Editor’s Note
This is the first issue of a quarterly newsletter, for free distribution to all licensed Colorado wineries in print form, and available for electronic download to anyone on the Western Colorado Research Center website after April 10, 2012. The site is located at http://www.colostate.edu/programs/wcrc/pubs/research_outreach/outreach.htm
It is intended to provide information for the Colorado wine industry, to encompass the area of enology topics and technology, especially those that deal with winemaking and the wine business in Colorado.

Issue 1 is devoted entirely to publication of a stand-alone Winery Laboratory Manual, in the remaining pages of this newsletter. This manual is written in very straightforward fashion and each step of each test is clearly stated (hopefully). This is the first Colorado draft, and does not contain diagrams for test equipment, which will be sent at a future date. This manual is also intended to serve as the procedures for the first “Colorado Winery Analysis Workshop”, to be held in Summer 2012 (TBA). This manual is derived from a hands-on course in small winery analysis that I have given while employed in two previous states. Please send any comments or suggestions on this manual or for topics in future newsletters to one of the following contact avenues.

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Dear Winemakers,

This manual contains just the text for carrying out various wine and juice analytical tests. It will be continually updated and expanded. The first expansion will be diagrams of test equipment for each test. Additional tests and detailed troubleshooting sections are planned for future updates. This manual will be the basis for a series of annual winery analysis workshops. The tests in this beginning manual include: initial wine or juice sensory evaluation, juice or wine pH, juice soluble solids by refractometer and hydrometer, juice yeast available nitrogen by formol titration, wine free and bound SO\textsubscript{2} (both = total SO\textsubscript{2}) by aeration-oxidation, juice or wine titratable acidity, wine residual sugar by Clinitest, volatile acidity by Cash still and colorimetric titration, and wine malolactic content by paper chromatography.

Sincerely yours,
Stephen Menke
INTRODUCTION

This manual is intended for use by small wineries, although most of the procedures contained herein are also used in many larger wineries. These tests are as accurate as possible while still being simple and cheap. The procedures are explained as simply and clearly as possible.

There are several reasons for consistently testing your wines and musts. The primary reason is for control of wine quality throughout the steps from grapes to bottled wines, so that no wine is either spoiled or of poor sensory or stylistic quality. The second necessary reason for testing is compliance with federal and state standards of chemical composition. The third reason is to identify potential problems, either particular or systemic, and to properly plan future winemaking activities.

This manual contains tests considered to be a basic repertoire of quality control for winemakers. Additional tests and techniques will be addressed in later additions to this manual. Not all tests need to be done on every wine, but the following should always be done on musts and wines:

Must or Juice

Soluble solids estimate
Refractometer (pp. 10-11)
Hydrometer (pp. 11-12)

pH (pp. 7-9)
Total titratable acidity (p. 22)
Nitrogen content, formol titration (p. 13)

Wine

pH (pp. 7-9)
Sulfur dioxide
Free (pp. 15-17)
Bound (pp. 17-18)
Residual sugar (p. 14)
Total titratable acidity (p. 22)
Volatile acidity (pp. 23-24)
Malolactic fermentation (pp. 18-21)
Expert sensory evaluation (pp. 3-6)
Sensory Evaluation
Wine can be evaluated by the senses of sight, smell, and taste. For most wines, a standard terminology has developed for the sensory experiences we undergo when 'tasting' wine. There are two types of sensory evaluation used by wine drinkers, experiential or "expert" and descriptive or "scientific".

Descriptive Sensory Evaluation
A consensus order of description has also been derived and descriptive palettes or 'wheels' have been developed as guidelines, by sensory researchers. Researchers use scientifically trained panels that are calibrated by exposure to standard solutions, containing the sensory character to be tested. These trained panels then test for these characters in a standardized environment, and statistical analysis is done on the data of the panelists. The end result is a sensory description of the samples tested, such as the Wine Aroma Wheel, developed by Dr. Ann Noble.

'Expert' Sensory Evaluation
The following is a very rudimentary introduction to the standard methodology of sensory evaluation by expert tasting. This is also the method most of us use for our own selection of wines, since it is very experiential in nature and thus more subjective.

This evaluation technique only requires training in detecting the components compared, and can be done by individuals or by a panel. Generally, more experienced tasters and a panel of a number of these expert tasters give the best results.

