**Table II. Summary of Swine Feeding Test**

| | Lot 1, Fed 27% Moisture Corn Stored in Sealed Bins | Lot 2, Fed 13% Moisture Corn Stored in Conventional Bins |
|---|---|---|
| Pigs per lot | 15 | 15 |
| Av. initial wt., lb. | 85.9 | 86.7 |
| Av. final wt., lb. | 191.1 | 188.4 |
| Av. daily gain, lb. | 1.50 | 1.45 |
| Av. daily feed, lb. | | |
| Corn | 5.06 | 4.52 |
| Supplement, 40% protein | 0.47 | 0.73 |
| Feed per 100-lb. gains, lb. | 368 | 362 |

19% moisture did not flow readily and the capacity of the auger elevator was reduced somewhat below that when handling dry corn.

The corn at 23 and 27% moisture was very difficult to handle with conventional equipment. It became so packed in storage that more than one man was required to insert a 5-foot sampling probe. The corn could not be moved with a grain scoop without first loosening it with a pick or probe. When unloading from the bottom of the bin, the corn bridged over the intake to the auger elevator and had to be probed loose.

The corn from the last three lots was dried in an experimental batch dryer over a 2- to 3-week period in the late summer. Although the bins were opened frequently to load the dryer, the undisturbed corn remaining in the bins did not heat or show any noticeable increase in mold growth.

### Feeding Tests

Hogs fed corn of 27% moisture stored in the sealed bin for two seasons remained healthy and made normal weight gains during a 10-week test period (Table II). Compared to the lot which received dry corn, those fed wet corn gained slightly more, consumed 12% more corn, but ate 36% less protein supplement. The cost per 100 pounds gain at 1952 prices was $12.63 and $12.18 for the lots receiving dry and wet corn, respectively. Nutritionally, the large difference in the consumption of protein supplement may be significant enough to warrant further study.

The wet corn fed was brown in color and that from some parts of the bin was somewhat moldy. Part of the time the corn placed in the self-feeders became completely covered with white mold within 24 hours. At other times, little additional mold growth appeared during the 3 to 4 days between times of filling the feeders. The amount of molding in the feeders was greater during wet weather. There was some caking of corn on the sides of the self-feeder, but in general no great difficulty was encountered in self-feeding the wet corn.

Whether or not hogs do well on wet corn will depend on the extent of damage, the type of mold present, and perhaps other factors.


### Acknowledgment

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**WINE PRODUCTION**

# Determination of Free and Total Sulfur Dioxide in White Table Wines


**M. A. JOSLYN**

**Department of Food Technology, University of California, Berkeley, Calif.**


THE CONCENTRATION OF SULFUR DIOXIDE in wine and its distribution in free sulfurous acid, bisulfite ions, and sulfite addition products determine the stability and palatability of wine. Of the many methods available for the determination of sulfur dioxide in wine (*5, 6, 12–14, 19, 21*), the direct iodine titration procedures and the colorimetric fuchsin procedures have been more frequently suggested for the determination of "free" and "bound" sulfur dioxide. The chemistry of sulfite addition products present in wine is still unknown, although there is evidence that acetaldehyde and glucose addition products are present (*5, 6, 11–14, 19, 21, 22, 28*). Deibner and Bénard (*7*) suggested recently that the acetaldehyde-sulfurous acid (α-hydroxyalcoyl sulfuric acid) could be separated by distillation, but found that dilution affected the stability of the compound more than any possible decomposition during distillation. The conditions affecting the equilibrium between free and bound sulfur dioxide and the rate of combination, liberation, and recombination have not been investigated extensively, although it is recognized that pH, temperature, alcohol concentration, concentration of glucose and acetaldehyde, and concentration, type, and method of adding sulfur di-

**Determination of free and total sulfur dioxide is necessary for the proper control of fermentation and storage deterioration of wine. Two rapid control methods, direct iodometric titration and colorimetric fuchsin-formaldehyde, were investigated to determine the factors affecting accuracy and reproducibility of the results. The concentration of starch indicator, the speed of titration, the concentration of alkali, and the period of storage affected the results. Nonsulfite iodine-reducing substances present in alkali-treated wines and in distillates from wine after acidification could be estimated better by addition of hydrogen peroxide as a specific sulfite oxidizing agent than by addition of formaldehyde as a sulfite binding agent. The concentration of sulfuric acid in the acid-bleached fuchsin reagent and the order of addition of the sulfite solution or wine to the stock reagent affected the accuracy of the colorimetric method. The modified direct iodometric method was more rapid and gave more reproducible results than the usual Ripper titration.**

oxide affect both the equilibrium conditions and the rate at which equilibrium is approached.

As part of the program of investigation of the chemistry and technology of the pretreatment and preservation of wines with sulfur dioxide and sulfites, data were obtained on the conditions affecting the Ripper method. Errors due to reduction of iodine by substances other than sulfur dioxide, although they are well established for other products [citrus juices (*4*, *10*); dehydrated fruit and vegetables (*23–27*)], have not been estimated or avoided in wine analysis.

The sensitive color reaction for sulfuric acid described by Steigman (*32*) and later by Grant (*9*) and Steigman (*31*) for the quantitative determination of sulfur dioxide has been applied to wines and beers, but with variable results. The effect of the composition of the acid-bleached fuchsin-formaldehyde reagent and conditions of use on the intensity of color formed and its applicability to the determination of free and combined sulfur dioxide content of white table wine was investigated.

### Iodometric Titration

The direct iodometric procedure of Ripper (*29*), in modified form, is used widely as a control procedure in wineries for white wines. Mills and Wiegand (*18*), by taking the end point in the direct titration as the first starch end point lasting for 1 minute, instead of several minutes as indicated in the earlier modifications, found that the Ripper (*29*) method agreed fairly closely with the Nichols and Reed (*20*) modification of the iodine distillation method.

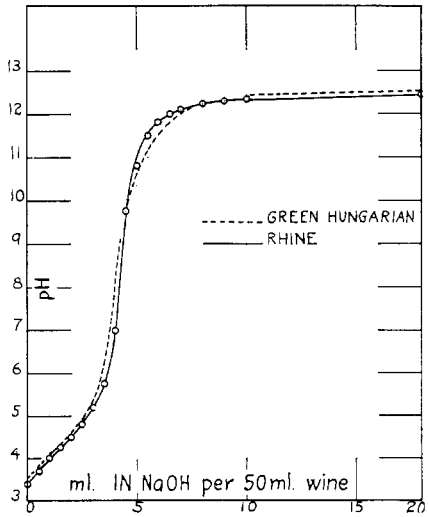
At the time that the Ripper method was developed (1892), the effect of pH on rate of association and dissociation of acetaldehyde and glucose bisulfite (more correctly α-hydroxysulfonate) addition compounds was not known. Ripper (*29*) suggested adding 50 ml. of wine to 25 ml. of 1*N* potassium hydroxide and allowing the mixture to stand at room temperature in a stoppered flask for 15 minutes before acidifying. It is now known that the glucose hydroxysulfonate dissociates completely at pH 6 at room temperature in 2 minutes in aqueous solutions, but in the presence of additional glucose (10 to 50%), the pH required for complete dissociation increases to ca. 10 (*14*). The acetaldehyde sulfonate dissociates practically completely at pH above 12. Alcohol is known to reduce the rate of combination of acetaldehyde with bisulfite (*11*), but its effect on the rate of dissociation is not known. Tomoda (*33*) reported that at pH 8 aldehyde bisulfite completely dissociates during titration with iodine, and this has been used as the basis of volumetric methods for determining acetaldehyde (*11*). Jaulmes and Espezel (*11*) carried out the iodometric titration at pH 10.5, but Lucas (*17*) preferred to add a measured excess of dilute iodine solution to the acetaldehyde sulfonate, add an excess of sodium bicarbonate, and titrate excess of iodine with arsenious acid solution at pH 7.5 to 7.7.

