turn-around time (values can be estimated within 10 minutes of sampling), fermentations can be adapted on the fly. An example of using the Harbetson-Adams Assay at Francis Coppola Winery has been published in [Vines & Wines, Oct 2010]



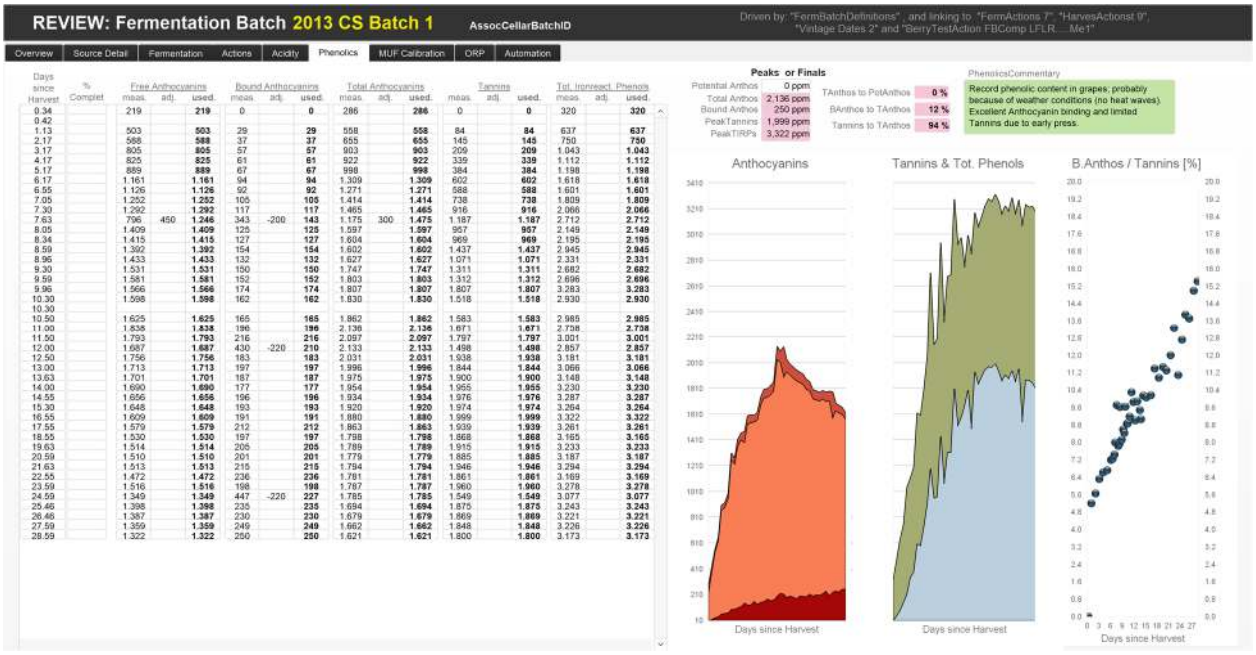
A second use is to **monitor the aging** of wines in the cellar. The chart (adapted from a WineXRay presentation) shows the level of anthocyanins over four years.



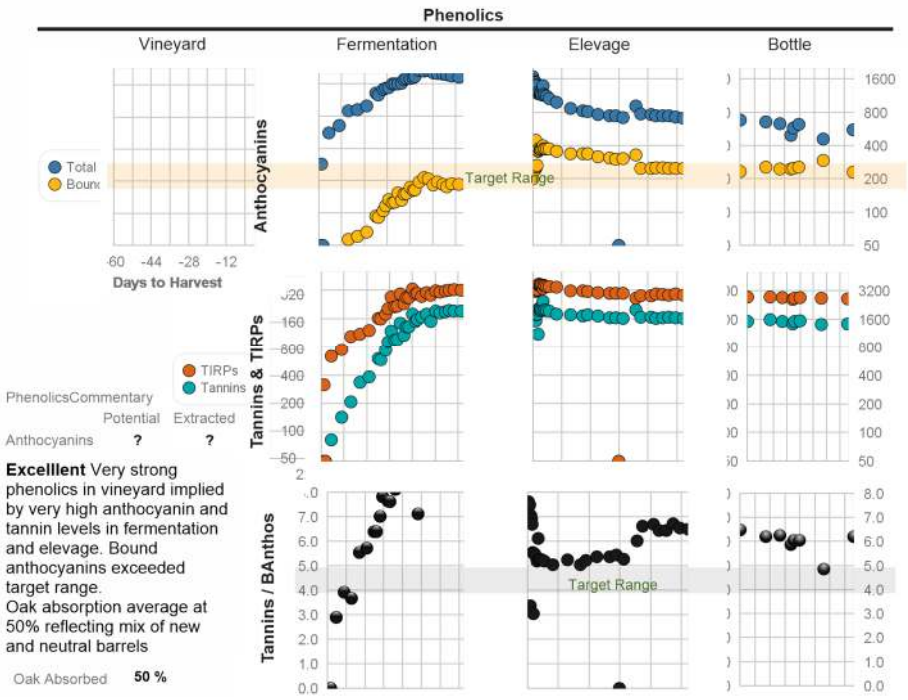
For the original research presented on this subject, read https://www.ajevonline.org/content/58/3/318.

Review of first results on our wines

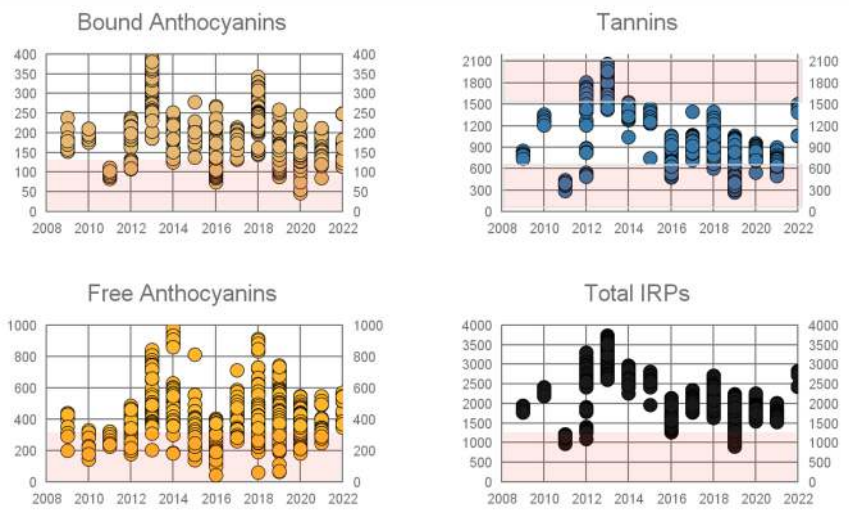
The following screenshot shows the phenolic data saved in our database for one of three 2013 fermentation batches. We measure phenolics every day. Note that this was a long fermentation that was not fully complete (0.5 Brix at the end). Note also some measurements had to be "manually" adjusted for obvious measurement errors.



The screenshot on the right shows the phenolic data for the entire life of the 2013 vintage: From fermentation through elevage and bottle maturation. Note we did not yet collect phenolic data in the vineyard in 2013. We started that only in 2014 – this is covered in the following section (measuring phenolics in grapes)



The last screenshot on the right shows all the phenolic measurements for all the vintages to date (2009) – 2022) during elevage. Note the ranges' differences each year, primarily due to weather patterns during the respective growing seasons.



