Manual for Wine Analysis and Laboratory Techniques
PREFACE

In order to assist the Ohio wine industry, this manual has been prepared for the third "Ohio Wine Analysis Workshop". This workshop is designed for winery personnel who would like to improve their laboratory techniques to help ensure the production of better quality wines. Knowledge of the chemical composition of wine and certain testing procedures is essential to the rational control of the winemaking process and wine quality. Furthermore, analysis for certain wine components is important when considering legal restrictions.

This manual provides several laboratory procedures which are considered basic to a quality control program for wineries. We sincerely hope that this material will be helpful to our industry. If you have any suggestions that will make it better, please share them with us.

James Gallander
Linn Briner
Judy Stetson
Jim-Wen Liu
Lynn Krielow
March 23, 1981
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SOLUBLE SOLIDS

Knowledge of sugar content is important to the winemaker in determining (1) the maturity of grapes, (2) the amount of amelioration needed, (3) the approximate alcohol yield, and (4) the completeness of fermentation.

At least 90% of the soluble solids of grapes are sugars. Therefore, measuring the soluble solids of a must gives a good indication of its sugar content. Soluble solids are determined using a Brix (Balling) hydrometer which measures the density of an aqueous solution. The Brix (Balling) hydrometer is calibrated in degrees corresponding to percent of sucrose (table sugar) in water at 20°C (68°F) or grams of sucrose per 100 grams of water at 20°C. For example, an 18% sucrose solution would have a Brix (Balling) reading of 18°.

Ethanol is less dense than water, so as the fermentation proceeds; the Brix (Balling) value will drop faster than expected considering actual sugar consumption. Most finished dry wines will have a negative Brix (Balling) reading due to the substantial amount of alcohol present. A sugar content in the range of 0.10 to 0.15% is common in dry wines. This residual sugar would have to be determined by a means other than hydrometry.

MATERIALS

Hydrometers, Brix or Balling - several will be needed, each calibrated over a specified range of sugar concentration.

Hydrometer cylinder

Thermometer (for temperature correction)

PROCEDURE

1. Fill a clean hydrometer cylinder approximately 3/4 full with sample.
2. Float a Brix (Balling) hydrometer in the sample. Tap the hydrometer gently and let it bob up and down a few seconds. When it is stationary, make a reading at the bottom of the meniscus.
3. Take a temperature reading of the sample, record it and make the necessary correction by referring to Table 1, p. 3.

NOTES

CAUTION: While preparing the sample be careful not to create air bubbles in the liquid.

A general method for estimating the expected ethanol yield is: degrees Brix (Balling) of the must times .55 equal vol. % of ethanol of the wine.

REFERENCES

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**TABLE 1. Corrections for Brix or Balling Hydrometers Calibrated at 20°C (68°F)**

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>°F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>15</td>
<td>59.0</td>
<td>0.20</td>
<td>0.22</td>
<td>0.24</td>
<td>0.26</td>
<td>0.28</td>
<td>0.30</td>
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<td>60.8</td>
<td>0.17</td>
<td>0.18</td>
<td>0.20</td>
<td>0.22</td>
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<tr>
<td>17</td>
<td>62.6</td>
<td>0.13</td>
<td>0.14</td>
<td>0.15</td>
<td>0.16</td>
<td>0.18</td>
<td>0.19</td>
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<td>18</td>
<td>64.4</td>
<td>0.09</td>
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<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
<td>0.13</td>
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<tr>
<td>19</td>
<td>66.2</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Subtract**

| 21            | 69.8 | 0.04 | 0.04 | 0.05 | 0.06 | 0.06 | 0.07 | 0.07 |
| 22            | 71.6 | 0.10 | 0.10 | 0.11 | 0.12 | 0.12 | 0.13 | 0.14 |
| 23            | 73.4 | 0.16 | 0.16 | 0.17 | 0.17 | 0.19 | 0.20 | 0.21 |
| 24            | 75.2 | 0.21 | 0.22 | 0.23 | 0.24 | 0.26 | 0.27 | 0.28 |
| 25            | 77.0 | 0.27 | 0.28 | 0.30 | 0.31 | 0.32 | 0.34 | 0.35 |
| 26            | 78.8 | 0.33 | 0.34 | 0.36 | 0.37 | 0.40 | 0.40 | 0.42 |
| 27            | 80.6 | 0.40 | 0.41 | 0.42 | 0.44 | 0.46 | 0.48 | 0.50 |
| 28            | 82.4 | 0.46 | 0.47 | 0.49 | 0.51 | 0.54 | 0.56 | 0.58 |
| 29            | 84.2 | 0.54 | 0.55 | 0.56 | 0.59 | 0.61 | 0.63 | 0.66 |
| 30            | 86.0 | 0.61 | 0.62 | 0.63 | 0.66 | 0.68 | 0.71 | 0.73 |
| 31            | 87.8 | 0.68 | 0.70 | 0.71 | 0.74 | 0.76 | 0.79 | 0.81 |
| 32            | 89.6 | 0.75 | 0.77 | 0.79 | 0.82 | 0.84 | 0.87 | 0.89 |
| 33            | 91.4 | 0.82 | 0.84 | 0.86 | 0.89 | 0.91 | 0.94 | 0.96 |
| 34            | 93.2 | 0.89 | 0.91 | 0.93 | 0.96 | 0.98 | 1.01 | 1.03 |
| 35            | 95.0 | 0.96 | 1.01 | 1.02 | 1.06 | 1.10 | 1.13 | 1.16 |

**Add**

From Amerine and Ough, 1974.
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TOTAL ACIDITY AND pH

Most dry table wines have total acidities (TA) of about 0.6-0.9% (Amerine and Ough, 1974). Sweet and dessert wines will range from 0.4-0.65% (Amerine and Ough, 1974). Proper acid level is important to the taste, color, and stability of wines.

Of the acids present in wine, tartaric and malic acid predominate. Acids are responsible for the fresh, tart taste of wine. Low acid levels result in flat and insipid wines, while high acid levels cause unpleasant tartness. In addition to the total acid, the particular balance of acids and the sugars present in a wine affect the degree of tartness.

Acid levels significantly influence wine pH which usually falls between 3.3 and 3.6. Total acidity and pH are important to both color and keeping quality. Orange-tinted rosés and purplish red wines indicate high pH values (low acidity). As pH decreases (T.A. increases) toward pH 3 the color of rosés and reds improve, becoming redder and brighter. Keeping quality of wines also improves as pH decreases. Spoilage bacteria are inhibited by higher acid levels. Problems with spoilage are likely at pH values approaching 4.0. Another reason for improved stability as the pH is lowered is the increased effectiveness of sulfur dioxide as an antimicrobial agent.

Grapes harvested in the eastern United States often have a high acid content, ranging from 0.5-1.6% (Beelman, 1973). During and after fermentation, the total acidity is reduced slightly. This decrease is due to the utilization of some of the acids by fermenting yeasts and precipitation of tartrates (during cold stabilization). In order to reduce excessive acidity, water (or sugar water) may be added to ameliorate the acids as long as the T.A. isn't reduced below 0.5% or the volume of water (or sugar water) added isn't greater than 35% of the resultant volume of the must. This amelioration process can reduce the quality of some wines by diluting their delicate bouquet, flavor, varietal character, body, and color.

Total acidity is determined by a direct titration procedure. The acids are titrated with a standardized sodium hydroxide (NaOH) solution to a phenolphthalein endpoint.

TOTAL ACIDITY

MATERIALS AND REAGENTS

2 beakers, 100 ml
Erlenmeyer flask, 250 ml, wide mouth
Erlenmeyer flask, 500 ml, wide mouth
Pipet, 1 ml, volumetric
Pipet, 5 ml, volumetric
Pipet, 10 ml, volumetric
Buret, 25 ml, Class A., calibrated in 1/10 ml divisions
Support, retangular base and clamp, buret
or, buret support
Stirrer, magnetic
Stirring bars
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Sodium hydroxide .1 N (N/10) -- measure 100 ml of 1 N sodium hydroxide in a 1 liter volumetric flask and bring to volume with distilled water or weigh 4.00g of sodium hydroxide pellets accurately, dissolve and bring to volume with distilled water in a 1 liter volumetric flask. Store solution in 500 ml reagent bottles.  
0.1 N sodium hydroxide may also be purchased.  
Sodium hydroxide .1 N (N/10) standardization -- after the .1 N sodium hydroxide has been prepared or used for several months, it should be checked to prove that the normality is correct. One method is to standardize the sodium hydroxide against a standard hydrochloric acid solution. The normality of the sodium hydroxide is determined by the following procedure:

1. Pipet 10 ml of .1 N hydrochloric acid and add approximately 50 ml water into 250 ml Erlenmeyer flask.
2. Add 3 drops of methyl red indicator.
3. Fill the 25 ml buret with sodium hydroxide solution.
4. Titrate the .1 N hydrochloric acid with the sodium hydroxide solution to a lemon yellow color.

The normality of the sodium hydroxide (NaOH) is calculated by using the following formula:

\[
\text{Normality of NaOH} = \frac{(V \times N)}{(V^*)}
\]

Where \( V \) = volume of hydrochloric acid, 10 ml.  
\( N \) = normality of hydrochloric acid, .1 N.  
\( V^* \) = volume of sodium hydroxide used for titration of hydrochloric acid, in ml.

Hydrochloric acid .1 N (N/10) -- This acid solution may be purchased.  
Methyl red indicator solution, .1% -- Weigh 1 g of methyl red powder, dissolve in 60 ml of 95% ethyl alcohol, and then add 40 ml of water. Store in 125 ml reagent bottle.  
Phenolphthalein indicator solution, 1% -- weigh 1 g of phenolphthalein powder, dissolve and bring to volume with 95% ethyl alcohol in a 100 ml volumetric flask. Store in 125 ml reagent bottle.

PROCEDURE

1. Place approx. 200 ml of boiling water in a 500 ml wide mouth Erlenmeyer flask.  
2. Add 1 ml of a 1% phenolphthalein indicator solution.  
3. Titrate the water with a 0.1 N NaOH solution to a faint but definite pink color.  
4. To the same flask add 5 ml of must or wine.  
5. Titrate the sample with a 0.1 N NaOH solution to a faint but definite pink color.
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NOTE

Red musts and wines will first turn green. Endpoint is a pinkish brown. Placing the flask in front of a white background or directing light through the solution may aid in the detection of color change.