Equipment and Solutions
Wine glass(es) of standard size and shape (usually 8 oz or 235 ml tulip)
Sunlight or other 'natural' light
Cup for spitting out wine and container for spent samples and spit cups
Glass or cup with water
Sample bottle(s) of wine in unmarked covering
Aroma wheel (and/or taste wheel)
Tasting Steps

1. Do tasting in a room that is as free as possible of odors and other sensory distractions, including your own personal care products. Do not eat strongly flavored food just before tasting. Taste when alert and taste at a standard time of day. Arrange glasses and bucket and water glass in standard manner. Have a white surface under glasses. Use aroma wheel (available at http://winearomawheel.com/ or other sources) to help generate wine descriptors.

2. Have wine at a standard temperature for the type of wine to be tested. When comparing wines, keep the temperature the same for all samples that are directly compared.

3. Open bottle and pour wine into glass(es) to about 1/3 height of glass bowl. When comparing wines, fill all glasses to same level and pour at the same time.

4. Look down through wine toward white table with adequately bright light present. Evaluate wine for clarity and color, and write down evaluation. Clarity is the ability of light to freely penetrate throughout the wine, and may range from brilliant to clear to hazy to cloudy. White wine color should range from clear to slightly yellow, depending on variety or style. Rosés or blushes should be pink and not orange. Red wines should range from a slightly purplish-red to a ruby-red for table wines up to several years of age, to a slightly brownish red for long-aged wines. Excessive purple usually indicates a high pH and low acid wine, which does not usually age well. Very brown wines indicate excessive oxidation, even though many people like some level of oxidized flavors. Purposely oxidized wines, such as sherries, ports, madeiras, angelicas, brandies, and cognacs will often be various golden or amber or brownish colors and represent a different standard of color. Fruit wines also are judged by their own unique color standards, but the clarity test is the same.

5. After color evaluation, put cover on wine glass(es)(use watch glass or paper sheet) and let wine stand covered for 30 seconds before evaluating aroma.
6. Lift the wine glass to your nostrils and sniff deeply. This will increase the flow of aromas over the smell receptors in your nose. This is a complex process that requires training, as you must not only detect any faulty odors, but also try to identify any desired odors. This is where the aroma wheel comes into play. By first identifying the inner circle of a wheel sector for a particular aroma, you can then further specify the aromas on the outer circles within that sector. Write down all odors you can identify. Cover glass with cap 30 seconds before re-sniffing.

7. Take a small sip with some air and let wine roll and slosh over tongue and palate, then spit it out. Evaluate for taste components: sweetness, acidity (tartness), bitterness, saltiness (not usually in wine). Body, or mouth-feel is a tactile effect, due to viscosity of ethanol and sugar and effects of tannins and other phenolics, as well as chemical warmth or irritation. Warmth is usually due to high ethanol content or extremely low pH. Astringency (mouth-pucker) is due primarily to effects of tannins.

While evaluating for taste, it is almost impossible to separate perception of aromas, released retronasally, from perception of tastes in the mouth cavity. This is flavor, the combination of taste and retronasal aroma released by interacting with salivary enzymes. For example, acidity may enhance freshness and intensity of some aromas. Synergies of taste and aroma may occur. The most common synergy is that sweetness and fruitiness are mutually enhanced or muted in intensity. Another synergy example is that the mouth-feel of alcohol may enhance the sweetness and fruitiness of the wine, or acidity and sweetness may complement each other, thus leading to the concept of acid-sugar balance. High astringency may mask bitterness. Bitterness and acidity enhance each other and are enhanced by the warmth of high alcohol. Tastes tend to linger differentially: i.e., sweet < acid < bitter. Finally, each person is genetically different for each aroma and taste. The important thing is to train yourself to be consistent and to
'taste' the same way each time. Write down your taste and secondary aroma descriptions.

WINERY LABORATORY MANUAL FOR WINE AND MUST ANALYSIS
Stephen Menke

SENSORY EVALUATION (Cont.)