Too little attention has been given to the conditions governing the starch iodine end point in the iodometric titration. The older literature on this has been extensively reviewed by Barger (*3*) and Kolthoff and others (*15*, *16*). Deibner (*5*) has stressed particularly the importance of attaining the proper concentration of potassium iodide in the solution to be titrated. The type of starch used, the concentration of starch solution, and the volume to be added to an aliquot are important. Kolthoff and Menzel (*15*) suggested 10 ml. of 0.2% starch solution per 100 ml. of solution to be titrated; Kolthoff and Stenger (*16*) recommended 2 to 5 ml. of 0.2% starch; and the Association of Official Agricultural Chemists (*1*, pp. 51, 472) recommended 5 ml. of 0.5% soluble starch or 1% potato starch. The sharpness of the end point, particularly in the presence of formaldehyde, is greatly influenced by the volume of starch solution used.

### Materials and Methods

Two commercial California white table wines were used in most of the tests. The first was a Rhine wine prepared from a blend of Riesling grape wines of the vintage of 1951 and the second was a white wine prepared from Green Hungarian grapes of the vintage of 1950. The Rhine wine contained 12% alcohol by volume, 0.615 gram of total titratable acidity per 100 ml., 0.085 gram of reducing sugar per 100 ml., 5.7 p.p.m. of iron, 0.57 p.p.m. of copper, and, when obtained, 190 p.p.m. of sulfur dioxide. The Green Hungarian wine was of similar composition. These wines were stored at 10° C. in glass until used during the period of December 1953 to November 1954. These wines were similar in titration characteristics, as shown in Figure 1, 50 ml. of wine requiring 3.6 and 4.0 ml. of 1*N* sodium hydroxide to raise their pH from 3.41 (R wine) and 3.55 (G H wine) to 7.0; 4 to 4.1 ml., to pH 8.0; 4.5 to 4.6 ml., to pH 10.0; and 6.5 to 6.9 ml., to pH 12.0. The pH value, as measured by the glass electrode, increased only slightly when

Figure 1. Alkali titration curve for white wines



10 ml. or more of alkali were added to 50 ml. of wine.

The approximately 1*N* sodium hydroxide solution was prepared by diluting 54 ml. of saturated carbonate-free sodium hydroxide to 1 liter; the 1*N* hydrochloric acid by diluting 89 ml. of C.P. concentrated acid to 1 liter. The 3% hydrogen peroxide was prepared by diluting 35% reagent grade solution and checking for strength. This solution was neutral to bromophenol blue. All other solutions used were prepared and standardized according to AOAC procedures (*1*). The standard direct titration procedure was to pipet aliquots of wine into 250-ml. glass-stoppered flasks and then quickly add the alkali from a free-running buret or graduate. After mixing, the solution was allowed to stand for the required period of time and then quickly acidified by addition of the required 1*N* hydrochloric acid or (1 + 3) sulfuric acid from a graduated cylinder. Modifications of this procedure are described below. At first the iodine titration was made using 0.5 to 1.0 ml. of 0.5% soluble starch solution; subsequently 5 ml. of 1% starch solution were added. The dilute iodine solution, approximately 0.01*N*, was run in rapidly from a 10-ml. microburet with vigorous shaking and then added dropwise until a blue color persisting for 0.5 minute was obtained. The sulfur dioxide content was determined also by distillation into dilute iodine as described by Nichols and Reed (*20*) and by the modified Monier-Williams procedure of Shipton (*30*), except that carbon dioxide was used as sweeping gas.

## Results and Discussion

**Ripper Titration.** In the original method, the 50-ml. aliquot of wine was pipetted into a flask containing 25 ml. of 1*N* potassium hydroxide (*2*). With the above white wines no significant difference was observed when the order of addition of alkali was changed, if the alkali was added rapidly and the wine and alkali were mixed quickly. Either sodium hydroxide or potassium hydroxide could be used without effect on final pH or volume of iodine reduced. The addition of alkali to 50-ml. aliquots of wine was preferable because agreement between replicates was improved. With the dilute iodine solution used, the speed of titration and the concentration of starch present affected the results more than order of addition of alkali and wine. With 1 ml. of a 1% solution of soluble starch, rapid titration gave results from 1 to 2 ml. of iodine less than with slow addition. With less starch or with a more dilute starch solution, more iodine solution was required and the end point was not so sharp. This was true also of water blanks acidified with hydrochloric acid. Typical of the data obtained are the following values for effect of volume of 0.5% starch indicator on volume of iodine solution required to obtain the first end point in a solution containing 25 ml. of water and 5 ml. of 1*N* hydrochloric acid:

| Starch Solution Added, Ml. | Ca. 0.01N Iodine, Ml. | End Point |
|---|---|---|
| 0.1 | 0.75 | Yellowish brown, indistinct |
| 0.2 | 0.35 | Faint blue |
| 0.3 | 0.35 | Light blue |
| 0.5 | 0.22 | Light blue |
| 1.0 | 0.21 | Deep blue |
| 2.0 | 0.19 | Deep blue |
| 3.0 | 0.18 | Deep blue |
| 5.0 | 0.14 | Deep blue |

The type of acid used (hydrochloric or sulfuric) had little effect on the volume of iodine required for the direct titration for total sulfur dioxide, although with hydrochloric acid the end point was somewhat better than with sulfuric acid. The type of acid used, however, did affect the results for free sulfur dioxide. With hydrochloric acid the agreement between replicates was better but the volume of iodine required in the titration was higher.

Thus, for 50 ml. of the Rhine wine titrated without added acid, 2.8 to 3.2 ml. of 0.0974*N* iodine solution were required, but the end point was poor and variable; in the presence of 5 ml. of (1 + 3) sulfuric acid, 1.04 to 1.20 ml. of iodine were required; in the presence of 2 ml. of 6*N* hydrochloric acid, 1.06 to 1.51 ml. (average 1.27); and in presence of 5 ml. of 6*N* hydrochloric acid, 1.28 to 1.50 ml.

In the original procedure, the directions specified that the titration with iodine be continued until the blue color persists for several minutes. It was difficult with this procedure to obtain agreement between replicates. By titrating to the first definite blue color that persists for 0.5 minute better agreement was obtained, but after the first end point faded, the wine reduced iodine even more rapidly than originally at the end point and this reduction continued. With a 50-ml. aliquot of wine treated for 15 minutes with 25 ml. of 1*N* sodium hydroxide, then acidified with 12.5 ml. of 6*N* hydrochloric acid, the first end point was obtained with 20.88 ml. of iodine; the second end point required an additional 0.5 ml. and so did the third. With a 50-ml. aliquot of the same wine treated with 10 ml. of 1*N* sodium hydroxide for 2 minutes and then acidified with 5 ml. of 6*N* hydrochloric acid, the first end point was obtained with 16.98 ml. of iodine; the second, third, and fourth end points required additional amounts of 0.8 ml. each.

The pH value of the mixture of 50 ml. of wine and 10 ml. of 1*N* sodium hydroxide was about 12.5 and was not significantly changed by increasing the volume of alkali added to 25 ml. or over. The pH of the mixture of 50 ml. of wine and 25 ml. of 1*N* sodium hydroxide changed from 12.4 to 1.0 when 10 ml. of (1 + 3) sulfuric acid were used to acidify it and to 1.15 when 5 to 6 ml. of 6*N* hydrochloric acid were used for acidification. The starch-iodide end points even in the strongly acid solutions (pH 1 to 1.1) were not very sharp and faded on standing. Even under these conditions, however, the iodine titration included an appreciable amount of nonsulfite iodine-reducing substances. These were equivalent to 1 to 2 ml. of 0.01*N* iodine when measured by addition of 1 ml. of formaldehyde to the acidified wine and 0.7 to 1.0 ml. of iodine when measured by addition of 1 ml. of formaldehyde to the acidified wine, and 0.7 to 1.0 ml. of iodine when measured by addition of 2 ml. of 3% hydrogen peroxide, as suggested by Potter for dehydrated vegetables (*24*). The end point in presence of formaldehyde was not so sharp or permanent as that obtained with hydrogen peroxide.