CALCULATION

Since tartaric acid is the major acid in grapes, it is customary to express total acidity as percent tartaric acid.

\[
\text{Tartaric acid, g/100 ml} = \frac{(V)(N)(75)(100)}{(1000)(v)} = \% \text{ T.A.}
\]

Where \( V \) = volume of sodium hydroxide solution used for titration of sample, in ml.
\( N \) = normality of sodium hydroxide solution
\( v \) = sample volume, in ml.

This simplified formula may be used if the procedure outlined above is followed.

\[
\% \text{ T.A.} = (\text{ml of sodium hydroxide used}) \times (0.15)
\]

Sample: 8 ml of .1 N sodium hydroxide was used to titrate a 5 ml sample of wine. What is the T.A. of the wine?

\[
\% \text{ T.A.} = (8), (0.15) = 1.2\%
\]
PH

MATERIALS

Buffer solutions, pH 4 and pH 7
4 beakers, 250 ml
pH meter
Bottle, washing

USE OF A pH METER

Always keep electrodes immersed in liquid. Failure to do so may result in irreversible damage.

Everyday before use, pH meter should be calibrated with two buffer solutions, one at pH 4 and one at pH 7.

Refer to manual accompanying pH meter for operating instructions.

REFERENCES


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ETHANOL

Most table wines have an alcohol content of 10-14% (volume %). A wine with a low alcohol content, say less than 10%, may have a "thin" character and will be more subject to microbial spoilage than wine with a greater alcohol content. Too much alcohol may give an otherwise delicately flavored wine a "hot" taste.

U.S. legal limits for ethanol are given in the following table:

<table>
<thead>
<tr>
<th>Wine Type</th>
<th>Ethanol, vol %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table wines (red rosé, white)</td>
<td>--- 14%</td>
</tr>
<tr>
<td>Dessert wines</td>
<td>18% 24%</td>
</tr>
<tr>
<td>Angelica, Madeira, Muscatel, Port</td>
<td>17% 24%</td>
</tr>
<tr>
<td>and Sherry</td>
<td></td>
</tr>
</tbody>
</table>

The ebulliometric method of alcohol determination is based on the boiling point of a mixture of ethanol and water. Ethanol lowers the boiling point of water. So, as the ethanol content becomes higher, the boiling point of an aqueous solution will be lower.

Since certain compounds, mainly sugars, influence the boiling point of a wine, the ebulliometer procedure is not usually used for sweet wines. For these type of wines, a distillation method is recommended, because it eliminates most of the interfering components. Although the method involves more time, its accuracy is such that it is often used in routine winery analysis. Both methods are described below.

EBULLIOSCOPIC METHOD

MATERIALS AND REAGENTS

Ebulliometer kit, includes:

- Ebulliometer
  - Special thermometer, calibrated from 90-100°C (0.1°) subdivisions
- Ebulliometer slide rule
- Alcohol lamp
- Graduated cylinder, 50 ml
- Graduated cylinder, 25 ml
- Denatured alcohol, for lamp

PROCEDURE

1. Rinse and drain boiling chamber (Fig. 1).
2. Fill condenser jacket with cold water.
3. Add 25 ml of water through the thermometer opening.
4. Insert the thermometer snugly into its opening.
5. Light the alcohol lamp and place it under the boiling chamber. Only a small flame is required.
6. Heat the water to boiling. As soon as a stationary temperature is reached, make a reading.
7. Set the observed temperature on the slide rule so that the boiling temperature of the water is opposite zero percent of alcohol. Tighten the set screw.
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8. Drain the condenser and boiling chamber.
9. Rinse out the boiling chamber with a small amount of the wine to be tested.
10. Fill condenser jacket with cold water.
11. Add 50 ml of wine through the thermometer opening.
12. Insert the thermometer and boil the sample. As soon as stationary temperature is reached, make a reading.
13. Locate the determined boiling point of the wine on the slide rule; the alcohol value opposite to it is the vol. % of ethanol of the wine sample (Fig. 1). Record this value.

NOTES

Change the water in the condenser jacket after each determination.

The boiling point of the water should be checked twice daily. Changes in atmospheric pressure will vary results.

Constant heat must be applied to the boiling chamber. Protect the instrument from drafts.

Handle the special thermometer with great care; it is fragile and expensive. Before using the instrument, check the thermometer for separation. If breaks in the mercury occur, try to remove them with gentle tapping or (slow) heating and cooling.

Occasionally, the boiling chamber should be washed out with a boiling 2% sodium hydroxide solution to rid it of scale which builds up with normal use. Be sure to remove all traces of sodium hydroxide by repeated and thorough rinsing.

For more accurate results, wines should be diluted before analysis to less than 5% ethanol and less than 2% sugar.

REFERENCES

Fig. 1 Ebulliometer with calculator
From Amerine and Ough, 1974
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DISTILLATION METHOD
( Hydrometer)

MATERIALS AND REAGENTS

Adapter, connecting, 24/40
Adapter, trap type bulb, 24/40
Boiling chips (or glass beads)
Condenser, Graham, 300 mm
Cylinder, graduated, 100 ml
Cylinder, hydrometer, 250 ml
Erlenmeyer flask, 250 ml
Flask, distillation (Kjeldahl), 800 ml
Flask, volumetric, 200 ml
Hot plate, electric
Hydrometer, alcohol, 10 to 15% by volume
Pipets, capillary, disposable
Stopcock grease
Support clamps and stands
Thermometer, Centigrade
Thermometer, Fahrenheit
Tubing, rubber, bore 6.4 mm, length 1.5 m
Wash bottle

PROCEDURE

1. Adjust temperature of wine sample to 20°C by placing bottle into an ice bath.

2. Transfer 200 ml of this sample to a 200 ml volumetric flask and check temperature (20°C). If temperature is not correct, adjust temperature to 20°C and bring volume to 200 ml with wine.

3. From the volumetric flask, pour wine into the distillation flask.

4. Rinse the 200 ml volumetric flask 3 times with a total of 50 ml of distilled water and transfer to distillation flask.

5. Add a few boiling chips or glass beads to the distillation flask.

6. Assemble the distillation equipment as shown in Figure 2.

7. Turn on condenser water. Water should enter condenser from bottom.

8. Apply heat and collect between 180 and 190 ml of distillate into a 250 ml Erlenmeyer flask.

9. With a few ml of distilled water, rinse tip of condenser into the Erlenmeyer flask.

10. Transfer distillate to the 200 ml volumetric flask.

11. Rinse the Erlenmeyer flask with a few ml of distilled water, and transfer to the 200 ml volumetric flask.

12. Adjust temperature to 20°C by placing volumetric flask into an ice bath and bring volume to 200 ml with distilled water.
PROCEDURE (cont.)

13. Pour distillate into a clean 250 ml hydrometer cylinder.

14. Float a special alcohol hydrometer (10 to 15% by volume) in the sample. When it is stationary, make a reading at the bottom of the meniscus.

15. Take a temperature reading of the sample, record it and make the necessary correction by referring to Table 2.

NOTE: For wines that contain a high volatile acidity (exceeding 0.100% expressed as g of acetic acid per 100 ml wine), neutralize the wine with 2N sodium hydroxide (NaOH) before distillation. The volume of 2N NaOH is calculated by using the following formula:

\[
\text{Volume of 2N NaOH, in ml} = \frac{(v)}{20} \times 40
\]

Where \( v \) = volume of 0.1 N NaOH (see section "Total Acidity and pH" in this manual), in ml.

REFERENCE

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<th>at 66°F</th>
<th>at 67°F</th>
<th>at 68°F</th>
<th>at 69°F</th>
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<th>at 72°F</th>
<th>at 74°F</th>
<th>at 76°F</th>
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</thead>
<tbody>
<tr>
<td>%</td>
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<td>%</td>
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<tr>
<td>1</td>
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<td>0.41</td>
<td>0.48</td>
<td>0.55</td>
<td>0.62</td>
<td>0.77</td>
<td>0.93</td>
<td>--</td>
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<tr>
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<td>0.42</td>
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<td>0.78</td>
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<td>0.96</td>
<td>1.10</td>
<td>1.25</td>
<td>1.40</td>
<td>1.70</td>
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<tr>
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<td>1.03</td>
<td>1.18</td>
<td>1.33</td>
<td>1.49</td>
<td>1.80</td>
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<td>1.26</td>
<td>1.42</td>
<td>1.58</td>
<td>1.90</td>
<td>2.22</td>
<td>2.54</td>
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<td>20</td>
<td>0.98</td>
<td>1.15</td>
<td>1.33</td>
<td>1.48</td>
<td>1.65</td>
<td>2.00</td>
<td>2.32</td>
<td>2.65</td>
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<td>21</td>
<td>1.02</td>
<td>1.20</td>
<td>1.38</td>
<td>1.54</td>
<td>1.72</td>
<td>2.06</td>
<td>2.41</td>
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<td>1.25</td>
<td>1.44</td>
<td>1.61</td>
<td>1.78</td>
<td>2.13</td>
<td>2.48</td>
<td>2.84</td>
</tr>
<tr>
<td>23</td>
<td>1.11</td>
<td>1.30</td>
<td>1.49</td>
<td>1.66</td>
<td>1.84</td>
<td>2.20</td>
<td>2.56</td>
<td>2.93</td>
</tr>
<tr>
<td>24</td>
<td>1.16</td>
<td>1.35</td>
<td>1.54</td>
<td>1.72</td>
<td>1.91</td>
<td>2.27</td>
<td>2.65</td>
<td>3.03</td>
</tr>
</tbody>
</table>
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FIG. 2 Distillation assembly for ethanol determination
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TOTAL AND FREE SULFUR DIOXIDE

Sulfur dioxide serves three major functions in winemaking: (1) control of undesirable microorganisms, (2) denaturation of browning enzymes, and (3) antioxidant. Usually 75-150 ppm (mg/liter) of SO₂ are added as soon as possible after crushing to assure proper control of fermentation.

The effectiveness of SO₂ is limited by its volatilization, its oxidation to sulfate, and its combination with various compounds to form what is referred to as bound sulfur dioxide. Uncombined SO₂ is termed free sulfur dioxide. In the bound state SO₂ is less effective as preservative, and in the oxidized form as sulfate, it is inert. After fermentation, periodically throughout the aging period, and prior to bottling, it is advisable to analyze for free and total SO₂. A free sulfur dioxide content of 20-30 ppm is usually sufficient to maintain the preservative effect.