Carry a pocket size wine notebook with you in the winery that records your systematic descriptions of wines. Also, record the wines you taste at home or away in another book. The winery tasting notes will help you to refine your analytical abilities and to remember what wines had what attributes at each stage of winemaking process. The outside notes will help you remember which wines you like. If you routinely taste your wines all the way from must to bottle, you will learn how to envision the final wine when you taste the grapes at harvest.
pH DETERMINATION

Determination of pH is the most useful test carried out in the winery lab. The pH of a must is the single best indicator of how it will ferment. The pH of a wine is important to sensory aspects of the wine and to its aging capabilities. The correct measure of pH in lab tests can be important, for example in the test for total titratable acids, having the test solution at pH 8.2 is important to determining the end-point color of the indicator. The pH meter is the most critical machine in the lab. It should be calibrated and maintained carefully.

Calibration Equipment and Solutions
pH meter and electrode, accurate to 0.01 or 0.02 units
Standard pH buffer standards: 3.00, 4.00, 7.00, 10.00
Thermometer, or temperature probe, accurate to 0.5 degrees
Squirt bottle with distilled or deionized water
Magnetic stir plate and stir magnet
Small beakers (50-100 ml) for standards, test solutions and rinse waste

Calibration Steps:
Calibration of a pH meter is one of the most important steps in pH measurement. In calibrating a pH meter, it is advisable to use the standard reference solutions whose pH values most closely bracket that of the solutions to be tested.
1. Connect electrode to meter and remove protective cap over small hole on upper side of electrode, and remove electrode from storage buffer solution.
2. Rinse electrode tip and surface in distilled water. Use squirt bottle, if possible, and rinse inside protective shell of electrode probe tip. Shake excess water out of tip cavity.
3. Take temperature of 7.00 buffer with thermometer (or temperature probe, if it comes with pH meter).
4. Immerse pH electrode in pH buffer 7.00, set the temperature control to that of the buffer 7.00.
Stir several times to ensure probe tip is in good contact with buffer. Allow sufficient time for the correct readout as indicated by pH meter manufacturer (usually 7.02). This will be when the readout is stable for several seconds. Adjust the Standardize control for the correct readout according to manufacturer’s recommendations (usually 7.02).

5. Rinse electrode with distilled water. Use squirt bottle, if possible, and rinse inside protective shell of probe tip. Shake excess water out of tip cavity.

6. Immerse pH electrode and thermometer (or temperature probe) in buffer 4.00 (or 3.00 / 10.00 if readings are under 4 or over 7.00), set the temperature control to that of the buffer and allow sufficient time for the electrode to stabilize. Adjust the slope control for the correct readout as indicated by manufacturer’s recommendations (usually 4.00 or 7.02 or 3.00 or 10.05).

7. Rinse electrode with distilled water. Use squirt bottle, if possible, and rinse inside protective shell of probe tip. Shake excess water out of tip cavity.

8. Immerse pH electrode in buffer 7.0 again to check accuracy over pH range. If it is not within 0.02 pH units of 7.02, repeat steps 3 through 8 until accurate.

For accuracy, perform calibration periodically and when replacing electrode.

**pH Test Equipment and Solutions**

- pH meter and electrode, accurate to 0.01 or 0.02 pH units
- Standard pH buffer standards: 3.00, 4.00, 7.00, 10.00
- Thermometer, or temperature probe, accurate to 0.5 degrees
- Squirt bottle with distilled or deionized water
- Must sample, wine sample, or reagent solution to be measured
Sample Reading Steps (after calibration or previous test):

1. Have sample in beaker on magnetic stir plate with stir bar in fluid.
2. Rinse electrode with distilled water. Use squirt bottle, if possible, and rinse inside protective shell of probe tip. Shake excess water out of tip cavity.
3. Standardize in buffer 7.00 and rinse in distilled water (as in steps 3 and 4 of calibration protocol).
4. Immerse electrode in sample, with stir bar in motion, and set temperature knob to the sample temperature. Allow time for probe to equilibrate to sample temperature. Allow reading to stabilize (reading does not fluctuate for at least ten seconds, or if it does it immediately returns to the previous reading). The readout indicates the pH of the sample.
5. Always rinse the electrode in distilled water after use or between samples. If not immediately testing another sample, immerse electrode in a storage buffer solution to avoid potential damage (unless it is a 'dry' electrode).
6. Repeat steps 1-5 with new sample.
The soluble solids of a must can be estimated by using the refractive index of a solution or refractometry. One instrument that uses this physical property and interprets the results in terms of dissolved solids is called a sugar/salt refractometer. The instrument is usually equipped with a \(^0\text{Brix}\) scale of 0 to 32 with 0.2 \(^0\text{Brix}\) divisions. Use a temperature compensated instrument. Accurate results can be obtained between 60° and 100°F.