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Next Page: Enotrex for measuring Phenolics in Grapes
Last updated: March 2, 2023

Enotrex for measuring Phenolics in Grapes

We measure Anthocyanins in grapes to evaluate their maturity and to anticipate the subsequent concentration of phenolics in wine. The goal is to measure the effect of the weather and viticultural practices and to improve harvest timing.

The process has been developed by Enotrex LLC (copyright 2010-2014) and provided by Gianni Colantuoni for research purposes. The central idea is that by pressing unripe grapes and exposing the pomace/skins to a warm alcohol solution, we can simulate the phenolic extraction process that the ripe grapes will undergo later, yet estimate their Anthocyanin levels now. This page has the following sections:

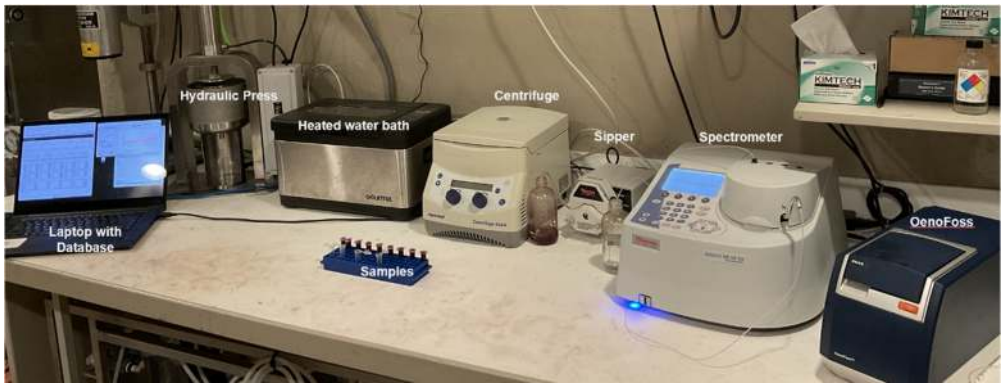
- Setup: describes the laboratory equipment required to prepare the simulated wine samples
- Preparing the phenolics extraction buffer: describes how to prepare the alcohol solution, which will extract the phenolics and create a simulated wine
- Process: describes the measurement process in detail
- Calculations: shows how to interpret the measurements

Setup

The setup consists of

1. A Phenolics Extraction Buffer, an alcohol solution, to create a model wine from grape samples.
2. A Press; we use a pneumatic laboratory press specially designed for this application
3. A temperature-controlled water bath
4. A standard Spectral Analysis Setup as used for phenolics measurements with WineXray

Here is an annotated picture:



Preparation of Phenolics Extraction Buffer

The materials needed are:

- Potassium Hydrogen Tartrate (KHTa; 99%; manufactured by SIGMA-ALDRICH; Catalog Number 243531)
- Ethanol (CH3CH2OH, i.e., EtOH; Alcohol, Anhydrous, Reagent; manufactured by J. T. Baker; Catalog Number 9401-22)
- De-ionized water (DI H2O)
- Hydrochloric Acid (HCl; 1.0N and/or 0.1N Volumetric Solution; manufactured by J. T. Baker; Catalog Numbers 5620-02, 5611-02)

The composition of the buffer is 5 g/l Potassium Hydrogen Tartrate, 12% Ethanol v/v; pH 3.3.

Follow these steps to prepare 500mL of the Phenolics Extraction Buffer:

1. Place 400 ml of de-ionized water in a 1000 ml graduated beaker.
2. Weigh and add 2.5 g of Potassium Hydrogen Tartrate.
3. Measure and add 80 ml of 100% Ethanol.
4. With a magnetic heater/stirrer, stir for five (5) minutes; do not worry if all the Potassium Hydrogen Tartrate does not dissolve.
5. Use a pH meter while stirring the solution to monitor its pH.
6. Allow the pH to stabilize, and then adjust the pH of the solution to 3.3 using Hydrochloric acid.
7. Bring the volume to 500 ml with de-ionized water.

Store the buffer at room temperature; it is to be used within 30 days

Process

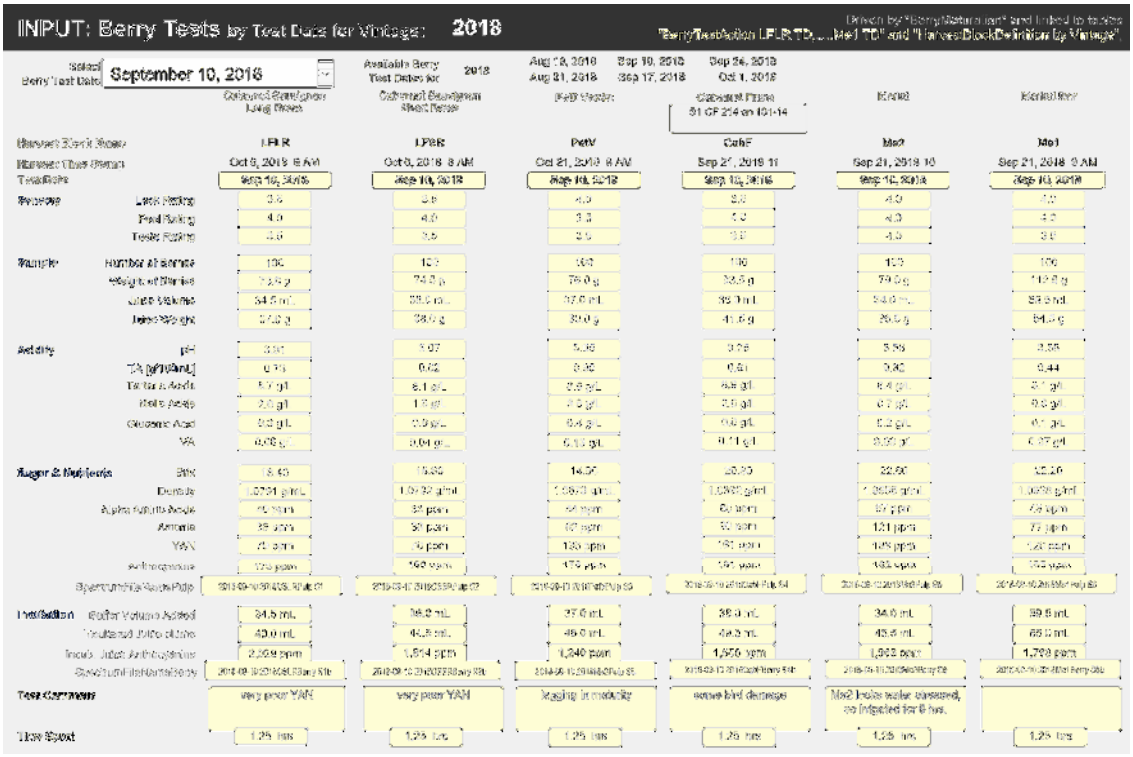
The measurement process has nine steps for each sample:

1. Collect 100 berries in a plastic bag. Count the Berries (NB = 100) and weigh them (WB in grams)
2. Crush the berries in the bag. Assure all berries are broken and the pulp and seeds are visibly separated from the skins.
3. Put the crushed berries sample into the press (calibrated for a 10-second close, 2-minute hold, and 10-second release). Activate the press.
4. Pour the pressed juice into a measurement container and measure the volume and weight of extracted juice: (VJ in mL, WJ in grams) and its approximate Sugar content per 100 g of solution (BX in Brix), pH (pH), and Titratable Acidity (TA in g/L).
5. Open the press and remove the compressed skins and seeds cake into a holding jar labeled "Extraction Jar" Then pour a given volume VJ of the "Extraction Buffer" into the Extraction Jar and mix well. Essentially replace the juice pressed out with the Extraction Buffer
6. Incubate the Extraction Jar for 2 hours in a water bath temperature controlled at 55 dC, then shake it and let it cool down for 15 minutes.