If too much SO₂ is added to must, fermentation may be delayed and incomplete and the resulting wine will have a high bound SO₂ content. In wine, excessive amounts of SO₂ may bleach the color and cause an objectionable, pungent odor. The maximum amount of total sulfur dioxide permissible in wines in the United States is 350 ppm.

Several methods are used for testing free and total SO₂ in wines. The Ripper method is widely used by wineries for its simple and time-saving procedure. However, the end point of this method is rather difficult to detect in red wines. For this reason, an aeration-oxidation method is the preferred test for red wines, because it has a clear end point and better accuracy for red wines. Both methods are described below for determining free and total sulfur dioxide in wines.

TOTAL SULFUR DIOXIDE
(Ripper Method)

MATERIALS AND REAGENTS

2 beakers, 100 ml
Erlenmeyer Flask, 250 ml, wide mouth
Pipet, 5 ml, volumetric
2 pipets, 10 ml, volumetric
Pipet, 25 ml, volumetric
Pipet, 20 ml, volumetric
Stopper for Erlenmeyer flask
Buret, 25 ml, Class A, calibrated in 1/10 ml divisions
Support, rectangular base and clamp, buret or buret support
Stirrer, magnetic
Stirring bars
Sodium bicarbonate
Sodium hydroxide 1 N — weigh 40 g sodium hydroxide pellets accurately, dissolve and bring to volume with distilled water in a 1 liter volumetric flask. Store in 500 ml reagent bottles. This solution may also be purchased.
Starch indicator solution, 1% — make a paste of 10 g soluble starch and a small amount of distilled water. Add the starch paste to 1 liter of boiling water. Cool and add 1 ml carbon tetrachloride as a preservative. Store in 500 ml reagent bottles.
MATERIALS AND REAGENTS (cont.)

Sulfuric acid solution, 1+3 -- carefully add 200 ml concentrated sulfuric acid to 600 ml distilled water. Store in 500 ml reagent bottles.

Iodine solution, .02 N -- measure 200 ml of .1 N iodine stock solution into a 1 liter volumetric flask. Bring to volume with distilled water. Store in 500 ml reagent bottles.

Iodine solution, .02 N standardization -- since the normality of iodine solutions change rapidly, it is necessary to restandardize the solution frequently. The normality of the iodine solution may be checked by the following procedure:

1. Pipet 10 ml of the iodine solution and add approximately 50 ml water into 250 ml Erlenmeyer flask.
2. Add 5 ml of starch indicator.
3. Fill the 25 ml buret with a standardized sodium thiosulfate solution (.02N).
4. Titrate the iodine solution with the sodium thiosulfate solution until the blue color disappears.

The normality of the iodine solution is calculated by using the following formula.

Normality of iodine = \( \frac{(V)}{(N)} \) \( \frac{(V^*)}{(V^*)} \)

Where:
- \( V \) = volume of sodium thiosulfate used for titration of iodine solution, in ml.
- \( N \) = normality of sodium thiosulfate, 0.2 N.
- \( V^* \) = volume of iodine solution, 10 ml.

Sodium thiosulfate, .02 N -- this standard solution may be purchased.

PROCEDURE

1. Pipet 20 ml of wine into 250 ml Erlenmeyer flask
2. Add 25 ml of 1 N NaOH solution
3. Mix, stopper the flask, and let stand for 10 minutes
4. Add a pinch of sodium bicarbonate to expel air
5. Add 5 ml of starch indicator solution
6. Add 10 ml of 1+3 sulfuric acid solution
7. Titrate rapidly with 0.02 N I\(_2\) soln. to a bluish darkening of the solution which persists for 30 seconds
8. Record the volume of iodine solution used in the titration
9. Calculate the concentration of total SO\(_2\) in the wine

CALCULATION

Total SO\(_2\), mg/liter or ppm = \( \frac{(V)}{(N)} \) \( \frac{(32)}{(1000)} \) \( \frac{(v)}{v} \)

Where:
- \( V \) = volume of iodine solution used for titration, in ml.
- \( N \) = normality of iodine solution
- \( v \) = volume of sample, in ml
CALCULATION (cont.)

This simplified formula may be used if the procedure outlined above is followed.

\[ \text{ppm Total SO}_2 = (\text{ml iodine solution used}) (32) \]

Sample: 4.7 ml of .02 N iodine solution was used to titrate a 20 ml sample of wine. What is the SO\(_2\)?

\[ \text{ppm total SO}_2 = (4.7) (32) = 150.4 \text{ ppm} \]

FREE SULFUR DIOXIDE
(Ripper Method)

MATERIALS AND REAGENTS

- 2 beakers, 100 ml
- Erlenmeyer flask, 250 ml, wide mouth
- 2 pipets, 5 ml, volumetric
- Pipet, 50 ml, volumetric
- Buret, 10 ml, Class A, calibrated in 1/20 ml divisions
- Support, rectangular base and clamp, buret or buret support
- Stirrer, magnetic
- Stirring bars
- Starch indicator solution, 1% -- see page 15
- Sulfuric acid solution, 1+3 -- see page 16
- Iodine solution, .02 N -- see page 16
- Sodium bicarbonate

PROCEDURE

1. Pipet 50 ml of wine into a 250 ml Erlenmeyer flask
2. Add 5 ml of starch indicator solution
3. Add 5 ml of 1+3 sulfuric acid solution
4. Add a pinch of sodium bicarbonate to expel air
5. Titrate rapidly with 0.02 N I\(_2\) solution to a faint blue which persists for 1-2 minutes
6. Record the volume of iodine solution used in the titration
7. Calculate the concentration of free sulfur dioxide in the wine

NOTES

The temperature of the solution being titrated should not exceed 20°C (68°F).

When testing red wines, the end point might be more clearly distinguished by passing (yellow) light from some direct source through the solution. End-point will be a bluish-purple.
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**CALCULATION**

Free $\text{SO}_2$ mg/liter or ppm = \( \frac{(V) (N) (32) (1000)}{(v)} \)

Where $V$ = volume of iodine solution used in titration, in ml  
$N$ = normality of iodine solution  
$v$ = volume of sample, in ml  

This simplified formula may be used if the procedure outlined above is followed.

ppm Free $\text{SO}_2$ = (ml iodine solution used) (12.8)

Sample: 3.4 ml of .02 N iodine was used to titrate a 50 ml sample of wine. What is the free $\text{SO}_2$ content of the wine?

ppm Free $\text{SO}_2$ = (3.4) (12.8) = 43.5 ppm.

**REFERENCES**


FREE SULFUR DIOXIDE
(Aeration-Oxidation Method)

MATERIALS AND REAGENTS

Semi-micro apparatus for sulfur dioxide determination (Fig. 2)
2 pipets, 10 ml, volumetric
Pipet, 20 ml, volumetric
Flask, 200 ml, volumetric
Microburner
Buret, 10 ml, calibrated in 1/20 ml divisions
Support, rectangular base, and clamps, buret support
Aspirator
Metering tube for air flow, 1.0-1.5 liter/min.
Gas washing bottle, 250 ml
Tygon tubing, bore 6.4 mm, wall thickness 1.6 mm, length 3.7 m
Rubber tubing, bore 7.9 mm, wall thickness 2.4 mm, and length 3.7 m
Rubber tubing, bore 4.8 mm, wall thickness 1.6 mm, and length 3.7 m
Hydrogen peroxide solution, 0.3% -- dilute 1 ml of a 30% hydrogen peroxide solution to 100 ml with distilled water.
Indicator -- dissolve 0.10 g methyl red and 0.05 g methylene blue in 100 ml of 50% ethyl alcohol
Phosphoric acid solution, 25% -- take 296 ml of 85% phosphoric acid and make to 1 liter with distilled water
Sodium hydroxide .1 N (N/10) -- measure 100 ml of 1 N sodium hydroxide in a 1 liter volumetric flask and bring to volume with distilled water or weigh 4.00 g of sodium hydroxide pellets accurately, dissolve and bring to volume with distilled water in a 1 liter volumetric flask. Store solution in 500 ml reagent bottles. .1 N sodium hydroxide may also be purchased.
Sodium hydroxide .1 N (N/10) standardization -- after the .1 N sodium hydroxide has been prepared or used for several months, it should be checked to prove that the normality is correct. One method is to standardize the sodium hydroxide against a standard hydrochloric acid solution. The normality of the sodium hydroxide is determined by the following procedure:

1. Pipet 10 ml of .1 N hydrochloric acid and add approximately 50 ml water into 250 ml Erlenmeyer flask.
2. Add 3 drops of methyl red indicator.
3. Fill the 25 ml buret with sodium hydroxide solution.
4. Titrate the .1 N hydrochloric acid with the sodium hydroxide solution to a lemon yellow color.

The normality of the sodium hydroxide (NaOH) is calculated by using the following formula:

\[
\text{Normality of NaOH} = \frac{(V)(N)}{(V^*)}
\]
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MATERIALS AND REAGENTS (cont.)

Where $V =$ volume of hydrochloric acid, 10 ml
$N =$ normality of hydrochloric acid, .1 N
$V^* =$ volume of sodium hydroxide used for
titration of hydrochloric acid, in ml

Sodium hydroxide $0.01 \text{N} (\text{N/100})$ -- measure 20 ml of 1 N sodium hydroxide
in a 200 ml volumetric flask and bring to volume with distilled water.
This volume is enough for approximately 16 analyses.

PROCEDURE

1. Pipet 10 ml of 0.3% hydrogen peroxide (trapping solution) into the 50 ml pear-shaped flask (Fig. 3, H).
2. Add 3 drops of indicator to turn the trapping solution purple.
3. Adjust the purple color to turquoise green with 0.01 N NaOH and
   connect the flask to the vacuum adapter (Fig. 3, G).
4. Pipet 20 ml wine and 10 ml 25% phosphoric acid into the 50 ml round bottom flask (Fig. 3, D) and connect to the Claisen adapter (Fig. 3 C).
5. Submerge the round bottom flask in an ice-water bath.
6. Turn on the aspirator to draw scrubbed air through the system
   for 10 min at a flow rate of 1.0-1.5 liter/min.
7. At the end of 10 min remove the pear-shaped flask and rinse
   the vacuum adapter tube into the flask.
8. Titrate the contents in the flask to the initial turquoise green
   with 0.01 N NaOH.
9. Record the volume of 0.01 N NaOH used in the titration.
10. Calculate the concentration of free $SO_2$ in the wine.