Soluble solids in grapes, which are those that are in the settled juice, are about 90% fermentable sugars. The balance is composed of organic acids, proteins, polysaccharides, pectins, and minor amounts of other chemicals. This is usually measured in \(^0\text{Brix}\), with each \(^0\text{Brix}\) = 1 gram sucrose/100 grams juice. The amount (concentration) of soluble solids of a certain density (weight/volume) and how the solids interact with the water solvent give the solution a defined amount of refraction, or bending of light. This refraction is dependent enough on concentration of the soluble solids that tables relating the two have been made. The practical effect is that at each concentration of soluble solids in a solution, the amount of refraction can be measured on a scale. Several scales exist, the most common one being \(^0\text{Brix}\).

**Equipment and Solutions**
- Refractometer, with Automatic Temperature Compensation (ATC)
- Juice sample, spun down or filtered from must
- Squeeze bottle with water

**Sample Reading Steps:**
1. Apply a drop of sample to the prism face and close the hinged prism lid. Be sure to close lid so that no bubbles are trapped under the lid.
2. Hold prism surface to strong light source and look through the eyepiece and read the scale. You will notice a line between contrasting dark and light areas on the scale. This line on the scale where the dark area ends and the light area begins is the point of reading. Value is read at the mark closest to the line between dark and light. Results are expressed in \(^0\text{Brix}\).
SOLUBLE SOLIDS BY REFRACTOMETER (Cont.)

3. Always rinse the prism lid and surface with distilled water, between samples and at the end of operation. After rinsing the apparatus make sure to dry it completely with soft cloth or soft paper that will not scratch prism or lid. Residues or leftover must or juice can affect further readings.

*Use of refractometer for wine is not recommended, because alcohol in wine interferes with readings.*

SOLUBLE SOLIDS BY HYDROMETER

Soluble solids in grapes, which are those that are in the settled juice, are about 90% fermentable sugars. The balance is composed of organic acids, proteins, polysaccharides, pectins, and minor amounts of other chemicals. This is usually measured in °Brix, with each °Brix = 1 gram sucrose/100 grams juice. The amount (concentration) of soluble solids of a certain density (weight/volume) and how the solids interact with the water solvent give the solution a defined specific gravity. This specific gravity is dependent enough on concentration of the soluble solids that tables relating the two have been made. The practical effect is that at each concentration of soluble solids in a solution, weight placed in the solution displaces a different volume of solution. This enables the use of a weighted bulb with a displaced-volume scale on its surface. This bulb is called a hydrometer. The hydrometer should not be used for wine, unless the alcohol has been distilled out of the wine and the volume reconstituted. Such a reading is called the extract.

**Equipment and Solutions**

Hydrometers, three scale, 0-35 °Brix, potential alcohol, and 0.98-1.16 specific gravity,

Hydrometer cylinder

Juice sample, settled, spun down or vacuum-filtered from must and at standard temperature for which hydrometer has been calibrated

Thermometer, or temperature probe, accurate to 0.5 degrees
Sample Reading Steps:
1. Carefully decant juice sample down inner wall of hydrometer cylinder, to avoid mixing air with the sample, fill cylinder to 3/4 full. Place hydrometer cylinder on flat, stable surface.

2. Immerse hydrometer gently into juice without allowing it to drop out of your fingers; give it a gentle spin and let go. When the hydrometer comes to a stop, read the line closest to the bottom of the meniscus; read while your eye is at same level as meniscus.

3. Take temperature reading of juice in hydrometer; note whether it is lower or higher than calibration temperature for hydrometer.

4. Look at table to obtain temperature correction value for °Brix read and temperature read. If the temperature read is below calibration temperature, subtract correction from °Brix read. If the temperature read is above the calibration temperature, add the correction factor to the °Brix read.

5. Wash hydrometer and cylinder immediately and wipe dry with clean lab tissue.
YEAST ASSIMILABLE NITROGEN BY FORMOL TITRATION

The formol titration procedure is a quick, easy, and fairly accurate way of determining nitrogen available for yeast to make the proteins it needs for good growth and health. The level of yeast assimilable nitrogen will help determine what nitrogen source to add to the must, if any, before fermentation.