The total sulfur dioxide content of samples of Rhine wine as determined by the Ripper method taken at different times during storage varied from 128 to 157 mg. per liter for the samples drawn earlier during the year, and dropped to 79.2 to 85.4 for samples that had been stored for 3 months at room temperature. At that time the sulfur dioxide content as determined by the Monier-Williams method was 70.2. When the original sample was resulfited, its sulfur dioxide content as determined by Ripper titration was 260.9, whereas by the iodine distillation method it was 371.2 and by the Monier-Williams method it was 400 mg. per liter. Similar differences between the three methods were observed with another lot of wine after storage for some time. The direct Ripper titration required 18.47 ml. of iodine solution and the nonsulfite iodine-reducing materials determined by treatment of acidified duplicates with hydrogen peroxide required 0.73 ml. of iodine solution, equivalent to 119.2 mg. of sulfur dioxide per liter (uncorrected) and 114.6 mg. (after correction for nonsulfite-reducing substances). The iodine distillation method on the same sample run at the same time gave a sulfur dioxide content of 146.1 and the Monier-Williams method gave 209.6.

The results on the Green Hungarian wine were similar. The direct Ripper titration on a 50-ml. aliquot required 13.06 ml. of iodine solution; the nonsulfite matter present determined in the presence of added hydrogen peroxide required 0.77 ml. of iodine solution, and in the presence of formaldehyde, 1.44 ml. The total sulfur dioxide content, uncorrected, was 84.4 mg. per liter and after correction (using the hydrogen peroxide values), 79.4. The iodine distillation method gave 114.6

and the Monier-Williams method 156.4.

**Effect of pH of Treatment.** The effect of varying the volume of 1*N* sodium hydroxide added to various aliquots of wine, which were allowed to stand 1, 2, and 5 minutes before acidification, on the volume of iodine reduced was determined a number of times for both wines. Because the sample of the wine used was not always the same, these show relative rather than absolute effects.

For a sample of the Rhine wine which had an uncorrected sulfur dioxide content of 134.2 mg. per liter by the regular Ripper method, the following values were obtained when 2 to 10 ml. of 1*N* sodium hydroxide were added to 50-ml. aliquots which were allowed to stand for 1 minute before acidification with sufficient 6*N* hydrochloric acid to allow an excess of 1.7 ml. after neutralization of alkali added:

| NaOH Added, Ml. | $SO_2$ Content, Mg./L. |
|---|---|
| 0 | 8.2 |
| 2 | 15.8 |
| 4 | 15.8 |
| 6 | 73.5 |
| 10 | 70.0 |

For a later sample of Rhine wine which contained 9.5 mg. per liter of free sulfur dioxide and 157.2 mg. of total sulfur dioxide (uncorrected) and 144.5 total (corrected with formaldehyde) by Ripper titration, 50-ml. aliquots treated with 10 ml. of 1*N* sodium hydroxide (sufficient to bring the pH value of the mixture to 12.35) for 1 to 10 minutes before acidifying with 5 ml. of 6*N* hydrochloric acid, the following values were obtained:

| Minutes Stored | $SO_2$ Content (Uncor.), Mg./L. |
|---|---|
| 1 | 131.8 |
| 2 | 119.2 |
| 4 | 128.0 |
| 10 | 140.0 |

A sample of the Rhine wine which had a sulfur dioxide content of 114.6 mg. per liter by the Ripper method (after correction for nonsulfite iodine-reducing matter with hydrogen peroxide), 146.0 by the iodine distillation, and 209.6 by the Monier-Williams method was treated with varying amounts of 1*N* sodium hydroxide for 2 minutes. Twenty-five-milliliter aliquots were mixed with 0 to 20 ml. of alkali, then acidified with 5 to 25 ml. of 1*N* hydrochloric acid and titrated directly with iodine solution. At the same time two other sets were prepared as above and titrated after addition of 1 ml. of formaldehyde and 2 ml. of 3% hydrogen peroxide, respectively. The results obtained are shown in Table I.

The above run was repeated in duplicate with Green Hungarian wine which contained 77.5 mg. of sulfur dioxide per liter by Ripper method (with hydrogen peroxide correction), 153.6 mg. by the Monier-Williams method, and 114.6 mg. by the iodine distillation method, with the results shown in Table II.

These and other data indicate that when an aliquot of white table wine is brought to slightly above pH 12, liberation of sulfur dioxide is sufficiently rapid to permit acidification and direct titration of the sample after 2 minutes with dilute iodine to obtain results comparable with the Ripper method. The results so obtained, however, are lower than those obtained by the distillation method.

**Effect of Period of Standing.** The effect of varying the period of standing in the presence of alkali was investigated by adding 2, 4, and 10 ml. of 1*N* sodium hydroxide to 25-ml. aliquots of Green Hungarian wine and allowing these to stand for 1 to 30 minutes before acidification with sufficient 1*N* hydrochloric acid to allow an excess of 5 ml. after neutralization of added alkali. The pH values before acidification were 7.22, 11.90, and 12.40, respectively; after acidification they were 1.05, 1.05, and 1.08, respectively. Fifty-milliliter aliquots of wine with 25 ml. of 1*N* sodium hydroxide were allowed to stand for 1 to 30 minutes at pH 12.40, then acidified with 10 ml. of diluted (1 + 3) sulfuric acid and titrated with iodine at pH 1.15; 20-ml. samples of wine treated with 25 ml. of 1*N* sodium hydroxide (at pH 12.135) were allowed to stand for 1 to 30 minutes before acidification with 10 ml. of (1 + 3) sulfuric acid and titration at pH 1.12. A duplicate lot was treated with 2 ml. of 1% hydrogen peroxide before titration and 5 ml. of 1% starch indicator was used. The nonsulfite iodine-reducing matter varied from 0.25 to 0.45 ml. of iodine for the 25-ml. aliquots of wine, 0.50 to 0.70 for the 50-ml. aliquots, and 0.25 to 0.40 for the 20-ml. aliquots. The results (corrected for the nonsulfite-reducing matter present) are shown in Table III.

In another comparative run, 50-ml. aliquots of the Green Hungarian wine after treatment with 25 ml. of 1*N* sodium hydroxide for 5, 10, and 15 minutes, required 12.15, 12.10, and 13.10 ml. of iodine solution. In a run made at the same time 25-ml. aliquots treated with 4 ml. of 1*N* sodium hydroxide for 5, 10, and 15 minutes, required 4.60, 4.70, and 4.50 ml. of iodine solution, respectively. In both instances there was an appreciable increase in iodine-reducing value with time, particularly between 10 and 15 minutes for the Ripper method, which was not accompanied by a noticeable increase in nonsulfite iodine - reducing material. In both these runs, the iodine-reducing values of aliquots made alkaline with excess 1*N* sodium hydroxide (25 ml.

**Table I. Effect of pH on Iodine Titration of Rhine Wine**

| 1N NaOH, Ml. | pH of Mixture | Iodine Solution Required, Ml. | | | $SO_2$ Content[a], Mg./L. |
|---|---|---|---|---|---|
| | | Direct | + HCHO | + $H_2O_2$ | |
| 0 | 3.4 | 1.42 | 0.55 | 0.55 | 22.6 |
| 2 | 7.0 | 1.30 | 0.95 | 0.60 | 18.2 |
| 2.5 | 10.8 | 4.53 | 1.07 | 0.63 | 101.8 |
| 3.0 | 11.8 | 4.50 | 1.25 | 0.64 | 100.6 |
| 3.5 | 12.1 | 5.25 | 1.35 | 0.68 | 119.6 |
| 4.0 | 12.25 | 5.50 | 1.19 | 0.75 | 123.8 |
| 4.5 | 12.3 | 4.95 | 1.64 | 0.73 | 110.0 |
| 5.0 | 12.35 | 4.80 | 1.25 | 0.72 | 106.4 |
| 6.0 | ... | 5.50 | 1.33 | 0.88 | 119.6 |
| 7.0 | ... | 5.70 | 1.22 | 0.84 | 125.2 |
| 8.0 | ... | 5.40 | 1.38 | 0.88 | 119.6 |
| 9.0 | ... | 5.70 | 1.25 | 0.95 | 123.8 |
| 10.0 | 12.45 | 5.70 | 1.37 | 0.95 | 124.0 |
| 20.0 | ... | 5.70 | 1.90 | 1.40 | 112.0 |

[a] Using $H_2O_2$ blanks for correction.