7. Empty the entire contents of the Extraction Jar into the press
8. Activate the press, and allow the juice to flow into a measurement container labeled "Incubated Juice." Measure the volume of the Exposed Juice (VEPB in mL). Discard the compressed seeds and skins cake.
9. Measure the phenolic compounds in the Extracted juice and in the Incubated Juice. Record Total Anthocyanins (JA and tEA in ppm Malvadin Equivalent) using the WineXray platform.

Calculations

Enter the measurements into the database using the "INPUT: Berry Test" layout.



The picture shows the measurements and results from the Berry Tests done on September 10, 2019, for all six vineyard sections. The following table shows for a different set of samples, the inputs (yellow fields) and result fields as calculated in the database (red fields) in weight per average berry [mg/Berry] or [% of average Berry Weight]

Berry Sampling and Measurements						
Berries	Number of berries in sample	NB		#	100
	Weight of berries in sample	WB		g	75.22
Extracted Fresh Juice	Volume of extracted juice	VJ		mL	27
	Weight of extracted juice	WJ		g	24.9
	Degrees Brix of juice	BX		g sucrose/100g juice)	18.8
	pH of juice	pH		pH units	2.9
	Titratable Acidity of juice	TA		g/l	13.5
	Anthocyanins in juice	JA		mg/l ME	
Incubated Juice	Vulume of buffer added	B		ml	100
	Volume of extracted juice after incubation	VEPB		ml	84.5
	Total extractable Anthocyanins	tEA		mg/l ME	745
Total extractable Anthocyanins		TEA	tEA* B/(1.5*VJ) + JA	mg/l ME	1840
Weight Composi-tion of sample	Berries Skins & Seeds weight (BSSW)	BSSW	WB - WJ	g	50.28
	Berries Acids weight (BAW)	BAW	TA*WJ/1000	g	0.34
	Berries Sugars weight	BSW	BX*WJ/100 - BAW	g	4.35
	Berries Water weight	BWW	WB-BSSW-BAW-BSW	g	20.25
Breakdown of Average Berry Weight	Berry Sugars Loading	BSL	BSW*1000/NB	mg/b	44
	Berry Acids Loading	BAL	BAW*1000/NB	mg/b	3.37
	Berry Water Loading	BWL	BWW*1000/NB	mg/b	203
	Berry Skins & Seeds Loading	BSSL	BSSW*1000/NB	mg/b	503
	Berry Anthocyanins Loading	BEAL	TEA/1000*VEPB*(1.5*V	mg/b ME	0.63
	TOTAL	TBL	BSL+BAL+BWL+BSSL+	mg/b	753
Breakdown of Average Berry Weight	Berry Sugars Content [%]	BSC	BSL/TBL	%	5.78%
	Berry Acids Content [%]	BAC	BAL/TBL	%	0.45%
	Berry Water Content [%]	BWC	BWL/TBL	%	26.9%
	Berry Skins & Seeds Content [%]	BSSC	BSSL/TBL	%	66.8%
	Berry Anthocyanins Content [%]	BEAC	BEAL/TBL	%	0.08%

The formulae for calculating the results are shown to the right of each variable. Note: if the volume of Buffer added is equal to the volume of Extracted Juice, then the estimated Total Extractable Anthocyanins TEA = JA + tEA (Anthocyanins in Juice + Anthocyanins extracted by buffer).

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Next Page: Accuro for ORP
Last updated: March 4, 2023

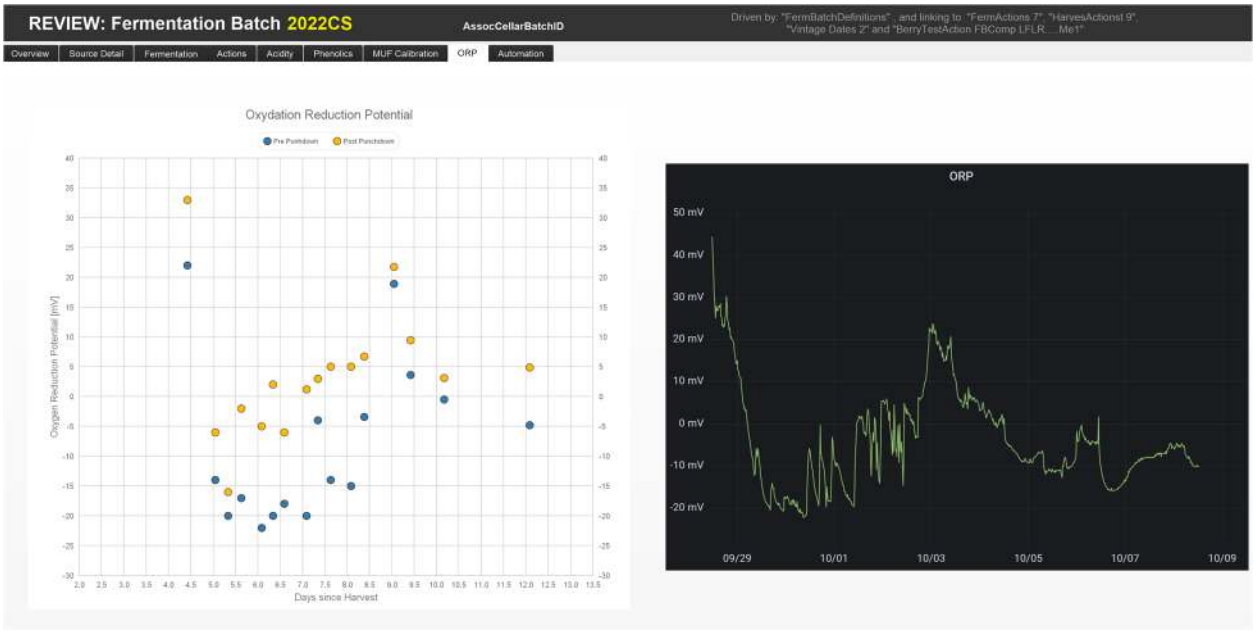
Accuro for ORP

Oxidation Reduction Potential "ORP" is a measure of the tendency of a molecule to acquire or lose electrons and thereby be reduced or oxidized (definition adapted from Wikipedia). Roger Boulton at UC Davis has advocated for a long time to measure the ORP in wine fermentations to understand the fermentation kinetics better. However, academic research and adoption of its use in the wine industry have taken off only recently.

We measure ORP with an electromechanical probe suspended in the fermenting must (not the cap). Many wineries in the US seem to use Hamilton probes with some oxygen management controller (e.g., FloTek, see https://flotekca.com/redox-fermentation-control-for-wine-industry). We found a more economical solution from Accuro (aka WineGrenade), a startup in New Zealand. The probe connects to a communications unit that transmits measured data to the cloud, from where it can be inspected/downloaded through an open-source data visualization tool (www.Grafana.net). The picture on the right shows the sieve from the fermentation tank, post-fermentation, with the probe hanging inside. The image on the left shows the control unit.

We record the ORP measurement once a day, right before and right after each punchdown in our database. The measurement before punchdown should be significantly lower than the measurement after. The increase is a result of oxygen added during the punchdown. A general rule is that the ORP should not drop below -70mV. However, we have observed fermentations that completed successfully while the ORP dropped below 110 mV. We have yet to figure out how to interpret the data. We aim to improve the timing and amount of oxygen injections.