Equipment and Solutions
100 ml sample of juice or clarified must
stir plate and small stirbar
2 x 200 ml graduated beakers
pH meter
two 25 or 50 ml titration burets, scale marked in 0.1 ml gradations, one containing 1.0N sodium hydroxide solution, and the other 0.10N sodium hydroxide 37% formaldehyde, reagent grade, neutralized to pH 8.0, w 1N sodium hydroxide. If stale, re-neutralize.

Sample Reading Steps
1. Place pH meter probe and stirbar in beaker w/100 ml juice or clarified must sample and start stirring
2. Neutralize stirring sample with 1N sodium hydroxide to pH 8.0
3. Add distilled water to 200 ml mark on beaker and stir well.
4. Transfer 100 ml of sample, and then transfer stirbar, into another clean beaker
4. Add 25.0 ml of neutralized formaldehyde to sample
5. Note scale mark on 0.10 N sodium hydroxide titration buret
6. Titrate stirring sample w/0.10N sodium hydroxide to pH 8.0
7. Note reading on 0.10N sodium hydroxide buret scale and find difference used between starting and ending readings
8. Calculate yeast available nitrogen, in mg/L = Amount of 0.10N sodium hydroxide used x 28
This derives from the full equation below;
N in mg/L = (ml of NaOH used) x (0.10 meq OH-/ml) x (1 meq N/meq OH-) x (200 ml/100 ml) x (10) x (14 mg N/mmol)
RESIDUAL SUGAR WITH CLINITEST® KIT

The Clinitest® kit is a quick estimation of reducing sugars in a wine. It is essentially the same copper reduction methodology involved in more complicated tests, such as the Rebelein or Lane-Enyon procedures. The kit is designed for use as a urine test, but it works equally well for wine. It is less accurate for sweet wines, and samples must be diluted to < 1% sugar to be accurately measured.

Equipment and Solutions
- Clinitest® kit
- Squeeze bottle with distilled water
- Graduated cylinder or volumetric pipets (for dilutions)

Sample Reading Steps
1. Follow directions in kit. Use 10-drop method. If you think the wine has sweetness, dilute it 5-fold before testing (1 part wine + 4 parts distilled water). If diluting, write down dilution ratio (5 times).

2. Read color chart in kit for % sugar. If the chart reads 2% or above, dilute the sample 5-fold, that is 1 part sample plus 4 parts water, and repeat test. Re-dilute if necessary, and keep track of dilutions. Remember to multiply the final reading by the total dilution ratio, to get the total sugar in the solution.

3. Rinse tube well with tap water and distilled water and completely dry tube before next test or storage in kit box.
Two measurements of $\text{SO}_2$ can be taken, free and bound. The principle is the same for both measures. The $\text{SO}_2$ is liberated from the wine sample and brought by vacuum pressure to a hydrogen peroxide trap solution, where it is trapped in the form of $\text{H}_2\text{SO}_4$ (sulfuric acid). The amount of sodium hydroxide needed to neutralize the trapped $\text{SO}_2$ is measured and a formula using this measurement gives the amount of $\text{SO}_2$ in the wine. Free $\text{SO}_2$ is liberated from unheated wine, whereas bound $\text{SO}_2$ is heated to liberate it. Free $\text{SO}_2$ + Bound $\text{SO}_2$ = Total $\text{SO}_2$

**Equipment and Solutions: Free $\text{SO}_2$**

- Sink and adjoining bench
- Aeration-oxidation distillation glassware assembly (see diagram).
- Water aspirator attached to faucet of sink (or vacuum pump)
- Ice bath, circulating or non-circulating
- Titration buret
- Volumetric pipettes, 10 and 20 ml
- Sodium hydroxide (0.01N)
- Hydrogen peroxide (3%), made fresh from refrigerated 30% solution
- Phosphoric acid (1+3 sol'n with distilled water, from 85% stock)
- Indicator solution (purchased ready-to-use)

**Sample Reading Steps: Free $\text{SO}_2$**

1. Assemble glassware as indicated in diagram (courtesy Vinquiry). All glassware must be rinsed clean from previous test.