**Table II. Effect of pH on Iodine Titration of Green Hungarian Wine**

| 1N NaOH, Ml. | pH | Iodine Solution, Ml. | | | $SO_2$ Content[a], Mg./L. |
|---|---|---|---|---|---|
| | | Direct | + HCHO | + $H_2O_2$ | |
| 0 | 3.55 | 1.85 | 1.05 | 0.70 | 15.0 |
| 2 | 8.20 | 2.80 | 1.25 | 0.70 | 27.4 |
| 2.5 | 10.35 | 5.40 | 1.30 | 0.75 | 60.6 |
| 3.0 | 11.65 | 6.55 | 1.35 | 0.85 | 74.4 |
| 3.5 | 12.10 | 6.40 | 1.25 | 0.85 | 72.4 |
| 4.0 | 12.30 | 6.35 | 1.10 | 0.95 | 70.4 |
| 4.5 | 12.40 | 6.73 | 1.55 | 0.80 | 77.4 |
| 5.0 | 12.45 | 6.40 | 1.70 | 0.82 | 72.8 |
| 6.0 | ... | 6.55 | 1.30 | 0.80 | 75.0 |
| 7.0 | ... | 6.80 | 1.30 | 0.85 | 76.6 |
| 8.0 | ... | 6.85 | 2.15 | 1.00 | 75.4 |
| 9.0 | ... | 6.85 | 1.75 | 1.10 | 75.0 |
| 10.0 | 12.55 | 6.85 | 1.30 | 1.00 | 75.4 |
| 20.0 | ... | 7.85 | 2.10 | 2.00 | 75.4 |

[a] Using $H_2O_2$ blanks for correction.

**Table III. Effect of Standing at Several pH Levels on Iodine Titration**

| Alkali, Ml. | pH | Storage Period, Minutes: 1 | 2 | 5 | 10 | 15 | 20 | 30 |
|---|---|---|---|---|---|---|---|---|
| | | Iodine solution required, ml., after times shown | | | | | | |
| 2 | 7.22 | 1.95 | 1.94 | 1.61 | 1.82 | 1.84 | 1.86 | 1.75 |
| 4 | 11.90 | 4.50 | 5.10 | 5.20 | 5.52 | 5.65 | 5.80 | 5.90 |
| 10 | 12.40 | 4.35 | 4.65 | 4.95 | 5.45 | 5.40 | 5.55 | 6.03 |
| 25 (50) | 12.40 | 8.35 | 9.50 | 10.35 | 10.40 | 11.80 | 11.75 | 12.50 |
| 25 (20) | 12.34 | 3.00 | 3.60 | 4.22 | 4.20 | 4.50 | 3.90 | 3.95 |

per 50 ml. of wine) were higher (11.80) than with aliquots containing less alkali (4 ml. per 25 ml. of wine), even though these (11.00) were at a pH value only slightly lower.

Fessler (*8*), at the author's suggestion, compared the effect of adding varying amounts of 0.1*N* sodium hydroxide to 10-ml. aliquots of Sauterne wines which were allowed to stand for 1 to 2 minutes, then diluted by addition of 50 ml. of water, acidified with 5 ml. of 6*N* hydrochloric acid, and titrated immediately with 0.025*N* iodine. His data for a Sauterne wine which contained 160 mg. per liter of sulfur dioxide by the iodine distillation method, corrected for nonsulfite iodine-reducing substances, were determined by adding 2 ml. of formaldehyde immediately after acidification and allowing the mixture to stand 10 minutes before titration:

| 0.1*N*, NaOH, Ml. | Time of Standing | $SO_2$ Content, Mg./L. |
|---|---|---|
| 10 | 45 seconds | 115 |
| | 2 minutes | 110 |
| 12 | 45 seconds | 120 |
| | 2 minutes | 120 |
| 15 | 45 seconds | 100 |
| | 2 minutes | 120 |
| 20 | 45 seconds | 110 |
| | 2 minutes | 120 |
| 50[a] | 15 minutes | 110 |

[a] Actually 2 ml. of 10% NaOH equivalent to Ripper method.

The titration for nonsulfite iodine-reducing matter was 0.13 ml. for all except the last case, where it was 0.16 ml. Using 10-ml. aliquots the end point in the iodine titration was sharp and stable, whereas with 50-ml. aliquots the end point faded badly, so that titrations were uncertain and time-consuming. Similar results were found subsequently with the Green Hungarian wine. Aliquots of 50, 25, and 10 ml., treated with 25, 4, and 2 ml. of 1*N* sodium hydroxide for 10 minutes, acidified with 1*N* hydrochloric acid to pH 1.0, gave iodine titration values (calculated on the basis of 100 ml. of wine) of 24.20, 18.80, and 22.0, respectively, but the end point improved as the aliquot was reduced.

On another sample of California Sauterne wine, which contained 100 mg. per liter of sulfur dioxide by distillation into iodine at the start of the runs and 95 at the end of the runs, but the distillate of which contained 20 mg. per liter of nonsulfite-reducing matter (determined by distillation into alkali, acidification, and titration with iodine solution before and after treatment with formaldehyde), Fessler obtained the following results on 10-ml. aliquots treated with 12 ml. of 0.1*N* sodium hydroxide and allowed to stand for 1 to 4 minutes before addition of 50 ml. of water and 5 ml. of 6*N* hydrochloric acid and titration with iodine:

| Minutes Stored | $SO_2$, Mg./L. |
|---|---|
| 1 | 70 |
| 2 | 70 |
| 4 | 70 |
| 15 (0.2 ml. 10% NaOH) | 80 |

With 50-ml. aliquots of this wine treated with 6 ml. of 1*N* sodium hydroxide, the following data for sulfur dioxide content corrected for nonsulfite iodine-reducing matters was found:

| Minutes Stored | $SO_2$, Mg./L. |
|---|---|
| 0.5 | 63 |
| 2.0 | 63 |
| 4.0 | 65 |

The Ripper method on 50-ml. aliquots of this wine gave a corrected value of 65 mg. per liter, which agreed closely with the iodine distillation value of 95 corrected for 20 mg. per liter of nonsulfite-reducing matter.

**Electrometric Titration.** Ingram (*10*) called attention to the fact that starch is not a satisfactory indicator of the end point in iodometric estimation of sulfur dioxide, particularly in citrus juices. The end points are ill defined and difficult to determine with any degree of precision because the naturally occurring pigments interfere with or mask the color change due to starch, substances like acetone added to remove the sulfur dioxide for the determination of reducing power of the juice alter the color change and result in dissimilar end points, and the end points tend to drift because excess of iodine combines with juice constituents or combined sulfite breaks down. Similarly, when wines are treated with the amounts of alkali originally specified, browning of white wines, which occurs during alkali treatment and is not completely reversed on acidification, tends to mask the end point, if insufficient starch or iodide is present. The fading of the end point constitutes a more serious cause of difficulty, but this is reduced by dilution of the wine. The effect of the sulfite binding agent, formaldehyde, on color change also is a factor, but this is reduced when sufficient starch is used. Formaldehyde was used in preference to acetone because the acetone-bisulfite complex is not stable and gives fleeting end points (*24*), so that results are dependent on speed of titration, speed of mixing, strength of iodine solution, etc. Furthermore, as reported by Potter (*24*) for dehydrated cabbage, the use of hydrogen peroxide to oxidize the sulfite to sulfate, instead of addition of a binding agent, results in a sharper and less fleeting end point. Ingram proposed electrometric titration using a bright platinum electrode and a silver–silver chloride reference electrode. Electrometric titration of the sulfur dioxide content of white wine was tried, using platinum-calomel cell electrodes. However, the change in potential in millivolts per milliliter of iodine solution per milliliter of wine was of the order of magnitude of 1, as against 10 to 30 found by Ingram for citrus juices, and the point of inflection was not sharp. For the white Rhine wine the end point in the titration of 25-ml. aliquots for free sulfur dioxide was 1 ml. of iodine solution but was too variable and not satisfactory. For total sulfur dioxide the end point for a 10-ml. aliquot of the same wine occurred at 2.75 ml. of iodine solution. As determined electrometrically, the wine contained 12.48 mg. per liter of free sulfur dioxide in comparison with 9.48 as determined with starch indicator, and 85.8 mg. per liter of total sulfur dioxide as compared with 130.2 by Ripper titration and 110.0 on a 50-ml. sample treated with 10 ml. of 1*N* sodium hydroxide for 2 minutes.