CALCULATION

Free $SO_2$, ml/liter or ppm = \( \frac{(V)(N)(32)(1000)}{v} \)

Where $V =$ volume of NaOH used for titration, in ml
$N =$ normality of NaOH
$v =$ volume of wine sample, in ml

For a 20-ml sample size and the suggested normality (0.01 N)
of NaOH, simply multiple the "$V$" by 16 to give the concentra-
tion of free $SO_2$:

\[ \text{ppm Free } SO_2 = (\text{ml sodium hydroxide}) \times 16 \]
Fig. 3  Semi-micro apparatus for $\text{SO}_2$ determination by aeration-oxidation

A) Pipette, Pasteur, sealed with O' ring
B) Adapter, Bushing, 14/20 -10/18
C) Adapter, Claisen, 14/20
D) Flask, Round bottom, 14/20
E) Condenser, Liebig, 14/20, 110 mm
F) Adapter, Connecting, 14/20
G) Adapter, Vacuum, 14/20
H) Flask, Pear shape, 14/20, 50 ml
I) Adapter, Bleed
TOTAL SULFUR DIOXIDE
(Aeration-Oxidation Method)

There are two methods to determine total SO\textsubscript{2} concentration: (a) use the original sample which has been used for free SO\textsubscript{2} determination; (b) use a new sample to determine the total SO\textsubscript{2}.

PROCEDURE

A. Original Sample:

1. After free SO\textsubscript{2} concentration has been determined, save the contents in the round bottom flask but discard the contents in the pear-shaped flask.
2. Pipet 10 ml of trapping solution (0.3% hydrogen peroxide), add 3 drops of indicator and adjust to turquoise green with 0.01 N NaOH.
3. Remove the ice-water bath, place a microburner flame under the flask and boil gently.
4. Turn on the condenser.
5. Turn on the aspirator to draw air through the system for 15 min at a flow rate of 1.0-1.5 liter/min.
6. At the end of 15 min, proceed as for free SO\textsubscript{2} (steps 7 through 9).
7. Calculate the concentration of bound SO\textsubscript{2} in the wine.

CALCULATION

\[
\text{Bound SO}_2 \text{ mg/liter or ppm} = \frac{(V) (N) (32) (1000)}{v}
\]

Where \( V \) = volume of NaOH used for titration, in ml
\( N \) = normality of NaOH
\( v \) = volume of wine sample, in ml

Sum of the concentrations of free and bound SO\textsubscript{2} equals the total SO\textsubscript{2} concentration in the wine.

Total SO\textsubscript{2} mg/liter or ppm = Free SO\textsubscript{2} + Bound SO\textsubscript{2}

B. New Sample:

1. Follow the steps in A except that the original sample is discarded from the round bottom flask and a new sample and 10 ml of 25% phosphoric acid are added.
2. Record the volume, in ml, of 0.01 N NaOH required for titration and calculate the total SO\textsubscript{2} concentration.

CALCULATION

\[
\text{Total SO}_2 \text{ mg/liter or ppm} = \frac{(V) (N) (32) (1000)}{v}
\]

Where \( V \) = volume of NaOH used for titration, in ml
\( N \) = normality of NaOH
\( v \) = volume of wine sample, in ml
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REFERENCES


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SULFUR DIOXIDE TREATMENT

I. TESTING WINE SAMPLES

The free sulfur dioxide content of a wine should be kept within 20 to 30 ppm. This range is usually sufficient to inhibit oxidation and growth of microorganisms.

To obtain a given level of free sulfur dioxide by calculation is rather difficult, for the ratio of free to total varies widely from one wine to another. The common method is to add various levels of sulfur dioxide to a series of wine samples and determine their free sulfur dioxide content. From these readings, one can determine the correct amount of sulfur dioxide necessary to produce the required free sulfur dioxide in a particular wine.

MATERIALS AND REAGENTS

4 beakers, 600 ml
5 wine bottles with caps, tenths
Cylinder, graduated, 500 ml
Pipet, 10 ml, volumetric
Pipet, 20 ml, volumetric
Pipet, 50 ml, volumetric
Flask, 250 ml, volumetric, Class A
Balance
Potassium metabisulfite, food grade, powder
Sulfur dioxide solution -- weigh 1.70 g potassium metabisulfite powder accurately, dissolve and bring to volume with distilled water in a 250 ml volumetric flask.

PROCEDURE

1. Place 400 ml of wine into each of 4, 600 ml beakers.
2. Add various amounts of the standard sulfur dioxide solution, so the wines will contain an additional 20, 40, 60, and 80 ppm sulfur dioxide.
3. These amounts added to 400 ml are as follows:

<table>
<thead>
<tr>
<th>Ml</th>
<th>g of St'd SO₂</th>
<th>g of K₂S₂O₅ added</th>
<th>g of SO₂ added</th>
<th>ppm SO₂ added</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.014</td>
<td>0.008</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>4.0</td>
<td>0.028</td>
<td>0.016</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>6.0</td>
<td>0.042</td>
<td>0.024</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>8.0</td>
<td>0.056</td>
<td>0.032</td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

4. Stir and transfer to tenth bottles, label.
5. Also, fill one bottle with the original wine, label 0 ppm SO₂.
6. Seal all wines and let stand for 5 days.
7. Analyze each wine for its free sulfur dioxide content, pp. 17.
8. Record free sulfur dioxide contents.
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Free Sulfur Dioxide Found (ppm) versus SO₂ added (ppm)

<table>
<thead>
<tr>
<th>SO₂ added (ppm)</th>
<th>Free Sulfur Dioxide Found (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>D</td>
</tr>
</tbody>
</table>

9. Plot the ppm of sulfur dioxide added versus the ppm of free sulfur dioxide found.
This page intentionally blank.
10. Draw a line between the points.
11. Determine the ppm of sulfur dioxide necessary to produce 30 ppm free sulfur dioxide in the wine.

<table>
<thead>
<tr>
<th>Wine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PPM of Sulfur Dioxide necessary to produce 30 ppm free SO₂</th>
</tr>
</thead>
</table>
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SULFUR DIOXIDE TREATMENT

II. TREATING BULK WINES

The first section of this procedure "I. Testing Wine Samples" outlined the steps in determining the ppm of sulfur dioxide necessary to produce 30 ppm free sulfur dioxide in a given wine. The next step is to determine the exact weight of sulfur dioxide to add in order to obtain the desired concentration of free sulfur dioxide (30 ppm). Since most small wineries use potassium metabisulfite, this section will cover the addition of sulfur dioxide in the form of potassium metabisulfite.

WEIGHTS, MEASURES, AND CONVERSIONS

1 lb. = 16 ozs. = 454 gms.
1 oz. = 28.4 gms.
1 gal. of wine = 8.2 lbs. = 131 ozs. = 3723 gms.
1 gal. of wine = 3.79 liters = 3790 mls.

<table>
<thead>
<tr>
<th>PPM</th>
<th>%</th>
<th>MULTIPLICATION FACTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000,000</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>100,000</td>
<td>10</td>
<td>.1</td>
</tr>
<tr>
<td>10,000</td>
<td>1</td>
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<td>1,000</td>
<td>.1</td>
<td>.001</td>
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<td>.01</td>
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<tr>
<td>10</td>
<td>.001</td>
<td>.00001</td>
</tr>
<tr>
<td>1</td>
<td>.0001</td>
<td>.000001</td>
</tr>
</tbody>
</table>

PERCENT SO₂ IN POTASSIUM METABISULFITE

Chemical Reaction:

\[ \text{K}_2\text{S}_2\text{O}_5 + \text{H}_2\text{O} \rightarrow 2\text{KOH} + 2\text{SO}_2 \]

Atomic wt. of K = 40
Atomic wt. of S = 32
Atomic wt. of O = 16

Molecular wt. of \( \text{K}_2\text{S}_2\text{O}_5 \) = 222
Molecular wt. of \( \text{SO}_2 \) = 64 × 2 = 128

\[ \% \text{SO}_2 \text{ in } \text{K}_2\text{S}_2\text{O}_5 = \frac{128 \times 100}{222} = 58\% \]

Therefore: 1 gm of \( \text{K}_2\text{S}_2\text{O}_5 \) = 0.58 gm of \( \text{SO}_2 \)
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OR: Converting grams of SO₂ to grams of K₂S₂O₅, one can multiply the weight of SO₂ by the factor 1.72

\[
\frac{1}{0.58} = \frac{\text{wt. of K}_2\text{S}_2\text{O}_5 (\text{unknown})}{\text{wt. of SO}_2}
\]

\[
1.72 = \frac{\text{wt. of K}_2\text{S}_2\text{O}_5}{\text{wt. of SO}_2}
\]

wt. of K₂S₂O₅ = 1.72 x wt. of SO₂

PROCEDURE

1. Determine the ppm of sulfur dioxide necessary to produce 30 ppm free sulfur dioxide in the bulk wine (see section I. "Testing Wine Samples").
2. Record ppm sulfur dioxide to be added.
3. Estimate the weight of the bulk wine by recording the volume and then referring to Table 3.
   Example: 50 gallons = 410 lbs. = 1164 ozs. = 186,140 gms.
4. Record the weight of the bulk wine.
5. Calculate the weight of sulfur dioxide to be added to the bulk wine in order to obtain 30 ppm.

   Example: \( Y \times Z = \text{weight of SO}_2 \)

   Where \( Y = \text{weight of bulk wine} \)
   \( Z = \text{ppm of SO}_2 \text{ to be added.} \)

   Sample A: 45 ppm of SO₂ was added to 50 gallons of wine to obtain 30 ppm of free SO₂.

   \[
   Y = 50 \text{ gallons} = 186,140 \text{ gms}
   \]
   \[
   Z = 45 \text{ ppm} \text{ (Multiplication Factor = .000045, see table under "Weights, Measures and Conversions")}
   \]

   Weight for SO₂ = 186,140 x .000045 = 8.4 gms.

   Sample B: 25 ppm of SO₂ was added to 1000 gallons of wine to obtain 30 ppm of free SO₂.

   \[
   Y = 233,000 \text{ ozs. or 3,722,800 gms.}
   \]
   \[
   Z = 25 \text{ ppm} \text{ (Multiplication Factor = .000025)}
   \]

   Weight of SO₂ = 233,000 ozs. x .000025 = 5.8 ozs.

6. Express the weight of sulfur dioxide in the form of potassium metabisulfite. See section under "Percent SO₂ IN POTASSIUM METABISULFITE".