2. Place the side-port flask into the ice bath flask.

3. Take the impinger apart and add 10 ml of 3% hydrogen peroxide and six drops of indicator to the impinger bottom (part #3). An initial violet color should result. If it does not, add 1 or several fine drops of some solution that is a thousand-fold dilution of the [phosphoric acid (1 + 3) ] solution until it just turns purple.
SO₂ BY AERATION-OXIDATION DISTILLATION (Cont.)

4. Titrate with a fine drop of 0.01N NaOH to reach a clear green-blue end point. Stir for a minute and if it gets cloudy, add another drop to get clear color that stays. Reassemble top and bottom of impinger.

5. Disassemble side-port flask from apparatus and leave in ice bath. Add exactly 20 ml of sample to flask. Then add 10 ml of phosphoric acid (1 + 3) solution. Replace flask onto apparatus.

6. Immediately begin aspiration with vigorous bubbling through sample for exactly ten minutes.

7. After exactly ten minutes of aspiration, shut off suction of aspirator and remove impinger with SO₂ trapped in hydrogen peroxide.

8. Take impinger to titration buret containing 0.01N NaOH. Read and write down the marked value at bottom of meniscus in buret.

9. Titrate purple solution in impinger drop-by-drop with 0.01 N NaOH until it turns clear green-blue. Read mark at bottom of meniscus. Stir for a minute and if it gets cloudy, add another drop to get clear color. Repeat until color stays clear. When color stays clear, read and write down mark at bottom of meniscus. Set impinger flask aside.

10. Find amount difference between starting reading and final reading on buret. This is ml NaOH used for titration.

11. Use formula to calculate SO₂ in mg/L (= ppm)

\[
SO₂ (\text{mg/L}) = \frac{\text{ml NaOH used} \times N \text{NaOH} \times 32 \times 1,000}{20 \text{ ml of sample}}
\]
12. **Save sample in side-port flask to do bound SO\(_2\)**

   Wash impinger with tap water and rinse several times with distilled water. Shake all water from impinger and replace on assembly.

**Equipment and Solutions: Bound SO\(_2\)**

   - Sink and adjoining bench
   - Aeration-oxidation distillation glassware assembly
   - Water aspirator attached to faucet of sink (or vacuum pump)
   - Microburner or heating mantle
   - Titration buret
   - Volumetric pipettes, 10 and 20 ml
   - Sodium hydroxide (0.01N)
   - Hydrogen peroxide (3%)
   - Phosphoric acid (1+3 sol'n with distilled water, from 85% stock)
   - Indicator solution (purchased ready-to-use)

**Sample Reading Steps: Bound SO\(_2\)**

   To do bound SO\(_2\), a condenser tube with water running through it is added between the side port flask (part #4) and the impinger set (parts #2 and #3). As well, the side port flask is heated by a heating mantle or other heat source. The sample is brought to boiling before aspiration begins.

   1. Assemble as indicated in diagram (courtesy Vinquiry), but add condenser as noted above. Run cold water through the condenser during the test.

   2. Place (or leave) the side-port flask, with **sample from free SO\(_2\) test**, onto assembly.

   3. Take the impinger apart and add 10 ml of 3% hydrogen peroxide and six drops of indicator to the impinger bottom (part #3). An initial violet color should result. If it does not, add 1 or several fine drops of some solution that is a thousand-fold dilution of the [phosphoric acid (1 + 3)] solution until it just turns purple.
4. Titrate with a fine drop of 0.01N NaOH to reach a clear green-blue end point. Stir for a minute and if it gets cloudy, add another drop to get clear color that stays. Reassemble top and bottom of impinger.

5. Use sample from free SO$_2$ test. Do not add more sample or more acid.

6. Heat flask to simmering boil with heating mantle or alcohol lamp. Then begin aspiration with vigorous bubbling through sample for exactly ten minutes.

7. After exactly ten minutes of aspiration, shut off suction of aspirator and remove impinger with SO$_2$ trapped in hydrogen peroxide.

8. Take impinger to titration buret containing 0.01N NaOH. Read and write down the marked value at bottom of meniscus in buret.

9. Titrate purple solution in impinger drop-by-drop with 0.01 N NaOH until it turns clear green-blue. Read mark at bottom of meniscus. Stir for a minute and if it gets cloudy, add another drop to get clear color. Repeat until color stays clear. When color stays clear, read and write down mark at bottom of meniscus. Put impinger flask aside.