### *Acid-Bleached Fuchsin-Formaldehyde Colorimetric Method*

**Materials and Methods** The sulfur dioxide solutions were prepared from C.P. sodium bisulfite or sodium bisulfite diluted with distilled water and standardized iodometrically not only as the stock solution containing approximately 100 mg. per liter of sulfur dioxide but also at all the levels of dilution used. Fifty-milliliter aliquots of the sulfur dioxide solutions were titrated with 0.01*N* iodine solution, using measured quantities of starch indicator and the titration values corrected for starch and water blank as described previously. All other chemicals used were of reagent grade. Several samples of white table wine were used, analyzed shortly before or at the time of use by direct iodometric titration, iodine distillation, or Monier-Williams methods.

The color reagent was prepared by the procedures given by Grant (*9*) and

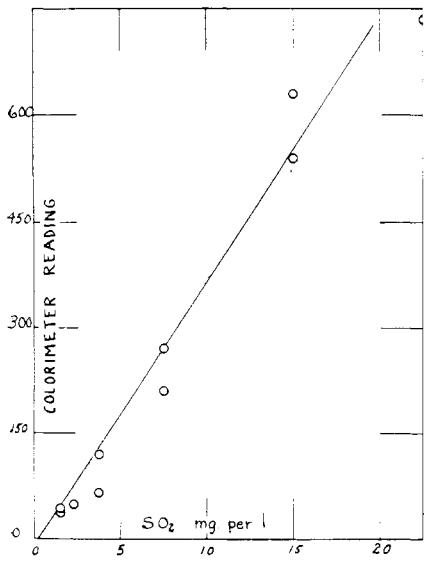



**Figure 2. Klett-Summerson colorimeter reading at various levels of sulfur dioxide content with undiluted Grant's reagent**

Steigman (*31*). Distilled water, C.P. sulfuric acid, and National Aniline & Chemical Co. basic fuchsin (lot NF60, 94% dye content) were used. In the early preparations the basic fuchsin was dissolved in 95% alcohol to make a solution containing 3 grams per 100 ml., but subsequently the basic fuchsin was either added directly to the diluted sulfuric acid or wetted with a few milliliters of alcohol and then added. More stable preparations of Steigman's reagent were obtained by the latter procedure. These were clear at the time of preparation and remained clear without any treatment after several months' storage at room temperature. At times better results were obtained with a freshly prepared diluted formaldehyde solution than with one stored for some time. The Grant color reagent clouded in a few hours and formed a dark sediment after one day. The sensitivity of the clear supernatant liquid was found to be less than that of the unclouded reagent, and because of this most of the data were obtained with the Steigman reagent. This was prepared at two levels of sulfuric acid concentration. Twenty-five and 50 ml. of concentrated (66° Bé.) sulfuric acid, respectively, were added to about 450 ml. of distilled water and after solution and cooling to room temperature, 8 ml. of 3% alcoholic basic fuchsin solution were added and the mixture was brought to 500 ml. in a volumetric flask. The basic fuchsin was added also as 0.100 gram wetted with 5 ml. of 95% ethyl alcohol, to a 500-ml. volumetric flask containing 25 ml. of solution of concentrated (66° Bé.) sulfuric acid in 450 ml. of water and then diluted to volume. Subsequently larger lots of this reagent were prepared by adding 1 gram of basic fuchsin wetted with 50 ml. of 95% ethyl alcohol to 200 ml. of concentrated (66° Bé.) sulfuric acid diluted to almost 2 liters in volumetric flask and bringing this to volume. The range in composition of color reagents used is shown in Table IV. Reagents as prepared remained perfectly clear and stable during over 10 months' storage.

The color reagents were added in various amounts and in various order to aliquots of sulfur dioxide solutions and of white wines, and their color was measured in a Klett-Summerson photoelectric colorimeter using No. 54 green filter.

## Results and Discussion

**Grant's Reagent.** The undiluted reagent, added in the proportion of 4 ml. to 1 ml. of sulfur dioxide solution, did not give satisfactory results with the Klett colorimeter. The reagent itself was too sensitive and the color values were too high for good reproducibility. The reproducibility, as shown in Figure 2, was poor. The blank reading, even with the freshly prepared reagent, was high (200 or over) and on standing the blank rapidly increased. The mixed Steigman reagent, undiluted, also had a high blank reading (4 ml. of reagent plus 1 ml. of water), which varied from 115 to 120 for the freshly mixed reagent to 484 to 500 for a 3-day-old reagent. The range of concentration of sulfur dioxide in which the reagent could be used and the reproducibility of the results were improved by pipetting 5 ml. of the freshly mixed reagent into a 25-ml. volumetric flask, adding 1 ml. of test solution, diluting to volume, and then transferring to a colorimetric tube. However, as shown in Figure 3, under these conditions the sensitivity of the reagent changed materially with storage time. When freshly prepared, the increase in colorimetric reading per increase of 10 p.p.m. of sulfur dioxide was 90 and after storage for 2 weeks or longer, it dropped to about 60. With reagent that was stored, even when only the clear supernatant liquid was used, the colorimeter readings did not increase linearly with increase in sulfur dioxide content of the test solution and the line of closest fit did not go through the origin. For these reasons the use of the Grant reagent was discontinued and most of the work reported was carried out with the Steigman reagent.

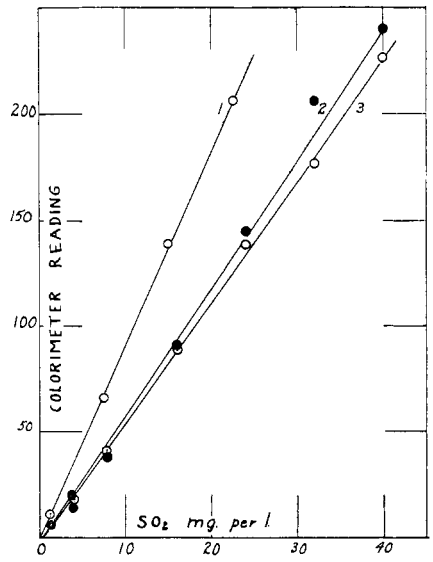



**Figure 3. Colorimeter reading at various levels of sulfur dioxide content with Grant's indicator**

Diluted 5 ml. → 25 ml., with $H_2O$
1. Freshly prepared reagent
2. Reagent after standing for 2 weeks
3. Reagent stored for 81 days

A similar variability with the Grant reagent was found with wine. One-milliliter aliquots of a white table wine added to 5 ml. of Grant reagent and then diluted to 25 ml. gave colorimeter readings varying from 52 to 103, corresponding to a range of 8.0 to 16.0 p.p.m. of free sulfur dioxide (average of five test runs was 11.5). When 10 ml. of this wine were diluted to 100 ml., a 1-ml. aliquot gave a colorimeter reading averaging 19, corresponding to 2.9 p.p.m. of free sulfur dioxide in the diluted wine or 29 in the original. The total

**Table IV. Range in Composition of Color Reagents Used**

| Reagent | Sulfuric Acid[a], N | Formaldehyde, G./100 Ml. | Basic Fuchsin, Mg./100 Ml. |
|---|---|---|---|
| Grant | 1.6 | 0.16 | 48 |
| Steigman | | | |
| I | 3.2 | ... | 48 |
| II | ... | 2.00 | .. |
| Mixed | 2.9 | 0.18 | 44 |
| A | 1.8 | ... | 90 |
| Mixed | 1.6 | 0.18 | 82 |
| B | 3.6 | ... | 90 |
| Mixed | 3.3 | 0.18 | 82 |
| C | 1.8 | ... | 20 |
| Mixed | 1.6 | 0.18 | 18 |
| D | 3.6 | ... | 70 |
| Mixed | 3.3 | 0.18 | 46 |

[a] Assuming that 66° Bé., 96% $H_2SO_4$ was 36*N* and not allowing for contraction in volume on mixing.