   Weight of K₂S₂O₅ = 1.72 x weight of SO₂

   Sample A:

   Weight of K₂S₂O₅ = 1.72 x 8.4 gms. = 14.4 gms.
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Sample B:

Weight of $K_2S_2O_5 = 1.72 \times 5.8$ ozs. = 10.0 ozs.

**TABLE 3. Weight of Several Volumes of Wine.**

<table>
<thead>
<tr>
<th>Volume (gallons)</th>
<th>Weight of Several Volumes of Wine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lbs.</td>
</tr>
<tr>
<td>1</td>
<td>8.2</td>
</tr>
<tr>
<td>50</td>
<td>410</td>
</tr>
<tr>
<td>1000</td>
<td>8,200</td>
</tr>
</tbody>
</table>
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VOLATILE ACIDITY

The volatile acid fraction of wine consists mainly of acetic acid. During normal fermentation, usually less than 0.03 grams of acetic acid per 100 ml of wine are produced. A spoiled, vinegary odor becomes apparent at about 0.07-0.10% of acetic acid. This sharp odor is due to both acetic acid and ethyl acetate which are produced simultaneously by spoilage organisms.

Volatile acidity (V.A.) is a good indicator of the soundness of a wine. A high V.A. in a young wine indicates that it may be contaminated with spoilage bacteria. These bacteria can convert wine to wine vinegar in a short time. A slight increase in V.A. usually occurs in older wines due to the natural aging process.

Federal law limits the maximum volatile acidity in wines. Values in the following table are expressed as grams of acetic acid per 100 ml of wine and are exclusive of sulfur dioxide:

<table>
<thead>
<tr>
<th>Wine Type</th>
<th>Maximum V.A. permitted in U.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Table</td>
<td>0.14</td>
</tr>
<tr>
<td>White Table</td>
<td>0.12</td>
</tr>
<tr>
<td>All Others</td>
<td>0.12</td>
</tr>
</tbody>
</table>

The volatile acidity is determined by passing a current of steam through the wine sample then collecting the distillate. The distillate is titrated with a standard sodium hydroxide solution to a phenolphthalein endpoint.

MATERIALS AND REAGENTS

2 beakers, 100 ml
Cash volatile acid assembly
Erlenmeyer flask, 250 ml, wide mouth
Pipet, 10 ml, volumetric
Pipet, 1 ml, volumetric
Buret, 10 ml, Class A., calibrated in 1/20 ml divisions
Support, retangular base and clamp, buret
or buret support
Stirrer, magnetic
Stirring bars
Hot Plate
Bottle, washing
Sodium hydorxide, .1 N -- see page 5.
Phenolphthalein indicator solution, 1% -- see page 5.

For sulfur dioxide correction:

Pipet, 1 ml, volumetric
Buret, 10 ml, Class A., calibrated in 1/20 ml divisions
Support, retangular base and clamp, buret
or buret support
Iodine, .02 N -- see page 16
Sulfuric acid, 1+3 -- see page 16
Starch indicator solution -- see page 15.
Potassium iodide crystals
PROCEDURE

1. Turn on condenser water. Water should enter condenser from bottom.
2. Add enough water through the water inlet to cover the heating coil (Fig. 4) in the boiler.
3. Turn on the heating coil.
4. Turn the two-way stopcock into the Sellier tube (open wine inlet) and pipette 10 ml of wine.
5. With a small amount of distilled water wash the wine into the Sellier tube.
6. Turn the two-way stopcock into the boiler (open water inlet).
7. Place a 250 ml Erlenmeyer flask under the condenser outlet. The flask should be marked (with a wax pencil) at 100 ml.
8. When steam issues from the water inlet, close the two-way stopcock (both water and wine inlets closed).
9. Collect 100 ml of the distillate. Remove the flask.
10. Clean the apparatus by turning off the electric heating coil, opening the two-way stopcock into the Sellier tube (open wine inlet) and adding a small amount of distilled water. Open the discharge clamp on the bottom of the unit and allow the contents of the boiler to flush out. Add two or more small portions of distilled water into the Sellier tube to clean it completely during the flushing operation.
11. Place the flask containing the distillate over medium heat on the hot plate. Watch it carefully. Bring it to a gentle boil. Do not allow it to boil vigorously. Do not let it boil for more than 30 seconds. Remove the flask from the hot plate.
12. Add 3 drops of phenolphthalein indicator solution.
13. Titrate the distillate with .1 N NaOH solution to a faint but distinct pink color (see note T.A. pg. 5).

Wines with either a high sulfur dioxide content or a volatile acidity approaching the legal limit should be corrected for sulfur dioxide (sulfurous acid) using the following procedure:

1. Immediately cool above distillate by holding the flask under running water.
2. Add 1 ml 1+3 sulfuric acid solution.
3. Add 2-3 ml freshly prepared starch solution.
4. Add a crystal of potassium iodide.
5. Rapidly titrate with .02 N iodine solution to a faint blue color which persists for 30 seconds.

CALCULATIONS

\[
\% \text{ V.A.} = \text{acetic acid, g/100 ml} = \frac{V(N)(60)(100)}{(1000)(v)}
\]

Where \(V\) = volume of sodium hydroxide used for titration, in ml
\(N\) = normality of sodium hydroxide solution
\(v\) = sample volume, in ml

This simplified formula may be used if the procedure outlined above is followed.

\[
\% \text{ V.A.} = \frac{(\text{ml of sodium hydroxide used})}{(.06)}
\]
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For sulfur dioxide corrections:

\[
\text{% sulfur dioxide (hydrated) = acetic acid, g/100 ml = } \frac{(V^*)(N^*)(32)(100)(60)(2)}{(1000)(v^*)(64)}
\]

Where \( V^* \) = volume of iodine used for titration, in ml
\( N^* \) = normality of iodine solution
\( v^* \) = sample volume, in ml

This simplified formula may be used if the procedure outlined above is followed.

\[
\text{% sulfur dioxide (hydrated) = (ml of iodine solution used) (.012)}
\]

To obtain volatile acidity exclusive of sulfur dioxide, subtract the value of \% sulfur dioxide (hydrated) from the \% volatile acidity previously determined.

Sample: 10 ml of white wine was titrated with 1.6 ml of .1 N sodium hydroxide according to the procedure outlined above. The sample was then checked for sulfur dioxide. .2 ml of .02 N iodine solution was used for the second titration. Does this wine meet the legal limit for volatile acidity?

\[
\begin{align*}
\text{% V.A.} & = (1.6) (.06) = .096\% \\
\text{% SO}_2 \text{ (hydrated)} & = (.2) (.012) = .0024\% \\
\text{% V.A. exclusive of SO}_2 & = .096\% - .0024\% = .0936\% \\
\end{align*}
\]

No, volatile acidity does not exceed the legal limit.

REFERENCES


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Fig. 4. Cash Still
From Amer. Soc. of Enol., 1972.
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Carbon dioxide is a major product of alcoholic fermentation. Although most of the carbon dioxide is lost during the winemaking process, a small amount remains in the wine even after bottling. For example, the carbon dioxide content in still wines range between 0.01 to 0.05 g/100 ml.

Since the quantity of carbon dioxide influences the quality of wines, the analysis of carbon dioxide is important to the winemaker. At levels between 0.06 and 0.10 g/100 ml, carbon dioxide may increase the freshness of white wines. However, above 0.10 g/100 ml, carbon dioxide may reduce the wine quality by producing a noticeable effervescence. On the other hand, sparkling wines require carbon dioxide for the display of bubbles and distinctive taste. Also, the law requires that wines containing at least a certain amount of carbon dioxide be classified as sparkling wines. These are taxed higher than other wine types, such as still wines.

Although there are several methods used to analyze for carbon dioxide in wines, the enzymatic method was selected because it seemed to be less tedious and time-consuming.

MATERIALS AND REAGENTS

Aspirator
Balance
4 Beakers, 125 ml
Bottle, reagent, 125 ml
Bottle, reagent, 1000 ml
Buret, 10 ml, Class A, calibrated in 1/20 divisions
Buret, 25 ml, Class A, calibrated in 1/10 divisions
Cylinder, graduated, 100 ml
Erlenmeyer filtering flask, 250 ml
Erlenmeyer flask, 25 ml
2 Erlenmeyer flasks, 250 ml
Flask, 1000 ml, volumetric, Class A
pH meter
Pipet, 5 ml, volumetric
2 pipet, 10 ml, volumetric
Pipet filler, automatic
2 pipets, Pasteur
Pipet, serological, Style I, 2 ml
Pipet, serological, Style I, 10 ml
2 Rubber bulbs for Pasteur pipets
Stirring bars
Stirrer, magnetic
2 stoppers, neoprene, No. 6
Tygon tubing, bore 6.4 mm, wall thickness 3.2 mm, length 2.0 m
Buffer solutions, pH 4 and pH 7
Carbonic anhydrase solution, 0.1% (w/v)--weigh 5 mg (0.005 g) of carbonic anhydrase and transfer to a 25 ml Erlenmeyer flask. Pipet 5 ml distilled water, dissolve and store in refrigerator.
Methyl red indicator solution, .1%--weigh 1 g of methyl red powder, dissolve in 60 ml of 95% ethyl alcohol, and then add 40 ml of distilled water. Store in 125 ml reagent bottle.
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MATERIALS AND REAGENTS (cont.)

Sodium hydroxide, .1 N (N/10) -- this solution may be purchased, or prepared on page 5.

Sodium hydroxide, 50% (w/w) -- transfer 200 g of reagent grade sodium hydroxide pellets to a 250 ml Erlenmeyer flask. To this flask, add 200 ml distilled water which was recently boiled and cooled to room temperature. Stir with glass rod until dissolved and set aside for at least 5 days. Pour clear solution into a clean flask and cap with rubber stopper (CAUTION: this solution causes severe burns, handle with care).

Sulfuric acid, 0.068 N -- transfer 1.9 ml of concentrated sulfuric acid (95%) by using an automatic pipet filler with a 2 ml serological pipet to a 1000 ml volumetric flask. Before adding the sulfuric acid, place approximately 200 ml distilled water into the flask. Mix and bring to volume with distilled water. Store solution in 500 ml reagent bottle.