The following screenshot shows the recorded measurements on the left and a picture of the Grafana screen on the right.



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Next Page: Measuring and adding SO2
Last updated: March 4, 2023

Measuring and Adding SO_2

Practically all wine is made with Sulfur Dioxide, SO_2 - repeatedly added in small doses to prevent spoilage. Still, wine can be made sulfur-free but requires rigorous sanitation and processing techniques (Frey Vineyards is a trailblazer in sulfur-free wines, http://www.freywine.com/). We stopped using sulfur in 2020, except at bottling.

Sulfur degrades certain enzymes that spoil the wine by oxidizing phenols; this is its role as an antioxidant. A specific form of SO_2 also kills bacteria and non-Saccharamyces yeasts by entering through their cell walls. There are four different instances when we consider adding SO_2:

- Right after grape sorting and before cold soak - if fermentation will be done with non-indigenous yeasts. The purpose is to kill off all indigenous yeasts first
- After the malolactic fermentation has finished.
- During cellaring, whenever barrels are topped up or racked.
- Just before bottling

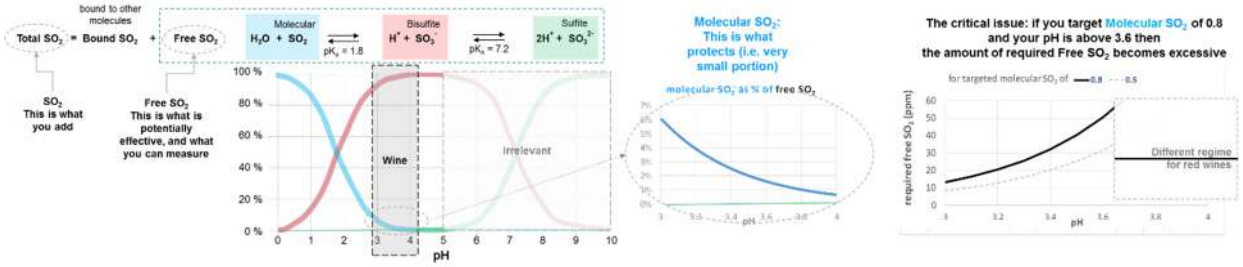
In 2016 we stopped using commercial yeast and relied instead on indigenous yeast to manage the fermentation; thus, we stopped adding SO2 before cold soak. In 2021 we stopped adding SO2 after malolactic fermentation to reduce SO2 additions further. We still add small amounts of SO2 before bottling.

On this page, we describe how we calculate the amount of SO_2 we added in each of the four instances and how we measure the concentration of SO_2 in the wine before and after.

Different forms of SO_2

The story is convoluted because only a portion of the "Total SO_2" added is effective; that portion is called "Molecular SO_2"; however, you can only measure and control "Free SO_2". Total SO_2 is the sum of Bound SO_2 and Free SO_2.

- **Bound** SO_2: When adding SO_2, a good portion of it becomes immediately bound to sugars, acetaldehydes, and phenolic compounds (called "Bound SO_2") and becomes ineffective; the remainder is called "Free SO_2."
- **Free** SO_2 exists in 3 forms and is the sum of Molecular SO_2 (SO_2), Bisulfite (HSO3-), and Sulfite (SO3=). Yair Margalit's Wine Concepts in Wine Chemistry 3rd edition explains how Free SO_2 demonstrates itself, pages 315-319, http://www.amazon.com/Concepts-Wine-Technology-Operations-Edition/dp/1935879804. The graphic below shows the relative percentage of each form in a solution depending on its pH. In very acidic environments (pH near zero). Molecular SO_2 dominates. As the pH rises, Bisulfite replaces Molecular SO_2 until the pH reaches around 4.5 and Molecular SO2 vanishes. When the pH increases further, Bisulfate is increasingly replaced by Sulfite. Thus, wine having a pH between 3 and 4, Free SO_2 is a mixture of mostly Bisulfite (HSO_3^-) and a small portion of molecular (SO_2).



Here is what matters: The amount of Molecular SO2 in Free SO2 is relatively small in wine. For example: if you want to have 0.8 ppm of Molecular SO_2, you need to provide 22 ppm of Free SO_2 when the pH is 3.2; whereas you need 43 ppm of Free SO_2 when the pH is 3.5. This is akin to breathing when you climb a very tall mountain: the higher you climb (in terms of pH), the more air you have to breathe to get your necessary oxygen (Molecular SO_2). The problem is: wine with a pH above 3.65 requires too high a Sulfur addition. Thus one either depresses the pH by acidifying the wine or switches to an entirely different regime.

How much SO_2 to add?

The accepted practice in the wine industry is to target a top Molecular SO_2- level of around 0.5 – 0.7 ppm for red wine. The idea is to add as little SO2 as possible while preventing spoilage.

- The upper bound is given by law (which sets a limit of 350 ppm Total SO_2) and, more importantly, by sensory degradation: Total SO_2 above 100 ppm can create a chemical

taste that covers up fruitiness; Molecular SO_2 above 0.7 ppm has a burnt match smell (sulfur).

- The lower bound is given by the effectiveness of Molecular SO_2 as an antioxidant and bacteria- and yeast-killer.

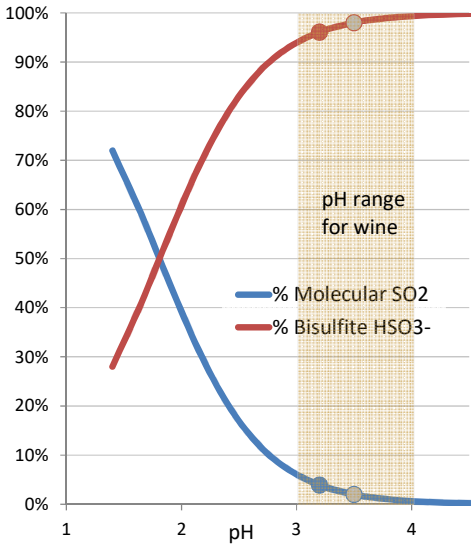
These boundaries create a challenge in wines with very low Acidity (high pH): the amount of Total SO_2 to be added to create enough Molecular SO_2 becomes too large. One response to this challenge is to increase the wine's Acidity artificially. Another is to be highly diligent with sanitation.

The compound most often used to add SO_2 to wine is not SO_2 itself but a powder, Potassium Metabisulfite $K_2S_2O_5$, "KMBS," which contains 57.4% SO_2. To calculate "A," the amount of KMBS (in grams) required, you need to know the following:

- The volume of wine to be treated (gallons): V
- The current level of "free SO_2" in the wine (in ppm) – see measuring "free SO_2": S
- The pH level of the wine: p
- The target level of "molecular SO_2" (e.g., 0.5 ppm): M

The formula to calculate the estimated required amount of KMBS (in grams) (derived in Margalit's book) is:

$$A= 0.0065712 * V * [M * (1+10^{(p-1.81)}) – S]$$



This formula approximates the balance between Bisulfite (HSO3-) and Molecular SO2 (SO2) in a liquid with a pH range between 1.5 and 4.5. The chart on the right shows the relationship. The constant 0.006572 = 3.785 / 1000 / 0.574 comes from converting gallons (3.785) into liters, ppm [i.e., mg/L into g/L (1000) and the fraction of Total SO_2 in KMBS (57.4%). Note the two markers on the curve: The amount of Molecular SO_2 in Free SO_2 is relatively small; i.e., the share of molecular SO_2 is only 3.9% at a pH of 3.2, and it drops to 2% at a pH of 3.5. In other words, at normal pH levels in wine (between 3 and 4), we will see only a small amount of free and potentially active Molecular SO_2.