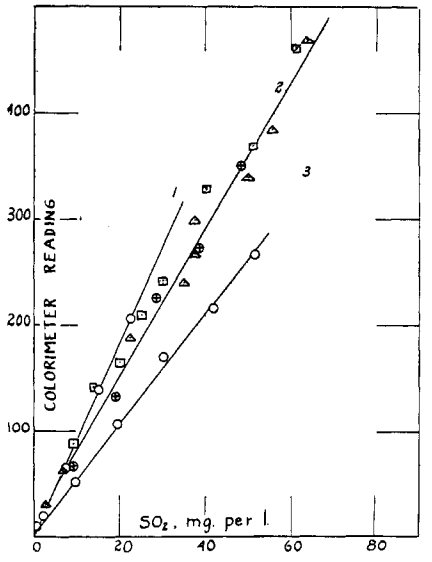



Figure 4. Colorimeter reading at various levels of sulfur dioxide content with Grant and Steigman's reagents

1. Grant reagent
2. Steigman reagent
   - ⊕ Original
   - △ Reagent A
   - □ Reagent A, prediluted
3. Steigman reagent B

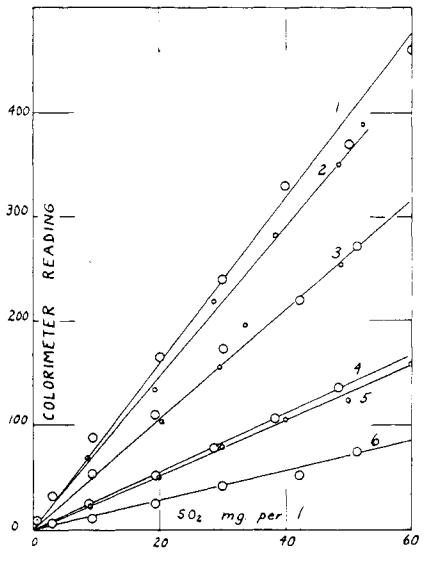



Figure 5. Effect of predilution with acid and water with modified Steigman reagents

1. Reagent C, prediluted with water
2. Reagent A, prediluted wth water
3. Reagent B, prediluted and not diluted with water
4. Reagent A, prediluted with $H_2SO_4$ of same strength
5. Reagent C, prediluted with $H_2SO_4$ of same strength
6. Reagent B, prediluted with $H_2SO_4$ of same strength

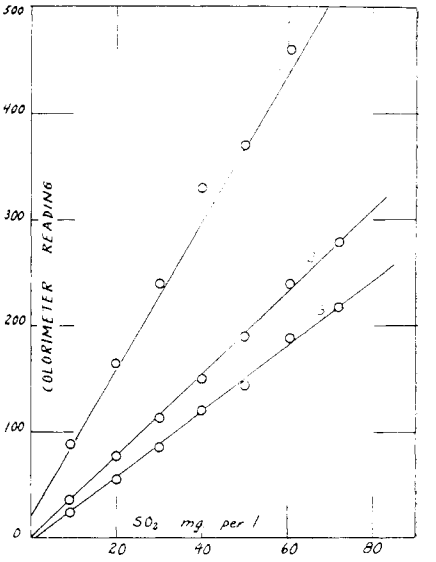



Figure 6. Effect of predilution with acid and water with reagent C

1. Reagent C, prediluted with $H_2O$
2. Reagent C, prediluted with $H_2SO_4$
3. Reagent C, diluted with $H_2SO_4$ after addition of $SO_2$

sulfur dioxide content of a 0.5-ml. aliquot of this wine, determined colorimetrically after treatment for 1 minute with 1 drop of 10% sodium hydroxide corresponded to a color reading of 570 or a sulfur dioxide content of 62.9 p.p.m. The total sulfur dioxide content of a 1-ml. aliquot of the wine diluted 1 to 10, determined after treatment with 0.2 ml. of 10% sodium hydroxide, corresponded to a color reading of 111 or a sulfur dioxide content in the original wine of 12.4 × 10 or 124 p.p.m.

**Effect of Concentration of Sulfuric Acid.** As shown in Table IV, the concentration of sulfuric acid in the mixed reagents used varied from 1.6 to 3.3*N* or in the test runs from 0.32*N* to 0.66*N*. The pH value of the undiluted reagents varied from 0.15 to 0.20 and of the reagent diluted 5 to 25 from 0.50 to 0.80. To determine the effect of this variation in acidity, 5-ml. portions of the reagents were transferred to 25-ml. volumetric flasks, and 1 ml. of standardized sulfur dioxide solution was added, allowed to stand for 5 minutes, then diluted to volume with distilled water and transferred to colorimeter tubes. The results obtained are shown in Figure 4, which also includes data for freshly prepared Grant's reagent. The sensitivity of the reagent decreases appreciably as the acidity is decreased. This effect is ever more pronounced when sulfuric acid of the same concentration as that in the reagent is used as the diluent. As shown in Figure 5, diluting the reagent with acid instead of water markedly decreases its sensitivity.

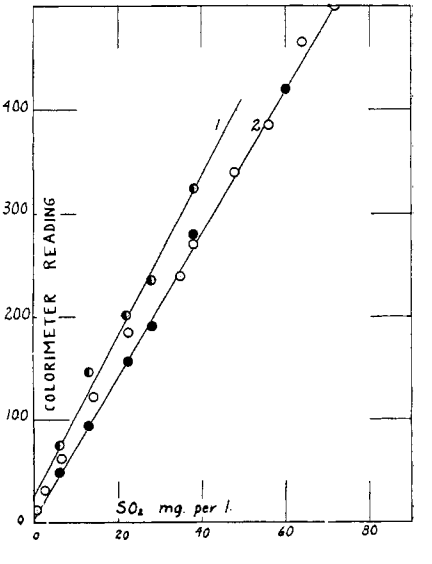
The data shown in Figure 4 were obtained by developing the color in the strongly acid color reagent and then diluting with water. In Figure 5, the color reagent was diluted first with water or acid to almost 24 ml., then 1 ml. of the sulfur dioxide solution was added and the volume was adjusted to 25 ml. Color was developed in less acid solution when water was used as diluent. This effect is shown more markedly with color reagent C in Figure 6. Diluting the color reagent with water before reaction had little effect on color development, but diluting with acid had a marked effect. Thus, developing the color in the reagent first and then diluting with acid reduced sensitivity less than diluting the reagent with acid and then developing the color. Dilution with acid also suppressed red pigment development in the blank. Thus, for reagent C the blank varied from 44 to 68 when dilution was made with water and from 20 to 25 when diluted with acid. The effect of predilution was tested in another run using freshly mixed Steigman's reagent, with the results shown in Figure 7. Here with higher initial acidity and higher fuchsin concentration predilution with water had no effect on color development.

**Effect of Formaldehyde Concentration.** The concentration of formaldehyde in the mixed Steigman reagent is approximately 0.18 gram per 100 ml. With the normal procedure of developing the color by adding 1 ml. of sulfur dioxide solution to 5 ml. of the reagent and then diluting, the slope of the linear plot of color reading *vs.* concentration of sulfur dioxide over the range of 0 to 90 p.p.m. of sulfur dioxide was 4.55. When the concentration of formaldehyde added was doubled, the slope increased slightly to 4.65. When the concentration of formaldehyde was increased tenfold, the mixture reagent itself became dark red in color and no longer was as sensitive to sulfur dioxide.