Sulfuric acid, 0.068 N standardization -- after the 0.068 N sulfuric acid has been prepared or used for several months, it should be checked to prove that the normality is correct. One method is to standardize the sulfuric acid against a standard sodium hydroxide solution. The normality of sulfuric acid is determined by the following procedure:

1. Pipet 10 ml of .1 N sodium hydroxide and add approximately 50 ml water into 250 ml Erlenmeyer flask.
   NOTE: 0.1 N sodium hydroxide may be purchased, or prepared on page 5.
2. Add 3 drops of methyl red indicator.
3. Fill a 25 ml buret with sulfuric acid solution.
4. Titrate the .1 N sodium hydroxide with the sulfuric acid solution to a lemon yellow color.

The normality of the sulfuric acid (H₂SO₄) is calculated by using the following formula:

\[
\text{Normality of } H₂SO₄ = \frac{(v) (N)}{(V*)}
\]

Where 
\[v\] = volume of sodium hydroxide, 10 ml.
\[N\] = normality of sodium hydroxide, .1 N.
\[V*\] = volume of sulfuric acid used for titration of sodium hydroxide, in ml.

WINE SAMPLE PREPARATION

Wine sample -- first, a bottled wine sample (750 ml) should be cooled to 3°C by placing in a refrigerator. Without agitation, open the bottle and transfer carefully 7 ml of 50% sodium hydroxide to the bottle. Recap the bottle and invert several times to mix the contents.

"Blank" degassed wine sample -- with a similar wine sample (750 ml) at room temperature, transfer approximately 50 ml to a 250 ml Erlenmeyer filtering flask. Insert a stirring bar into the flask and place the flask on a mag-
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WINE SAMPLE PREPARATION (cont.)

netic stirrer. Then, cap the flask with a rubber stopper, and connect the sidearm of the flask to a vacuum source (27 to 28 inches of vacuum). Under this amount of vacuum, agitate the sample by stirring for 5 minutes.

PROCEDURE

Wine Sample

1. The preparation of the "Wine Sample" is described under the section "WINE SAMPLE PREPARATION".

2. Pipet 10 ml wine sample into a 125 ml beaker.

3. Add 40 ml distilled water and a magnetic stirring bar.

4. Place on a magnetic stirrer and immerse electrodes of a pH meter (calibrated previously at pH 4 and pH 7).

5. Adjust the pH between 10 and 11 by adding 50% (w/w) sodium hydroxide with a Pasteur pipet equipped with a rubber bulb.

6. Then, add 3 drops of carbonic anhydrase solution.

7. Fill a 10 ml buret with the standardized 0.0682 N sulfuric acid and titrate with constant stirring to pH 8.6.

8. Refill the buret with the standardized 0.0682 N sulfuric acid and titrate with constant stirring to pH 4.0.

9. Record the volume of sulfuric acid (0.0682 N) used to titrate from pH 8.6 to 4.0.

Blank (Degassed Wine Sample)

1. The preparation of the "Blank" is described under the section "MATERIALS AND REAGENTS".

2. Pipet 10 ml of the degassed wine sample (blank) into a 125 ml beaker.

3. Add 40 ml distilled water and a magnetic stirring bar.

4. Place on a magnetic stirrer and immerse electrodes of a pH meter (calibrated previously at pH 4 and pH 7).

5. Adjust the pH between 10 and 11 by adding 50% (w/w) sodium hydroxide with a Pasteur pipet equipped with a rubber bulb.

6. Then, add 3 drops of carbonic anhydrase solution.
Blank (Degassed wine sample)(cont.)

7. Fill a 10 ml buret with the standardized 0.0682 N sulfuric acid and titrate with constant stirring to pH 8.6.

8. Refill the buret with the standardized 0.0682 N sulfuric acid and titrate with constant stirring to pH 4.0.

9. Record the volume of sulfuric acid (0.0682 N) used to titrate from pH 8.6 to 4.0.

CALCULATION

\[
\text{Carbon dioxide, mg/100 ml} = \frac{(A-B) (N) (44) (100)}{V}
\]

Where

A = volume of sulfuric acid used for titration of wine sample, in ml.  
B = volume of sulfuric acid used for titration of the blank (degassed wine sample), in ml.  
N = normality of sulfuric acid  
V = sample volume, in ml

The following simplified formula may be used if N = 0.0682, and V = 10 ml.

\[
\text{Carbon dioxide, mg/100 ml} = (A-B) (30)
\]

Sample: 3.60 ml of 0.0682 N sulfuric acid was used to titrate a 10 ml wine sample. For the blank, 2.00 ml of 0.0682 N sulfuric acid was used to titrate a similar volume. What is the carbon dioxide concentration of the wine sample?

\[
\text{Carbon dioxide, mg/100 ml} = (3.60-2.00) (30) \\
= (1.60) (30) \\
= 48 \text{ mg/100 ml}
\]

NOTE: Since 7 ml of NaOH was added to the wine sample, a correction must be made by multiplying the calculated concentration by 1.01.

REFERENCES


Since the consumer usually demands a clear wine, the clarification of wines by fining has become a routine operation. Fining refers to a process in which various agents are added to the wine to promote clarification. The undesirable materials to be removed may range from large particles to colloidal substances. In addition to clarifying the wine, fining is used to aid in stabilizing the wine and to change the sensory properties of the wine. The following offers some general information regarding some of the more widely used fining agents.

Gelatin: Since gelatin is a protein, it is composed of several amino acids linked together by carboxyl and alpha amino groups (peptide bond). When gelatin is added to the wine, it appears that bonding occurs between the phenolic hydroxyls of the tannin and the peptide bond of the protein. This gelatin-tannin complex in the wine carries down other suspended particles upon settling. In addition to this clarification action, gelatin fining reduces the tannin content and color of the wines. In red wines the color is lightened and astringency reduced to a lower level. For white wines and some reds, which may be deficient in tannin, the best clarification is accomplished by adding tannin prior to the gelatin preparation. Tannin is usually added a few days in advance of gelatin application and at a level equal to that of gelatin. Generally, the amount of gelatin ranges between 1/8 to 1 pound per 1000 gallons for white wines and 1/4 to 2 pounds for red wines.

Bentonite: Most of the bentonite used by the wine industry is mined in Wyoming and has a tremendous ability to swell in the presence of water. Wyoming bentonite is the sodium form of bentonite, which is different from calcium bentonite mined in other locations. Sodium bentonite is available in the granular form, which is more easily mixed than is the powder. Bentonite in wine carries a strong negative charge and is attracted to the positively charged substances, such as protein. When neutralization occurs, flocculating particles are formed and upon settling clarifies the wine. In addition, bentonite fining has been used to stabilize wine against protein flocculation, copper cloudiness, and browning. One disadvantage of bentonite is that it produces large and loose lees which are difficult to remove. Generally, bentonite is added at the rate of 2 to 10 pounds per 1000 gallons of wine.

Sparkolloid: This material is a proprietary fining agent which is a mixture of polysaccharides and diatomaceous earth. Upon treating the wine, the constituents of Sparkolloid combined with normal ions of the wine to form a coagulum. When this coagulum settles out, it carries other finely divided particles which are responsible for wine cloudiness. This product is recommended for fining hard-to-clarify wines. Generally, Sparkolloid is added at the rate of 1 to 3 pounds per 1000 gallons of wine.

Since numerous factors influence the effectiveness of fining agents, no single material will satisfy all the conditions and wine types. Before using any fining material, it is important to test each agent in the laboratory. Also, it would be advisable to check with the United States IRS concerning their regulations for use of fining agents in wines.
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MATERIALS AND REAGENTS

6 beakers, 250 ml
Graduated cylinder, 100 ml and 250 ml
Gelatin solution, 2.4% -- sprinkle 2.4 g of gelatin into 100 ml of water with stirring. Allow the gelatin to soak for 15 minutes. Then stir and apply indirect heat to raise the temperature to 70°C to 85°C to dissolve the gelatin. Cool to room temperature and add to the wine samples.
Bentonite suspension, 2.4% -- sift 2.4 g of bentonite into 100 ml of water with stirring. Stir this mixture until smooth and free of clots. Then age the mixture for a few days and stir again before adding to the wine samples.
Sparkolloid suspension, 2.4% -- add 2.4 g of Sparkolloid to 100 ml of boiling water. Stir in the Sparkolloid and keep simmering for 30 minutes. Stir frequently and replace water lost by evaporation. Mix well and hold mixture hot until addition to the wine samples.
Tannin solution, 2.4% -- add 2.4 g of food grade tannic acid into 100 ml of water and dissolve with stirring.
Balance
Hot plate, electric
Stirrer, magnetic
Stirring bars
Pipets, regular serological, Style I, 1 ml and 10 ml
Thermometer, centigrade
Bottles, clear glass with caps, approximately 200 ml.

PROCEDURE

1. Place 200 ml of wine into each of 5, 250 ml beakers.
2. Select a fining agent (gelatin, bentonite, or Sparkolloid) which you want to evaluate.

See "NOTES" for gelatin fining of white and some red wines.

3. Each fining agent was prepared as a 2.4% suspension (see MATERIALS AND REAGENTS).
4. When various amounts of the 2.4% fining agent suspension or solution are added to 200 ml of wine, the resulting concentration is as follows:

<table>
<thead>
<tr>
<th>Volume of 2.4% Suspension in 200 ml wine</th>
<th>Pounds per 1000 gallons</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 ml</td>
<td>equals 1/4</td>
</tr>
<tr>
<td>0.50 ml</td>
<td>equals 1/2</td>
</tr>
<tr>
<td>1.00 ml</td>
<td>equals 1</td>
</tr>
<tr>
<td>2.00 ml</td>
<td>equals 2</td>
</tr>
<tr>
<td>5.00 ml</td>
<td>equals 5</td>
</tr>
<tr>
<td>10.00 ml</td>
<td>equals 10</td>
</tr>
</tbody>
</table>
This page intentionally blank.
5. Pipet various amounts of the 2.4% fining agent suspension or solution into the 200 ml of wine samples with stirring so the wines will contain the following levels:

<table>
<thead>
<tr>
<th>Pounds per 1000 Gallons</th>
<th>Gelatin</th>
<th>Bentonite</th>
<th>Sparkolloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4</td>
<td>1</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>2</td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

6. Transfer to clear glass bottles (200 ml), label.
7. Also, fill one bottle with the original wine, label 0 pounds per 1000 gallons.
8. Seal all wines and let stand for a few days.
9. Observe the clarity of each level of fining agent. The greatest degree of clarity with the minimum amount of fining agent is the best level.
10. Record the amount of fining agent for the best clarification.