So here are the **guidelines** we have followed for SO_2 addition in practice:

1. In the years we use industrial yeasts for fermentation, we kill off all non-saccharomyces yeasts before cold soak or fermentation by adding an amount of KMBS, which creates a molecular SO_2 level of 0.5 ppm. Since 2016 we have used the naturally occurring indigenous yeasts and no longer add any KMBS upfront.
2. While in the barrel, we measure the pH of the wine and the Free SO_2 level every three months and compute the corresponding Molecular SO_2 level. If Molecular SO_2 has fallen below 0.35 ppm, we top up with KMBS to target a Molecular SO_2 level of 0.45 ppm. This was the process we followed until 2019. In 2020 we stopped using SO2 in the cellar altogether. Having improved our sanitation significantly, we felt we could take the risk of no longer using SO2 as a preventative against contaminations.
3. Before bottling, we top up with KMBS to a target Molecular SO_2 level of 0.5 ppm

Further Resouces: Good websites:

- http://www.mbhes.com – distributor of SO_2 measurement tools
- http://www.practicalwinery.com/janfeb09/page5.htm - easy-to-read article
- http://www.santarosa.edu/~jhenderson/Sulfur%20Dioxide.p°F – excellent lecture
- http://fermsoft.com/sulphite.html - sulfite calculator from fermsoft.com

Measuring Free SO_2

Unitil 2017, we measured Free SO_2 by the "Aeration - Oxidation" method. First, the SO_2 is removed by a stream of air passing through the sample solution, and the SO_2 gas is trapped in a hydrogen peroxide solution which oxidizes it to sulfuric acid. Then the amount of sulfuric acid created is measured by titration with NaOH. The process takes four steps::

Step 1: Put the following into the round bubble flask (and close it)

- 10 milliliter of 25% Phosphoric Acid (use rubber ball at the end of pipette!)
- 20 milliliter of wine to be analyzed

Step 2: Fill the straight flask into which the vapor goes with

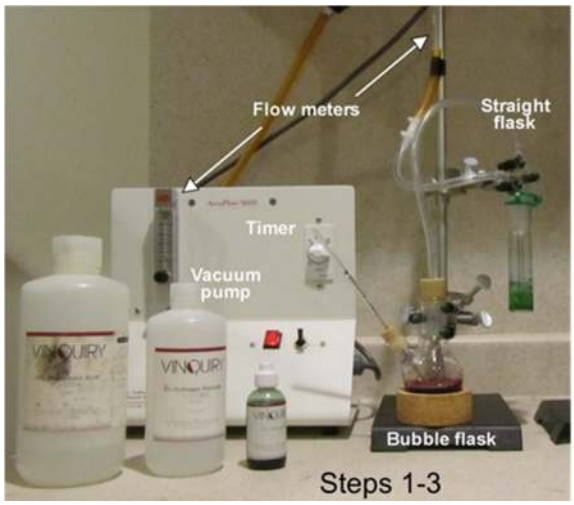
- 3% Hydrogen Peroxide up to the line and
- Add three drops of green indicator solution (in the fridge)

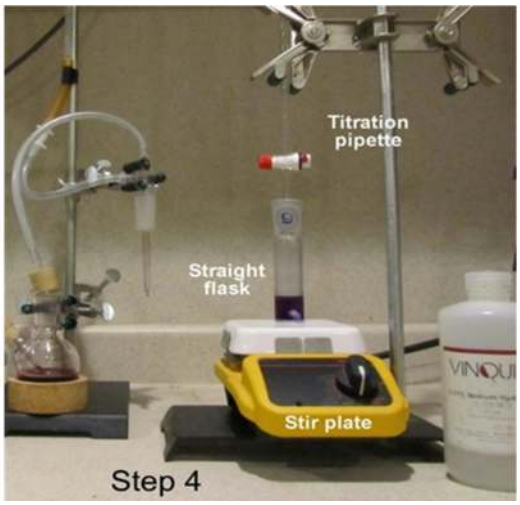
Step 3: Set the pump timer to 10 minutes, turn on and put volume control so that the indicator shows midlevel volume. Wait till done. The liquid in the straight flask should turn purple

Step 4: Fill the titrator with 0.01 Sodium Hydroxide up to an easily identifiable Starting point

- Put straight flask onto stirring table with stirrer inside and turn stirrer on
- Slowly drip Sodium Hydroxide into purple liquid and stop when it turns green

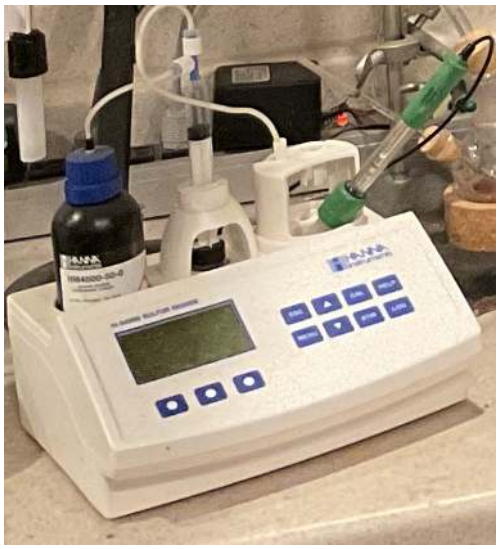
Result: Free SO_2 [in ppm] = (Start point [in milliliter] – Endpoint [in milliliter]) * 16





Note: a research paper posted in the November 2015 issue of Cornell University's Appellation Cornell Newsletter (http://grapesandwine.cals.cornell.edu/sites/grapesandwine.cals.cornell.edu/files/shared/Research%20Focus%202015-4.pdf) argues that the above formula to calculate the amount A of required KMBS addition is approximative at best and tends to overestimate the needed addition. This is because the constant 1.81 (chemists call it the "pKa") used in the formula above depends on the alcohol content and the temperature of the wine. A pKa of 1.81 is correct for water; in wine, at 14% alcohol and 68 ºF, the pKa is more like 1.95. The paper then suggests Headspace-Gas Detection, a new method to measure SO2 more accurately. For the time being, we will stick to the established industry practice described above.

Since 2017, we have simplified the process using an automated titrator from Hanna Instruments. Since 2020 we hardly ever measure SO2 anymore as we use indigenous yeasts for fermentation, and we no longer rely on sulfur for sanitation.



Previous page: Measuring Volatile Acidity
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Next Page: Measuring Dissolved Oxygen
Last updated: March 31 2023

Measuring Dissolved Oxygen

Definition and Relevance

Oxygen supply is essential during the fermentation of must; it keeps the yeast growing. But, once yeast cells stop multiplying, excess oxygen encourages the growth of microorganisms. In barrels during elevage, extra oxygen can lead to accelerated wine aging. So we add oxygen during the early fermentation phases and control the wine's exposure to oxygen once it is in the barrel.

Oxygen dissolves in wine, as it does in water. Wine can dissolve oxygen up to around 8 ppm or mg/L at 60 °F (significantly more at lower temperatures). Measuring DO is tricky because sampling can expose the wine to air.

Measurement

In the past, we tried to measure DO electromechanically (taking a sample and measuring a charge difference across a membrane). The instrument required is relatively inexpensive, but the measurement turned out unreliable because a sample must be extracted and probed, exposing it to oxygen in the air. We also experimented measuring DO with a Portable Dissolved Oxygen Meter HI9146 from Hanna (see http://www.hannainst.com/usa/prods2.cfm?id=004002&Pro°Code=HI%209146). Neither led to valuable insights, so we stopped experimenting with electromechanical probes.