**Rate of Color Development.** The

Figure 7. Effect of addition of sulfur dioxide before and after dilution

Steigman's indicator
1. Before dilution
2. After dilution



effect of time of standing on development of color with the Steigman reagent at three levels of sulfur dioxide concentration (6.9, 14.4, and 35.2 p.p.m.) was determined by adding 1 ml. of the sulfur dioxide solution to 5 ml. of the reagent and allowing it to stand for various periods of time before diluting to 25 ml. and measuring color. With 6.9 p.p.m. of sulfur dioxide solution, maximum color was reached in 2 minutes and remained constant up to 10 minutes; with 14.4 p.p.m. of sulfur dioxide maximum color formation also was reached in 2 minutes, but with 35.2 p.p.m. of sulfur dioxide 3 minutes were required. When 5 ml. of the reagent were prediluted to ca. 24 ml. with water and 1 ml. of sulfur dioxide solution was added, maximum color development was slower, requiring 5 minutes at the two lower concentrations and 7 minutes at the higher level. Once the maximum color development was reached it was stable for 10 to 20 minutes. Similar results were obtained with color reagents B and C.

**Table V. Effect of Volume of Reagent Added on Colorimeter Reading**

| Reagent, Ml. | Color Reading: Water dilution | Color Reading: Acid dilution |
|---|---|---|
| 1 | 324 | 86 |
| 2 | 296 | 87 |
| 3 | 270 | 96 |
| 4 | 259 | 98 |
| 5 | 242 | 91 |
| 10 | 213 | 124 |

**Effect of Volume of Reagent.** To determine the effect of varying the volume of mixed reagent, 1 to 10 ml. of reagent were pipetted into 25-ml. volumetric flasks, 1 ml. of 43.6 p.p.m. sulfur dioxide solution was added, and after development of color the solution was brought to volume with water or acid solution of the same strength as in the reagent. The color readings, corrected for blanks which varied from 11 to 33 for water dilution and from 11 to 41 for acid dilution, are shown in Table V. The color readings decreased as the volume of reagent added was increased with water as diluent but increased with acid as diluent, although the increase in the range of 1 to 5 ml. of reagent was not so marked or so regular as the decrease in the case of water.

To investigate this change in color formation with increase in volume of reagent, 5 ml. of sulfur dioxide solution were added to 10 and 20 ml., respectively, of reagent and diluted to 100 ml. with water. The resulting color values found are shown in Table VI.

The color reading did not vary consistently with volume of reagent used, being the same at lower concentrations of sulfur dioxide, higher in the range of 17 to 35 p.p.m., and lower at higher levels.

**Effect of Volume of Sulfur Dioxide Solutions.** One to 20 ml. of a solution containing 4.7 p.p.m. of sulfur dioxide were added to 5 ml. of color reagent and after standing for 5 minutes the solution was diluted to volume with water and transferred to colorimeter tubes. The color was compared with that found for 1-ml. portions of sulfur dioxide solutions varying in concentration from 0 to 100 p.p.m. and its equivalent concentration was calculated. This was also done with 1- to 10-ml. portions of an 8.2 p.p.m. sulfur dioxide solution. As shown in Table VII, the concentration found agreed closely with that calculated when 15 ml. or less of solution were added but varied appreciably at higher levels for the more dilute solution. The agreement was poorer with the more concentrated sulfur dioxide solution.

**Application to Wine.** In applying the colorimetric method to wine, color reagent D was used and 1-ml. aliquots were added to 5 ml. of this reagent and then diluted to 25 ml. with water, or 1- or 5-ml. aliquots were added to 20 ml. of reagent and diluted to 100 ml. The calibration curves used are shown in Figure 8. The usual procedure for determining free sulfur dioxide content colorimetrically was to pipet 1 ml. of white table wine into a 100-ml. glass-stoppered volumetric flask, add 20 ml. of reagent freshly mixed with diluted formaldehyde solution, allow to stand for 5 minutes, then dilute to volume, transfer to a colorimeter tube, and read. The colorimeter was adjusted to 0 with a blank of diluted color reagent. The sulfur dioxide content was then taken from a calibration made with a solution of known concentration under conditions used in the test run. The total sulfur dioxide content was determined by adding 3 ml. of 0.1*N* sodium hydroxide to 1 ml. of wine, allowing the mixture to stand for 2 minutes, and then adding 3 ml. of 0.1*N* hydrochloric acid followed by the color reagent. The free and total sulfur dioxide content was determined at the same time by the Ripper or modified Ripper titration. Table VIII is typical of the data obtained.

Figure 8. Effect of volume of sulfur dioxide solution and extent of dilution on colorimeter reading with Steigman reagent D

1. 5 ml. $SO_2$, 20 ml. reagent → 100 ml.
2. 5 ml. $SO_2$, 20 ml. reagent → 100 ml.
3. 1 ml. $SO_2$, 5 ml. reagent → 25 ml.
4. 1 ml. $SO_2$, 20 ml. reagent → 100 ml.

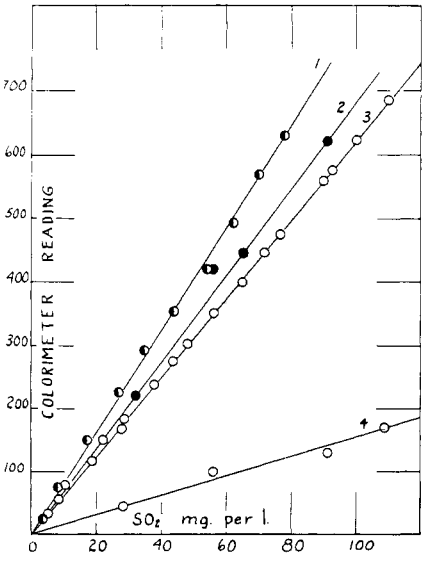



**Table VI. Effect of Reagent Volume on Color Development at Various Levels of Sulfur Dioxide**

| $SO_2$, P.P.M. | Reagent Volume, Ml. 10 | Reagent Volume, Ml. 20 |
|---|---|---|
| 0 | 9 | 21 |
| 0 | 0 | 0 |
| 3.24 | 38 | 37 |
| 7.95 | 78 | 78 |
| 16.95 | 130 | 146 |
| 26.50 | 179 | 224 |
| 34.60 | 241 | 279 |
| 43.70 | 364 | 344 |
| 53.00 | 461 | 421 |
| 61.00 | 509 | 483 |
| 69.50 | 581 | 570 |
| 78.30 | 674 | 630 |
| 88.00 | 781 | 674 |

**Table VII. Effect of Volume of Sulfur Dioxide Solution on Sulfur Dioxide Content**

| $SO_2$ Solution Added, Ml. | Equivalent $SO_2$ Content, P.P.M. A[a] Calcd. | A[a] Found | B[b] Calcd. | B[b] Found |
|---|---|---|---|---|
| 1 | 4.7 | 4.7 | 8.2 | 8.2 |
| 2 | 9.4 | 9.5 | 16.4 | 18.5 |
| 3 | 14.1 | 14.0 | 24.6 | 27.5 |
| 4 | 18.8 | 18.8 | 32.8 | 37.0 |
| 5 | 23.5 | 26.0 | 41.0 | 46.5 |
| 10 | 47.0 | 52.0 | 82.0 | 91.0 |
| 15 | 70.5 | 72.0 | | |
| 20 | 94.0 | 102.0 | | |

[a] With 4.7 p.p.m. of $SO_2$.
[b] With 8.2 p.p.m. of $SO_2$.