10. Find amount difference between starting reading and final reading on buret. This is ml NaOH used for titration.

11. Use formula to calculate SO$_2$ in mg/L

$$SO_2 \text{ (mg/L)} = \frac{\text{ml NaOH used}}{\text{20 ml (sample size)}} \times N \text{ NaOH} \times 32 \times 1,000$$

= ppm

12. Add free SO$_2$ and bound SO$_2$ to get total SO$_2$
DETECTION OF MALO-LACTIC FERMENTATION

It is often desirable to perform a malolactic secondary fermentation on fermented wine, either to reduce excess acidity (ex., some cool-season grapes or some varieties of grapes) or to impart beneficial flavors or mouth-feel (Chardonnay and Chardonel). Some problems with malo-lactic fermentation can be: excessively high pH, or unwanted flavors. The standard method for detection of malolactic conversion of malic acid to lactic acid is by chromatography and visualization of malic and lactic acids on the chromatography paper. Other times, you may want to check to see if a malolactic fermentation is going on without your knowledge. Or you may want to check to see when to stop the malo-lactic fermentation when partially done.

Equipment and Solutions

One gallon wide-mouth jar w/lid
Chromatographic paper, cut into 20 cm x 30 cm sheets, do not touch with bare hands, or at least only at top edge
Chromatographic solvent (Can be purchased, as well)
  100ml n-Butanol
  100ml distilled water
  10.7 ml formic acid
  15 ml of solution, made from 1 g Bromcresol green in 100 ml distilled water
Separatory funnel and stand (If making own solvent)
Storage jar for solvent (if making own solvent)
0-20 ul pipettors or glass 10 ul micropipets
1-gallon glass or labware plastic jar w/wide mouth or similar width glass dish
Wine acid standards
tartaric, malic, lactic, 0.3% solution of each
Chromatographic paper
Wine sample
Paper towels

Sample Reading Steps

1. Make chromatographic solvent several days ahead of time by mixing thoroughly, and pouring into a separatory funnel for 1-2 days, and discarding lower phase and saving upper phase. Put upper phase in air-tight bottle and store
out of light and heat until use. It will keep for several weeks. Use solution only once or twice before discarding.

2. Cut chromatographic paper to size with paper cutter or scissors, and do not touch with bare hands. Keep paper clean and dry until use.

3. Using a pencil (not ink), draw a baseline parallel to bottom edge of paper, about 2.5 cm from bottom edge of paper. Use pencil to mark where spots of standards and samples will go (just under baseline) with letters or numbers. Keep spots at least 2 cm equally apart.

4. Using the pipettor or micropipettes, put about 10 ul spots on the paper, just below the pencil baseline, of the standards and the sample. Make sure spots are in a line parallel to bottom edge of paper. Let dry for a minute or two and respot. Repeat drying and respotting. Do not make each spot larger than 1 cm in diameter, or spots on developed chromatograph may be fuzzy. Let paper dry.

5. Put about 70 ml of chromatographic developing solvent in jar, on flat surface. Depth of solvent should be a little less than 1 cm. Put lid on jar until paper insertion.

6. Staple or clip paper into circle, but do not overlap edges.

7. Place paper circle into jar with solvent, with baseline edge of paper immersed in solvent. Cover jar with lid.

8. Let stand until solvent has migrated nearly to top of paper. Remove paper from jar and stand on paper towel to dry for several hours or overnight.

9. When dry, you will see yellow spots on a blue background. They are identified by your marks on the paper (sample lanes) and by the relative distance (Rf) they have migrated from baseline to top edge of solvent. Rf values = malic 0.50, lactic 0.75, tartaric 0.25. Your sample should have spots that have migrated roughly equally to one or more of the standards, and thus can be identified.
Absence of malic + presence of lactic in sample is indicative of malolactic conversion.
TOTAL TITRATABLE ACID

Total acid in a grape can be critical to the decision to buy grapes. Total acid in a must is a big factor in how fermentation goes and whether malolactic fermentation is used. Total acid in a wine is a factor in proper aging and in the sensory impression of a wine. Thus it is important to check total acidity at all stages of winemaking.