In 2019 we bought an optical DO meter from Flotek. To experiment with measuring DO in three situations:

1. In must before and during fermentation. Yeast growth and replication depend on and absorb a fair amount of oxygen; that is one reason for vigorous punch-downs and/or macro-oxidation (injection of air or pure oxygen) in the must during the first half of the fermentation. We thought the DO measurement before and after each punch-down or injection should allow us to manage better the intensity of punchdowns and the diffusion of oxygen. It turns out that measuring the Oxidation Reduction Potential ("ORP," see earlier page) is more promising for monitoring yeast health.

2. In wine, during cellaring: The goal is to minimize the dissolved oxygen. Oxygen (except when added in tiny doses, i.e., controlled micro-oxidation in steel tanks) ages the wine prematurely and increases the chance of spoilage through microorganisms. Wine is exposed to oxygen each time we open the barrel for topping up or racking. Consequently, we measure DO before each topping up, before and after racking, and most importantly, before bottling. We stopped measuring DO in 2020 when we introduced a new top-up process which eliminated having to open up the barrels.

3. In wine, to measure its oxygen absorption capacity: The more oxygen and the faster a wine can absorb oxygen, the longer its expected future life and, thus, the ability to mature further. We experimented with measuring the oxygen absorption capacity in test bottles by forcefully increasing the dissolved oxygen level to its maximum (by vigorously shaking the bottle, thus dissolving air), measuring the DO, then capping the bottle and measuring the DO over time recording how fast and to what level it falls (i.e., how fast the dissolved oxygen gets absorbed)

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Next Page: Measuring Dissolved CO2
Last updated: March 30, 2023

Measuring Dissolved CO2

Definition and Relevance

Carbon Dioxide, CO2, dissolves in water and wine up to around one g/L at room temperature and normal air pressure. The amount decreases with rising temperatures and falling ambient pressure.

Fermentations create a lot of CO2, as sugar is converted into alcohol. CO2 bubbles up and forms a blanket on the top of the must in the fermentation tank, preventing the growth of microorganisms on the cap. When the fermentation is complete, no further CO2 is produced, the amount of dissolved CO2 stabilizes, and the wine must be protected by other means from contact with oxygen.

Small amounts of dissolved CO2 give the wine a fresh taste. Particularly in Bordeaux, winemakers often adjust the level of dissolved CO2 to 500-800 ppm in red wines and a bit higher in whites. Adding dry ice is the easiest way to add CO2 to wine at low cellar temperaures before bottling. A good rule of thumb is that about half the dry ice added will dissolve in the wine, and the other half will bubble up. So, the amount of dry ice to be added can be calculated as follows:

Dry Ice Add [in gram] = Amount of wine [in Liters] * Desired Increase in Dissolved CO2 [in ppm] * 0.002

Measurement

We measure dissolved CO2 with a Cabodoseur – an old-fashioned French mechanical tool that measures the amount of CO2 that escapes wine when it is vigorously agitated. The following picture shows the instrument

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Next Page: Measuring Phenolics in Wine
Last updated: March 31, 2023

Brix measured with Refractometers & Hydrometers

Brix is a measure of sugar content by weight in an aqueous solution. 1 degree Brix is equivalent to 1 gram of sucrose in 100 grams of water at a temperature of 20 degrees Celsius. We measure Brix in berries in the vineyard (to track their maturity) and in the must during fermentation (to track the conversion of sugars into alcohol).

The following paragraphs explain how we measured Brix before we purchased the OenoFoss Analyzer.

Refractometer

We used a simple Optical Refractometer (picture) to measure the sugar content of grapes. The instrument measures the optical refraction of sunlight as it passes through a thin layer of sugary water. The measurement is reasonably accurate under normal temperatures around 20 °C; more sophisticated digital instruments can compensate for temperature deviations. We continue to use a refractometer to measure Brix in the field occasionally.

Optical Refractometer

Hydrometer

Hydrometer

We used Hydrometers (picture) to measure the sugar content of must during fermentation. They measure the relative weight of the sugary alcoholic solution vs. a standard. We used two hydrometers – one at the beginning for high sugar content and the other at the end of fermentation to get better accuracy at low sugar contents. Because the must contains suspended particles, the measurement is not immediate; the suspended particles must be given

time to settle out in a fridge before the measurement is taken. This makes hydrometer measurements cumbersome.

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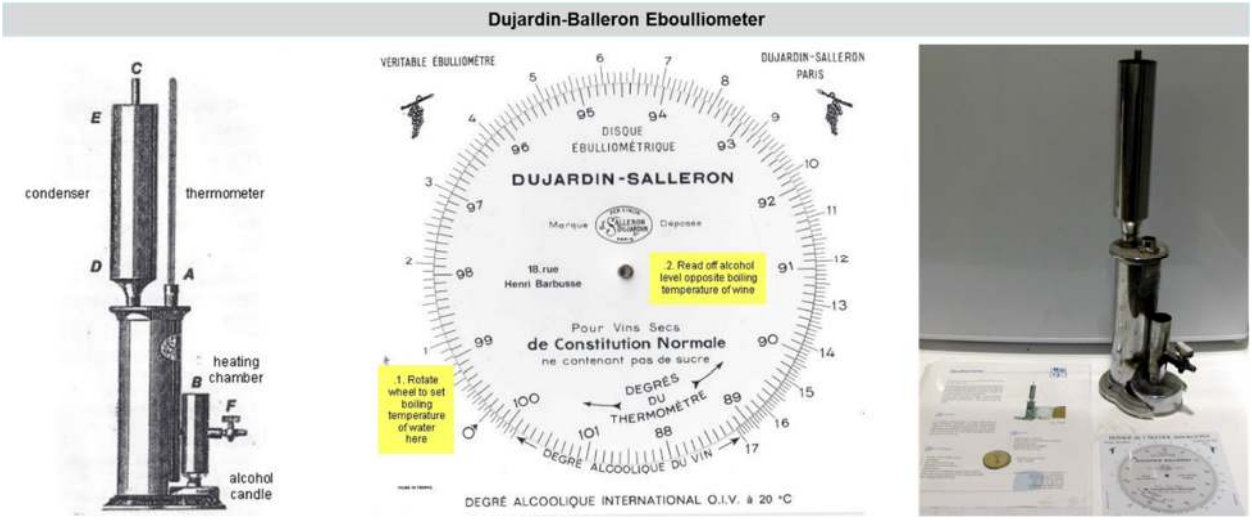
Next Page: Measuring Alcohol Content
Last updated: March 31, 2023

Alcohol measured with Eboulliometer

Up to 2017, we measured the alcohol content of wine with an Ebulliometer, which compares the boiling temperature of the wine with the boiling temperature of water at a given atmospheric pressure point. The procedure is

Step 1: Rinse the boiler/heating chamber: Rinse the cooling tank by pouring some water into the big opening on top, and then fill the boiler by pouring water into the small opening C. Open the tap, incline the instrument to 45 degrees to the front and empty the boiler. Blow out the remaining water in the boiler by blowing into C while obstructing hole A for the thermometer with a thumb.