<table>
<thead>
<tr>
<th>Wine</th>
<th>Fining Agent</th>
<th>lbs/1000 gal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTES:

The addition of tannic acid prior to the gelatin treatment is advisable for the clarification of white and some red wines. This extra tannin will ensure the formation of a suitable coagulum with the gelatin. For testing, the tannic acid (2.4% solution, see MATERIALS AND REAGENTS) is added first to each of the 200 ml wine samples at same level of the gelatin solution. Then, the samples are treated with the gelatin solution (see step #5, Procedure).

All fining agent suspensions and solutions should be thoroughly mixed before using. Also, when adding these agents to the wines, including the test samples, the wines should be constantly stirred. In addition, the temperature of the test samples should be close to the temperature of the bulk wines.

REFERENCES

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DETECTION OF MALO-LACTIC FERMENTATION

The bacterial conversion of malic acid to lactic acid and carbon dioxide in wine is termed malo-lactic fermentation. This fermentation is caused by growth of certain lactic acid bacteria and often occurs after the alcoholic fermentation. However, the prediction of this secondary fermentation is often difficult. This is due to the fact that malo-lactic fermentation is influenced by many factors such as temperature, aeration, alcohol, pH, time of racking, sulfur dioxide, bacterial strain, and amount of inoculum.

The main effect of malo-lactic fermentation is deacidification which may be desirable depending upon the initial acidity of a particular wine type. This fermentation may also cause a favorable or unfavorable change in wine flavor by adding small amounts of certain bacterial metabolic products. In addition, biological stability is another aspect which is often considered in determining the benefits of malo-lactic fermentation. A non-sterile wine with malic acid present is considered unstable.

Consequently, it is often important to monitor and determine whether or not malo-lactic fermentation has occurred. The detection of this fermentation would be of value to the winemaker by helping him appraise those factors which tend to influence malo-lactic fermentation.

A rapid, easy, and inexpensive method for the detection of malo-lactic fermentation has been described by Kunkee (1). This technique uses the principle of paper chromatography and reveals the presence or absence of malic acid. The absence of malic acid in wine is considered evidence of the occurrence of malo-lactic fermentation.

MATERIALS AND REAGENTS

- Chromatographic paper: The paper should be designated for "chromatography" and cut into 20 x 30 cm rectangles. Whatman No. 1 or Schleicher and Schuell No. 2043 chromatographic paper is suitable for this technique.
- Micropipettes: Glass capillary tubes, I.D. 1.1-1.2 mm. are used to spot the wines on the chromatography paper.
- Separatory funnel: A 500 ml funnel is used in preparing the chromatographic solvent.
- Graduate cylinders: 100 and 25 ml graduated cylinders are used in measuring reagents for the chromatographic solvent.
- Chromatographic jar: A "one-gallon mayonnaise" jar with lid is suitable for this technique.
- Stapler: A common desk stapler is used in preparing the paper chromatogram.
- Indicator solution: This indicator solution is used in the chromatographic solvent and is prepared by dissolving 1 g of water-soluble bromocresol green in 100 ml water.
- Solvent: To prepare the solvent for this chromatographic technique, transfer the following into the separatory funnel: 100 ml water, 100 ml reagent grade n-butanol, 10.7 ml reagent grade concentrated formic acid and 15 ml indicator solution. Then, thoroughly shake the solvent mixture and allow two layers to form. The lower layer is discarded (aqueous phase) and the upper phase is saved to develop the paper chromatogram. Although this solvent can be used more than once, it is advisable to transfer the solvent to the separatory funnel periodically to remove any additional aqueous phase.
PROCEDURE

1. Obtain the chromatography paper (20 x 30 cm) and draw a pencil line parallel to the length of the longest side of rectangle (30 cm) about 2.5 cm from the edge.

2. Draw into the micropipette by capillary action a sample of wine.

3. Touch the pipette to the paper on the pencil line and make a spot about 1 cm in diameter.

4. Repeat this step for each wine to be tested, about 2.5 cm apart.

5. When the spots are dry, staple the short edges of the rectangle to form a cylinder, the edges should not overlap.

6. After adding 70 ml of the solvent to the chromatography jar, place the paper cylinder into the jar with the spotted edge towards the bottom and secure the lid.

7. When the solvent has ascended to near the top edge of the paper cylinder, remove the paper and place it in a well-ventilated area.

8. Leave undisturbed until the paper is thoroughly dry, yellow spots on a blue background.

9. In order to identify these spots as to specific organic acids, the Rf value of each acid should be determined.

10. Measure the distance that the solvent traveled, from the pencil line to the solvent front.

11. Then measure the distance between the center of each acid spot and the pencil line.

12. The Rf value is calculated by dividing the measured distance of the solvent front into that measured for the acid spot.

13. Each organic acid will have a different Rf value, such as lactic acid (0.75), malic acid (0.50) and tartaric acid (0.26).

REFERENCE

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FREEZE-DRIED CULTURES FOR MALO-LACTIC FERMENTATION

Since efforts to initiate a natural malo-lactic fermentation is not always successful, a recent trend has been directed toward pure culture inoculation. In addition to providing a more rapid and predictable malo-lactic fermentation, pure culture inoculation offers a better chance in producing wines without "off" flavors and odors. These defects are sometimes associated with a natural malo-lactic fermentation.

Although a number of species of bacteria in the genera Lactobacillus, Pediococcus, and Leuconostoc have been shown to cause malo-lactic fermentation, most of the attention has been directed toward Leuconostoc oenos ML-34. This strain was isolated from a wine made in California and has been extensively studied for pure culture inoculation, particularly in red table wines.

More recently, Beelman isolated and characterized another strain of Leuconostoc oenos. This organism was isolated from a Pennsylvania wine and was designated as PSU-1. Studies comparing this bacterium with ML-34 have shown that PSU-1 is better in encouraging malo-lactic fermentation in eastern high acid wines than the California strain.

With this in mind, freeze-dried cultures of PSU-1 are now commercially available for inoculating wines. These cultures are suited for use in small wineries and if used correctly will help induce malo-lactic fermentation in red table wines.

MATERIALS AND REAGENTS (direct method)

Pipet, 25 ml, volumetric
Flask, 100 ml, volumetric, Class A
Balance
Peptone solution, .1% -- weigh 0.10 g of peptone powder, dissolve and bring to volume with distilled water in a 100 ml volumetric flask.
Bacteria, PSU-1, freeze-dried culture

PROCEDURE (direct method)

1. Pipet 25 ml of 0.1% peptone solution into the serum bottle containing the freeze-dried culture.
2. Wait 10 minutes for rehydration. Each ml contains about $1 \times 10^{11}$ cfu/ml, or $25 \times 10^{11}$ cfu in 25 ml.
3. Add the entire 25 ml to approximately 60 gallons of fermenting wine. The resulting bacterial population is about $1 \times 10^7$ cfu/ml, which is sufficient to induce malo-lactic fermentation.

NOTES

It is recommended that the sulfur dioxide content of the fermenting wine be kept to a minimum. The sulfur dioxide treatment at the time of crushing should be less than 50 ppm.
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MATERIALS AND REAGENTS (expanded culture method)

Bacteria, PSU-1, freeze-dried culture, LS-5A, direct inoculum for 5 to 10 gallons of wine
Balance
Beaker, 2000 ml
Buffer solution, pH 4 and pH 7
Calcium carbonate
Carboy, glass, 5 gallon
Cylinder, graduated, 1000 ml
pH meter
Pipet, 25 ml, volumetric
Potassium metabisulfite
Stirrer, magnetic
Stirring bars
Weighing boat, medium
Yeast culture, Montrachet #522

PROCEDURE (expanded culture method)

1. Place 0.5 gallons (1892 ml) of free-run juice into a 2000 ml beaker.
2. Place approximately 10 g of calcium carbonate (CaCO₃) into a small container.
3. Weigh container and record the weight.
4. Immerse the pH electrodes into the free-run juice.
5. With constant stirring, add the calcium carbonate until pH stabilizes at 3.8.
6. Calculate the amount of calcium carbonate used to obtain pH 3.8 by subtracting the weight of the container from the original weight (see step #3). Record this weight.
7. Place 3.5 gallons of free-run juice into a clean 5-gallon glass carboy.
8. Transfer the 0.5 gallon of pH 3.8 juice to the glass carboy, total volume equals 4 gallons.
9. Multiply the weight of calcium carbonate recorded in step #6 by 7, and weigh this amount into a small container.
10. With constant stirring, add this amount of calcium carbonate to the juice and let stand for 30 minutes.
11. Check pH, and adjust to 3.8 if necessary.
12. Add 15 ppm sulfur dioxide in the form of potassium metabisulfite to the juice.
13. Inoculate with 5 g of active-dry yeast, Montrachet #522, which was rehydrated with a small volume of juice.
14. After 6 hours, inoculate with the freeze-dried PSU-1 culture. This culture size inoculates 5 gallons of wine. Rehydrate with 25 ml distilled water and wait 10 minutes before inoculation.
15. Place carboy at 22°C.
16. During fermentation, check for the complete disappearance of malic acid by using the procedure for the detection of malo-lactic fermentation (see page 41).
17. When the malic acid disappears, this culture is ready to be used as a inoculum for 200 gallons of fermenting wine.
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NOTE

This method is recommended for red wines with the sulfur dioxide kept at a minimum. The sulfur dioxide treatment at the time of crushing should be about 50 ppm.