Equipment and Solutions
- pH meter and standard solutions
- Magnetic stir plate and stir bar
- 250 ml beaker
- 50 ml titration burette
- Volumetric pipettes, 5 ml
- Sodium hydroxide (0.1N)
- Distilled water
- Degassed (if possible) must (clarified) or wine sample

Sample Reading Steps
1. Standardize pH meter.
2. Set up buret on rack and fill w/ sodium hydroxide (0.1N). Put buret tip 6 inches above stir plate.
3. Put 100 ml boiled distilled and cooled water in a 250 ml beaker on a stir plate, with stir bar in it. Put pH probe in beaker (careful to keep above stir bar). Turn on stir bar.
4. Put 2-3 ml of sample in beaker. Titrate w/0.1N NaOH to pH 8.2.
5. Look at buret w/ sodium hydroxide and read volume at meniscus.
6. Use 5 ml volumetric pipet to transfer degassed sample (room temperature) into beaker.
7. Write down meniscus level of buret. Titrate solution w/0.1N NaOH to pH 8.2. Write down meniscus level of buret after titration.
8. Calculate results by this equation:
   \[ \text{Titratable acidity (g/L tartaric)} = \frac{(\text{ml NaOH}) \times (N \text{ NaOH}) \times (0.075) \times (1,000)}{\text{ml sample}} \]
VOLATILE ACIDITY DISTILLATION BY CASH STILL

Volatile acidity, most of which is acetic acid, can be a problem in wine. Pronounced volatile acidity to the nose usually indicates that the bacterium Acetobacter has contaminated the wine, changing some ethanol to acetic acid by an oxidation process. As wine ages and undergoes slow oxidation, some ethanol is oxidized to acetic acid or acetaldehyde, affecting the sensory impression of the wine. Most people do not mind very low levels of acetic, but high levels are not well tolerated. There is a legal limit for volatile acidity, though most wines are unfavorably judged before the legal limit is reached. Thus, at bottling, or if Acetobacter contamination is suspected, a volatile acid test is done. The easiest and most accurate methods are chromatographic or enzymatic, but require extremely expensive equipment. Though a steam distillation still is also a little expensive, it is much less expensive and does an adequate job. The most common one used is the Cash still.

Equipment and Solutions
Cash volatile acidity still assembly, w/condenser, hooked up to faucet and drain.
Regulated power supply (Variac or similar)
10 ml volumetric pipettes
250 ml graduated Ehrlenmeyer flask
50 ml titration burette and stir plate and stir bar
Volumetric pipettes, 5 and 10 ml
Sodium hydroxide (0.1 N)
Distilled water
Phenolphthalein 1% indicator solution
1 g of phenolphthalein + 70 ml of 95% ethanol +
titrated w/.0.1 N NaOH until light pink +
distilled water to make solution of 100 ml.
Wine sample

Sample Reading Steps
1. Assemble Cash still, condenser and receiving flask
   (250 ml flask)
2. Turn on cooling water for condenser.
3. Fill boiling chamber bulb of Cash still about 1/2 full with distilled water, either through bottom hoses or by funnel. Make sure bottom drain is closed off.

4. Adjust stopcock by funnel to deliver sample to sample chamber. Use volumetric pipette to deliver 10 ml of wine sample to sample chamber. Rinse funnel into chamber w/ distilled water and then close stopcock by funnel.

5. Plug heater into regulated power supply and bring water in steam chamber to moderately strong boil. If wine sample foams excessively, add anti-foam or reduce power to heater coil. Boil until flask below condenser tube contains 100 ml of distillate. Remove flask.

6. Turn off power to still and unplug.

7. Self-clean sample chamber by opening Venturi-type valve in still assembly. Close again

8. Open stopcock to sample chamber, put in 15 ml of distilled water, close stopcock, and repeat step 7. Repeat step 8.

9. Take receiving flask to stir plate, read meniscus of burette, and titrate solution w/.0.1 N NaOH to a pink end-point that lasts at least 10 seconds. Read meniscus and calculate total used by the following formula:

\[
\text{Volatile acidity (g/L acetic) = } \frac{(\text{ml NaOH}) (N \text{ NaOH}) (0.060) (1,000)}{\text{ml wine}}
\]

Legal limits are: 1.40 g/L for red, 1.20 g/L for white. California and export standards are more strict.