Step 2: Determine the water boiling temperature: Fill the graduated glass to the mark "Eau" and pour it into the boiler through the top hole C. Do not put water into the cooling tank! Place the thermometer into its hole A. Light the alcohol lamp and place it under the boiler shaft. After a few minutes, steam comes from the top, and the thermometer is steady at the water boiling point. Put that temperature on the sliding wheel directly opposite the zero mark on the fixed part of the calculator

Step 3: Determine the boiling point of the wine. Empty the boiler and rinse it with some wine poured into the top opening C, fill it completely, empty it, and close the tap. Fill the graduated glass with the wine sample up to mark "vin" and pour it into the boiler. Put the thermometer into its place. Fill the cooling tank with cold water. Light the alcohol lamp and place it under the

boiler shaft. After 5 minutes, the mercury will rise and stop; wait 30 seconds, then read the temperature. Extinguish the heater flame. Read off the alcohol % directly opposite the boiling temperature of the wine on the moveable disk.

Since 2017 we have measured alcohol, and its related indicator, Density, with OenoFoss.

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Next Page: Measuring pH
Last updated: May 16, 2023

Measuring pH

Definition

The pH is a logarithmic scale for hydrogen ions (H+) concentration in a liquid solution: pH = -log[H+]. It is a measure of a solution's Acidity (low pH) or alkalinity (high pH). Pure water has a pH of ~7, and acidic wine has a pH of 3 to 4. A pH of 4 indicates 0.0001 mol/L of ions, while a pH of 4 indicates ten times more ions, 0.001 mol/L.

Relevance

From the winemaker's perspective, pH may be the most critical chemical parameter in premium wine production as it is known to have significant effects on the following:

- *Biological stability:* The lower the pH, the less chance for bacterial growth, and the higher the pH, the greater opportunity for bacterial spoilage
- *Color:* At higher pH values, the color tends to be in the blue range and highly unstable with the precipitation of pigments. At pH 3.0, approximately 40% of free anthocyanins are in ionized colored form, while at pH 4.0, only 11% are in the colored state.
- *Oxidation rate:* Higher pH wines tend to be poised toward oxidation.
- *Protein stability:* Concerning protein, lower pH tends to foster more rapid precipitation of unstable fractions.
- *Bitartrate stability:* Potassium ions play a critical part in the distribution of tartaric acid into its various forms of bitartrate and tartrate.
- *Overall palatability:* High pH wines generally can be described as flat, while overly acidic wines are too "tart."
- *Sulfur interactions:* At lower pH values, you need far less sulfur to protect wine, while at higher pH readings, more SO_2 is necessary to increase the molecular fraction with anti-microbial activity.

We adapted these bullet points from information on Enartis's website (www.enartis.com). The pH of must or wine can be adjusted downward to increase its stability by adding tartaric acid. Alternatively, it can be adjusted upwards through the addition of potassium bicarbonate.

Measurement

Until 2017 we used a portable pH meter to measure the pH of juice, must, or wine, and we used a variety of benchtop pH meters to track changes in pH during a titration. Since 2017 we have used OenoFoss, which constantly eliminated the need to calibrate the pH meters with reference solutions.

Handheld measurement

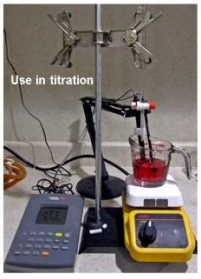
For handheld measurements, we used a HANA 92128. It is essential to recalibrate the instrument if it has not been used for more than 24 hours. To calibrate, follow either of these procedures:

- Single point calibration with buffer 4.01: When the meter is on, press and hold the start button until REC appears on the screen and place the meter into the 4.01 buffer. Wait until the message OK appears. Now the meter is ready for measurement.
- Two-point calibration: Place the meter into a 7.01 buffer and calibrate as in a single point. After the first calibration point has been accepted, the "Ph 4.01 USE" message appears, and you have 12 seconds to place the meter into a 4.01 buffer. When the second point is accepted, the display shows the value with the "OK 2" message. Now the meter is ready for measurement

For calibration video, see http://www.youtube.com/watch?v=cPbBZqgT4KQ

Hanna handheld pH meter

Beckman-Coulter benchtop pH meter



Benchtop measurement

We used a Beckman-Coulter Series 500 benchtop pH meter or a Hana Edge meter for benchtop measurement. Again, if the instrument has not been used for a few days, it must be recalibrated. (https://www.beckmancoulter.com/wsrportal/wsr/research-and-discovery/products-and-services/electrochemistry/500-series-benchtop-meters/index.htm?i=A58748)

Since 2017 we have used OenoFoss to measure pH.

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Next Page: Measuring Total Acidity
Last updated: May 16, 2023

Measuring Total Acidity / Titratable Acidity

Definition

Total Acidity "TA," also called Titratable Acidity, is the total amount of all the available Hydrogen ions in a solution, i.e., ions that are both free in solution (as H+) and those that are bound to undissociated acids and anions (e.g., H_2T and HT- for tartaric acids and anions). In grapes, must, or wine, TA is often expressed as grams of tartaric acid per 100 mL of wine or % weight. We prefer to represent it in parts per million [ppm]. Note this measure expresses Acidity in terms of tartaric acid, although we know that the wine contains a mixture of acids (mostly tartaric, malic, lactic, citric, and acetic acids). Some regulatory bodies prescribe a minimum TA (like 0.45 grams tartaric /100mL for red wine in the EU). A general range for TA in red wine is 0.6 to 0.8 [600-800 ppm]; for red wine must, it is 0.7 to 0.9 [700-900 ppm].

Relevance

During the final weeks of grape development, their TA drops from over 1500 ppm to around 700 ppm while the sugar content increases from 12 to 24 Brix. If the TA is not in a 700 – 900 ppm range by harvest time, adjustments should be considered by adding either tartaric acid to increase TA or potassium bicarbonate to reduce TA.

Although TA and pH are interrelated, they are not the same thing. A solution containing a specific quantity of a relatively weaker acid, such as malic, will have a different (higher) pH than a solution containing the same amount of a stronger acid, such as tartaric

Measurement

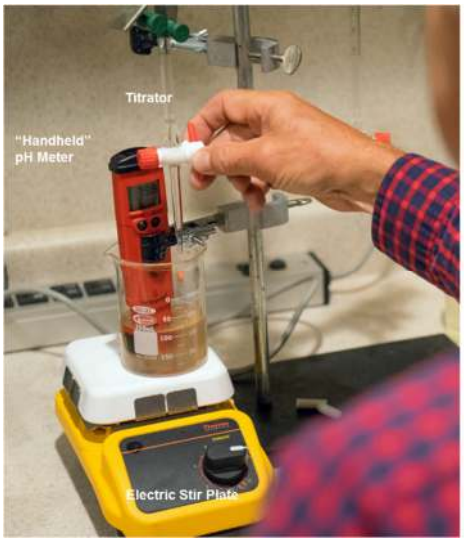
Until 2017 we measured TA with a titration procedure: we start with a given amount of must or wine and slowly add and measure the amount (titrate) of a given alkaline solution it takes to bring the pH of the mixture to 8.2.

The titration procedure has five steps:

Step 1: Calibrate the benchtop pH meter first with a buffer solution of pH 7, then pH 4. This makes sure that the instrument measures correctly

Step 2: Fill the titration pipette to a given Start Point with the 0.1 normalized Sodium Hydroxyde (0.1 N NaOH), the alkaline solution.

Step 3: Fill a beaker with 100 mL of purified water, put it on a stir plate, and dip the electrodes of the pH meter into the water. Ensure the electrodes are adequately submerged, do not touch the beaker wall, and have a safe distance from the magnetic stirrer.