REFERENCES


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SUPPLIES, SUPPLIERS AND COSTS

ITEM AND SPECIFICATIONS

Cash volatile acid assembly or volatile acid still

<table>
<thead>
<tr>
<th>APPROX. COST</th>
</tr>
</thead>
<tbody>
<tr>
<td>$185</td>
</tr>
</tbody>
</table>

**Suppliers**
Scott Industries
860 S. 19th Street
Richmond, CA 94804
Phone: (415)232-8288

VWR Scientific, Inc.
PO Box 855
Columbus, OH 43216
Phone: (614)445-8281

**Ebulliometer**

<table>
<thead>
<tr>
<th>APPROX. COST</th>
</tr>
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<tbody>
<tr>
<td>$225</td>
</tr>
</tbody>
</table>

**Suppliers**
Presque Isle Wine Cellars
9440 Buffalo Road
North East, PA 16428
Phone: (814)232-8228

Scott Laboratories
860 S. 19th Street
Richmond, CA 94804
Phone: (415)232-8288

**Fining Agent - Bentonite**

<table>
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<tr>
<th>APPROX. COST</th>
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<td>$0.20/lb.</td>
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</tbody>
</table>

**Suppliers**
American Colloid Company
5100 Suffield Court
Skokie, ILL 60076
Phone: (312)585-0499

Scott Laboratories
860 S. 19th Street
Richmond, CA 94804
Phone: (415)232-8288

**Fining Agent - Gelatin**

<table>
<thead>
<tr>
<th>APPROX. COST</th>
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<td>$1.50/lb.</td>
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</table>

**Suppliers**
George A. Hormel & Co.
8550 W. Bryn Mawr Ave.
Suite 100
Chicago, IL 60525
Phone: (312)693-6460

American Colloid Company
5100 Suffield Court
Skokie, ILL 60076
Phone: (312)585-0499

Germantown Mfg. Co.
505 Parkway
Broomall, PA 19008
Phone: (215)544-8400

**Fining Agent - Sparkoloid**

<table>
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<th>APPROX. COST</th>
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<td>$2.00/lb.</td>
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</tbody>
</table>

**Suppliers**
Scott Laboratories
860 S. 19th St.
Richmond, CA 94804
Phone: (415)232-8288

**Malo-Lactic Bacteria (PSU-1), freeze-dried culture**

<table>
<thead>
<tr>
<th>APPROX. COST</th>
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<td>$6.00/btl.</td>
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**Supplier**
TRI BIO Laboratories, Inc.
1400 Fox Hill Road
State College, PA 16801
Phone: (814)355-1541
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ITEM AND SPECIFICATIONS

<table>
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<tr>
<th>Item Description</th>
<th>Approx. Cost</th>
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<tr>
<td>Peptone</td>
<td>$13.00/lb.</td>
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<td><strong>Supplier</strong></td>
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<tr>
<td>ICN Life Science Group</td>
<td></td>
</tr>
<tr>
<td>26201 Miles Road</td>
<td></td>
</tr>
<tr>
<td>Cleveland, OH 44128</td>
<td></td>
</tr>
<tr>
<td>Phone: (216)831-3000</td>
<td></td>
</tr>
</tbody>
</table>

Semi-micro apparatus for SO₂ analysis

A. Pipette - Pasteur (K883350-0009) $16.00/720 pipettes
B. Adapter Bushing 14/20-10/18, Size 21 (K273500-0021) $7.00
C. Adapter Claisen, 14/20 (K273750-0000) $16.50
D. Flask, Round-bottom, 14/20, 50 ml (K294000-0050) $6.25
E. Condenser, Liebig 110 mm, 14/20 (K282210-0000) $19.20
F. Adapter, Connecting, 14/20 (K275050-1420) $13.50
G. Adapter, Vacuum, 14/20 (K276750-0000) $11.75
H. Flask, Pear shape, 14/20, 50 ml (K294250-0050) $6.80
I. Adapter, Bleed (K273410) $5.00

**Supplier**

Kontes Glass Inc.
Vineland, New Jersey 08360
Phone: (609)692-8500

Yeast - Montrachet #522 $3-6/lb.

**Suppliers**

Universal Foods Corp. Scott Laboratories
433 East Michigan St. 860 S. 19th St.
Milwaukee, WI 53201 Richmond, CA 94804
Phone: (414)271-6755 Phone: (415)232-8288

Presque Isle Wine Cellars
9440 Buffalo Road
North East, PA 16428
Phone: (814)232-8228

Aspirator $12.00
Adapter, Connecting 24/40 $10.00
Adapter, Trap Type bulb, 24/40 $15.00
Balance $1000-1500
Beaker, 100 ml, 250 ml and 600 ml $10-12/doz.
Bottles, clear with caps, 200 ml $10/doz.
Bottles, reagent 125 ml and 500 ml, pyrex $3-8/doz.
Bottle, Washing, polyethylene $1.00
Bottles, wine with caps, 1/10 gal. $2/doz.
Bromocresol green, water soluble $15/5 gram
Buffer Solution, pH 4, potassium acid phthalate $6/quart
Buffer Solution, pH 7, monobasic potassium phosphate $6/quart
and sodium hydroxide
Buret, 10 ml, Class A, calibrated in 1/20 ml divisions, teflon stopcock $45.00
Buret, 10 ml, Class A, calibrated in 1/20 ml divisions, teflon stopcock $45.00
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ITEM AND SPECIFICATIONS

Buret support and clamp (double) $35.00
Butanol, reagent grade $6/pint
Carbon tetrachloride $3/pint
Carbonic anhydrase, freeze-dried $5/50 mg
Carbon, glass, 3 gallon $3.00
Chromatographic paper, Whatman No. 1 or Schliecher and Schuell No. 2403 $92/100 sheets
Condenser, 24/40 $15-20
Cylinder, graduated, 25 ml, 100 ml and 500 ml $7-12
Cylinder, hydrometer, 100 ml or 150 ml $10
Erlenmeyer flask, 250 ml and 500 ml, wide mouth $18-24/doz.
Erlenmeyer filtering flask, 250 ml $4.00
Ethanol or alcohol, 95% $10/gallon
Flask, 100 ml, 250 ml and 1 liter, volmetric, Class A $5-12
Formic acid, concentrated, reagent grade $4/pint
Hot plate, electric $40-50
Hydrogen peroxide, 30% $6.75/100 ml
Hydrochloric acid, .1 N $2/pint
Hydrometers, alcohol $15/each
Iodine, .1 N $9/liter
Iodine, .1 N concentrate $6/liter
Kjeldahl flask, 800 ml, 24/40 $4.00
Methyl red powder $3/10 gm
Microburner $8.00
pH meter $300.00
Phenolphthalein powder $3/qtr. lb.
Phosphoric acid, reagent grade $15.50/liter
Pipets, capillary, disposable $5/1000
Pipets, serological, Style I, 1 ml and 10 ml $3-4/each
Pipets, volumetric, Class A, 1 ml, 5 ml, 10 ml, 20 ml, 25 ml and 50 ml $3-6/each
Potassium iodide, crystals $10/lb.
Potassium metabisulfite, food grade, powder $5/lb.
Separatory funnel, 500 ml, pear-shaped or globe $25.00
Sodium bicarbonate, Baker analyzed $5/lb.
Sodium hydroxide, .1 N $8/gallon
Sodium hydroxide, .1 N, concentrated $5/liter
Sodium hydroxide, 1 N $8/gallon
Sodium hydroxide pellets $7/lb.
Sodium thiosulfate, .02 N $3/qt.
Starch, soluble potato, powder, for iodometry $7/lb.
Stirrer, magnetic $75-150
Stirring bars $2-3/each
Stopper, neoprene, No. 8 $3/dozen
Sulfuric acid, reagent, A.C.S. $4/pint
Tank, about 60 gallons $60.00
Tannic acid $10/lb.
Thermometer, centigrade $4.00

Suppliers
Arthur H. Thomas Co. VWR Scientific, Inc.
PO Box 779 PO Box 855
Philadelphia, PA 19105 Columbus, OH 43216
Phone: (215)627-5600 Phone: (614)445-8281
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Suppliers (cont.)
Fisher Scientific Co.
26401 Miles Ave.
PO Box 7030
Cleveland, OH 44128
Phone: (216)292-7900

Sargent-Welch Scientific Co.
4620 Midwest Ave.
Cleveland, OH 44125
Phone: (216)587-3300

Kontes Glass, Inc.
Vineland, NJ 08360
Phone: (609)692-8500

Fischer Scientific Co.
5481 Creek Road
Cincinnati, OH 45242
Phone: (513)793-5100

Sargent-Welch Scientific Co.
10400 Taconic Terrace
Cincinnati, OH 45215
Phone: (513)771-3860

Presque Isle Wine Cellars
9440 Buffalo Rd.
North East, PA 16428
Phone: (814)232-8228

Sigma Chemical Co.
PO Box 14508
St. Louis, MO 63178

References to commercial products or trade names are for educational purposes only. No discrimination is intended and no endorsement is implied for specific product names.
Appendix A

CLEANING GLASSWARE

The insides of glassware should be clean enough that an even film remains after they have been filled with distilled water and emptied. It is usually sufficient to scrub with commercial detergent then rinse several times with tap water. If glassware does not come clean with detergent, a cleaning solution may be purchased from most laboratory suppliers which is a highly corrosive mixture of sulfuric acid and sodium dichromate. Cleaning solution should be handled with great care; it burns skin and destroys clothing. Any drop spilled on any surface should be neutralized with sodium bicarbonate then rinsed several times with water. Depending on how dirty the glassware is, cleaning solution requires from a half hour to overnight contact to clean. As long as it retains its red color, cleaning solution may be used over and over. When it turns green it has lost its cleaning power and should be discarded.

Appendix B

USING A BURET

Burets should be cleaned thoroughly after use, filled with distilled water, and stored upright, if possible. When preparing a buret for titration, it should be rinsed several times with small portions of the solution to be placed in it. Fill the buret almost full with the titrating solution then open the stopcock until all the air bubbles have been removed from the tip. Remove a drop clinging to the buret tip by touching it against the side of the waste solution beaker. Make a reading at the bottom of the meniscus formed by the solution. A piece of white paper held behind the buret will make it easier to establish the correct level for the meniscus. Remember to keep your eye level with the meniscus (Fig. 5). Record the solution level reading, then proceed with titration.

Appendix C

USING A VOLUMETRIC PIPET

A clean pipet will have no droplets remaining on the inner surface after the final rinsing with distilled water. The fragile pipet tip should be protected from breakage during storage. If moisture is present from the cleaning process, the pipet should be rinsed a couple of times with the solution to be pipetted.

Insert the pipet into the solution and fill it above the calibration line by mouth suction. Quickly cap the pipet with the index finger of the hand holding the pipet—do not use the thumb. Holding the pipet over a waste solution beaker, slowly release the pressure upon the index finger to adjust the liquid level so that the bottom of the meniscus is exactly even with the calibration mark. Remove a drop clinging to the pipet tip by touching it against the side of the waste solution beaker. Keeping the pipet almost vertical, place the tip against the wall of the intended container and allow the liquid to drain freely. DO NOT BLOW OUT THE LIQUID REMAINING IN THE TIP. Volumetric pipets are calibrated to deliver (TD) a definite volume of liquid which does not include the drop remaining in the tip of the pipet.

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Fig. 5. Reading a buret
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