Step 4: Pipette precisely 10 mL of juice, must, or wine into the beaker. To increase accuracy, you may want to degas the sample by heating it to a boil for a few seconds and then letting it cool down to room temperature.

Step 5: Turn on the stir plate and slowly drip the alkaline solution into the beaker. Stop when the pH has risen to 8.2. Note that the pH will rise only slowly at the beginning but then rise fast after reaching a pH of 6. When 8.2 is reached, note the End Point and calculate the amount of 0.1N NaOH used (in mL)

The following equation calculates the TA in grams per 100 mL: TA = 75 * V * N / S

V = ml of sodium hydroxide solution used for titration (i.e., EndPoint – StartPoint)
N = Normality of sodium hydroxide solution (e.g., 0.1)
S = Sample juice/must/wine volume (ml) (e.g., 10)

So, if, for example, you used 8 mL of 0.1N NaOH to titrate a wine sample of 10 mL, then the TA of that sample would be 0.6 (g/100 mL) = 75 * 8 * 0.1 / 10.

Since 2017 we have measured TA with OenoFoss

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Next Page: Measuring Volatile Acidity
Last updated: May 16, 2023

Volatile Acidity measured with Cash Still

Definition & Relevance

Volatile Acidity refers to the steam-distillable acids present in wine (mostly acetic acids). In small, barely detectable amounts below 600 mg/L [600 ppm], Volatile acids are believed to add complexity, but above 1g/L [1000 ppm], they are considered to spoil the wine (vinegar).VA is a natural byproduct of fermentation and tends to increase over time due to microbial activity. In concentrations above 900 mg/L, it affects the nose negatively: either ethyl acetate esters (acetone), which smell like nail polish or remover, or acetic acids, which smell like vinegar.

Early detection of rising VA is crucial as it indicates excessive exposure to oxygen and harmful microbial activity. While excessive VA can be removed (see http://chateauhetsakais.com/filtering-reverse-osmosis), it is essential to treat its root causes – the earlier, the better.

Measurement

Until 2017 we measured VA with a Cash Still manufactured by Research & Development Glass Products & Equipment, Berkeley, CA. The procedure is as follows:

Step 1: Fill the boiler. Fill the boiler reservoir to the middle of the round flask with distilled water through the funnel; the green handle on the funnel stopcock must point down. This takes about 500 mL.

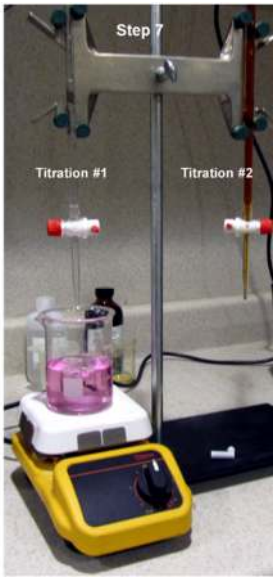
Step 2: Start the Condenser. Connect the cooling water to the condenser's lower end (inlet). Connect the upper end (outlet) of the condenser to the upper end (inlet) of the aspirator and connect the cooling water discharge hose to the lower end (outlet) of the aspirator. Turn on the cooling/tap water and adjust the flow to create suction in the aspirator. (Add a little distilled water to the sample chamber [through the funnel, with the red handle on the funnel-stopcock down] to check the suction. Then open the aspirator-stopcock. If the water in the sample chamber is sucked out, the water flow is sufficient).

Step 3: Add the sample. Turn the red handle on the funnel-stopcock down. Close the aspirator stopcock and add 10 mL of the wine sample through the funnel to the sample chamber. Add two drops of Antifoam B and rinse the funnel into the sample chamber with a few mL of distilled water.

Step 4: Degass the sample. For this, the red handle of the funnel stopcock needs to stay down. Turn the electric power to the heating coil on. Place a 300 mL Erlenmeyer flask under the delivery end of the condenser. Wait 20 seconds after the water starts boiling, and then close the funnel-stopcock (these 20 seconds allow the sample to be degassed from CO2)

Step 5: Distill the sample. Collect approximately 125mL of distillate in the Erlenmeyer flask for the subsequent titration.

Step 6: Clean and ready for the next sample. Open the aspirator-stopcock to suck out the remains of the wine sample. Close the aspirator-stopcock. Turn the red handle on the funnel-stopcock down and add distilled water to cleanse the sample chamber. Open the aspirator-stopcock to flush out. Repeat until the sample chamber is clean. Before going back to Step 3 for the next sample, check the water level in the boiler reservoir. It must be well above the heating coil. The reservoir may break if it overheats due to a lack of water. Never add water to a dry and hot reservoir – instead, turn the power off and wait until the glass has cooled down.



Step 7: Titration. Take the 125 mL of distilled sample (S), add three drops of 1% Phenolphthalein, and titrate in Titrator #1 with 0.1N NaOH until the distillate turns pale pink and stays pink for 15 seconds. Calculate the amount of NaOH used (AN = Startpoint less Endpoint). Then add 1 mL of Starch Indicator and 5 mL of 25% Sulfuric Acid and titrate in Titrator #2 with 0.02N Iodine to a faint blue color. Calculate amount of 0.02N Iodine used (AI = Startpoint less Endpoint)

Result: VA [mg/L] = 600 * AN[mL] - 12 * AI [mL]

http:// https://www.youtube.com/watch?v=eJdq9jkdZ_0

Since 2017 we have measured VA with OenoFoss. This cut down laboratory time from 1 hour/sample to less than 3 minutes per sample.

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Last updated: May 16, 2023

Measuring Malic & Lactic Acids

We measure the concentration of Malic Acids in wine to monitor the malolactic fermentation. In malolactic fermentation, malic acids are converted to lactic acids – this helps shape a wine style and character and its aging potential. We used two methods to confirm the successful completion of the malolactic fermentation:

Paper Chromatography, which is time-consuming,

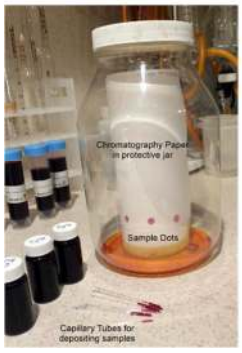
Chemical Test, which is only very approximative, and

Another accurate measurement of malic acids is possible with enzymatic test kits, which use spectral analysis (similar to what we use for measuring phenolics) – however, we never tried it.

Paper Chromatography

Paper chromatography has been employed for decades to visualize molecules of different weights in a solution. First, tiny dots of the sample wines are placed along the bottom of a chromatography paper; then, the sides are clipped together to form a vertical cylinder. The cylinder is placed into a jar holding a chromatography solution and capilar forces push the solution up to the top of the cylinder, dragging the malic acid and lactic acid molecules with them. Malic acids, heavier than lactic acids, will not travel as far up the paper. After a day of standing in the chromatography solution, the paper is removed from the jar and dried. Once dried, traces of malic and lactic acids become visible at different heights of the paper. When there is no longer a trace of malic acids, the malolactic fermentation is complete.

Paper Chromatography



Chemical Test

Chemical Test

Accuvin (www.accuvin.com) sells an economical kit to get approximative measurements of Malic Acids in wine. The kit includes sampler tips and test strips, which change color within 5 minutes of applying a 20-micro-liter sample. Matching that color with a reference chart allows us to estimate the remaining malic acid concentrations [>500, 100-500, 30-100, and <30 mg/L]. The goal is to reach <30mg/L. The cost of the kit with five samples is around $30.

Since 2019, we have used OenoFoss to measure Malic and Lactic Acids

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