**Table VIII. Colorimetric and Iodometric Determination of Sulfur Dioxide in Wine**

| Wine | Free $SO_2$, P.P.M. Colorimetric | Free $SO_2$, P.P.M. Iodometric | Total $SO_2$, P.P.M. Colorimetric | Total $SO_2$, P.P.M. Iodometric |
|---|---|---|---|---|
| A | 17.0 | 9.5 | 100.0 | 130.0 |
| B | 95.0 | 66.5 | 208.0 | 189.0 |
| C | 5.0 | 6.0 | 97.5 | 67.0 |
| D | 66.7 | 30.0 | 183.5 | 371.2 |
| E | 14.3 | 3.8 | 41.6 | 103.8 |

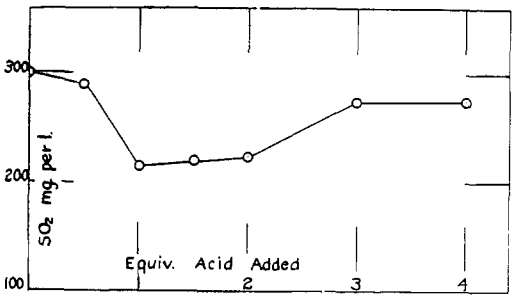



**Figure 9. Effect of addition of hydrochloric acid to wine after treatment with alkali before addition of Steigman reagent and development of color**

The free sulfur dioxide content in all white table wines tested was considerably higher by the colorimetric than by the iodometric method; the total was lower in some cases and higher in others. The recovery of added sulfur dioxide, however, was of the order of magnitude of 90 to 95%. To determine whether this variation was caused by neutralization of the added alkali, several runs were made in which the aliquot of wine used was not acidified or was partly acidified. With wine E the addition of 0, 0.5, and 1 equivalent of acid to the aliquot before addition of color reagent gave values of 64.5, 59.0, and 41.6 p.p.m., respectively. It was surprising that variation of 0 to 3 ml. of 0.1*N* acid (0 to 0.3 meq. of acid) in comparison with 20 ml. of color reagent containing 3.6*N* sulfuric acid (3.678*N* by actual titration) or 73.6 equivalents, would have this effect. This was observed repeatedly. To investigate it in more detail, 1-ml. portions of a freshly standardized sulfur dioxide solution were transferred to 100-ml. volumetric flasks, 3 ml. of 0.1*N* sodium hydroxide were added, followed after 2 minutes by 0 to 5 ml. of 0.1*N* hydrochloric acid and 20 ml. of the color reagent. The colorimeter readings after development of color and dilution to volume (solution A contained 49.9 p.p.m. and B, 206.5 p.p.m.) are shown in Table IX.

The addition of acid resulted in a noticeable decrease in observed sulfur dioxide content in both cases, apparently owing to interference in color development. Higher recoveries were observed without any acidification, and alkali treatment caused appreciable oxidation of sulfur dioxide, particularly at higher levels. The effect of adding varying amounts of 0.1*N* hydrochloric acid to a freshly sulfited white table wine is shown in Figure 9, which is typical of several similar runs. The addition of acid in amounts equivalent to alkali used in hydrolyzing the bound sulfur dioxide caused a marked decrease in sulfur dioxide content found colorimetrically, but this decrease was less as more acid was added. There was a noticeable change in tint of the reagent, but this effect was not investigated in detail.

**Table IX. Effect of Addition of Acid to Sulfur Dioxide Solutions before Development of Color**

| Alkali Added, Ml. | Acid Added, Ml. | Colorimeter Reading A | Colorimeter Reading B | $SO_2$ Content A | $SO_2$ Content B |
|---|---|---|---|---|---|
| 0 | 0 | 79.0 | 315 | 51.4 | 209.5 |
| 3.0 | 0 | 76.0 | 282 | 49.4 | 183.3 |
| 3.0 | 1 | 57.0 | 278 | 37.1 | 181.5 |
| 3.0 | 2 | 53.0 | 240 | 34.5 | 156.0 |
| 3.0 | 3 | 49.0 | 176 | 31.9 | 114.5 |
| 3.0 | 4 | 51.0 | 276 | 33.2 | 179.5 |
| 3.0 | 5 | 38.0 | 255 | 24.7 | 165.8 |

A contained 49.9 p.p.m., and B, 206.5 p.p.m. of $SO_2$.

## Summary and Conclusions

Several of the factors affecting the reproducibility of the Ripper titration for total sulfur dioxide content of white table wine were determined. The end point in the titration was found to be objectionably fleeting even under conditions that were not expected to lead either to an appreciable content of unchanged residual sulfite-addition products or to appreciable rate of hydrolysis of α-hydroxysulfonic acids. The concentration of starch present during titration was found to affect the sharpness of the end point. The speed of titration with dilute iodine also noticeably affected the reproducibility of the results. Dilution of the aliquot used improved the end point. The amount of alkali used and the period of storage could be greatly reduced without noticeably affecting results. Sufficient alkali to adjust the pH of the mixture to 12 resulted usually but not always in liberation of practically all the sulfur dioxide in 2 minutes. Under these conditions oxidation of the liberated sulfite tending to give low results and production of nonsulfite iodine-reducing substances tending to give high results could be reduced. Nonsulfite iodine-reducing substances were found to be present in both the alkali-treated wines and distillates from wines after acidification with hydrochloric acid. These could be estimated by the addition of formaldehyde as a sulfite binding agent or hydrogen peroxide as a sulfite oxidizing agent. The latter gave sharper and less fleeting end points. The direct iodine titrations, however, did not correspond with the direct distillation into iodine or with reflux distillation into hydrogen peroxide. The latter procedure on the two wines examined gave unexpectedly high results. Modified electrometric titration was not found to be an improvement over iodine titration when starch was used as indicator.

Several factors affecting the use of acid-bleached fuchsin for the colorimetric determination of sulfur dioxide were investigated. The concentration of sulfuric acid in the reagent had the greatest effect on sensitivity. For use with the Klett photoelectric colorimeter, the undiluted reagent limited the range of sulfur dioxide content that could be determined and both the range and reproducibility of the reagents could be improved by 1 to 5 dilution after development of color. The Grant reagent, containing added formaldehyde, was not so stable as the Steigman reagent and this was simplified by addition of weighed amounts of fuchsin after wetting with alcohol. The reagent so prepared was stable for several months. The results obtained were noticeably influenced by conditions of use, but when the order of addition of the sulfur dioxide and reagent and condition of color development were controlled, reproducible results could be obtained. As applied to wine, however, the colorimetric method gave appreciably higher values for free and lower values for total sulfur dioxide.

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**WINE PRODUCTION**

# Skin Pigments of the Cabernet Sauvignon Grape and Related Progeny

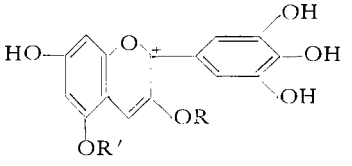
**A. H. BOCKIAN[1], RICHARD E. KEPNER, and A. DINSMOOR WEBB**

**Department of Chemistry and Department of Viticulture, University of California, Davis, Calif.**

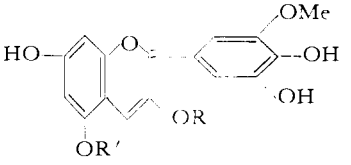
The skin pigments of the Cabernet Sauvignon grape have been separated by paper chromatography and identified as malvidin, two glucosides of malvidin, a petunidin glucoside, a delphinidin glucoside, and a complex diglucoside of malvidin. Evidence is presented to indicate that the two malvidin glucosides are the 3-glucoside and the 3,5-diglucoside, and that the delphinidin and petunidin pigments are the 3,5-diglucosides. The malvidin diglucoside was the most abundant. The pigments of progeny of Cabernet Sauvignon and Carignane grapes were qualitatively identical with those of the Cabernet Sauvignon. The order of appearance of the anthocyan pigments in the maturing Cabernet Sauvignon grape was: malvidin diglucoside, malvidin monoglucoside and delphinidin glucoside, petunidin glucoside, and free malvidin, contrary to the hypothesis that in the developing fruit or flower anthocyanins are formed at the expense of the corresponding anthocyanidin.

THE VISUAL APPEAL of many table grape varieties and many wines depends upon the attractive red colors of the anthocyan pigments present. The term "anthocyan" is used here to refer to both anthocyanin- and anthocyanidin-type pigments. Among the many factors influencing the shade and intensity of the red color, perhaps the most important is the composition of the pigment mixture in the grape or in the wine. Classical analytical techniques have often given incomplete information concerning the pigment mixtures. A detailed knowledge of the composition and inheritance pattern of the skin pigments in grapes is of particular value to the geneticist in the development of new grape varieties most likely to show desirable color characteristics.

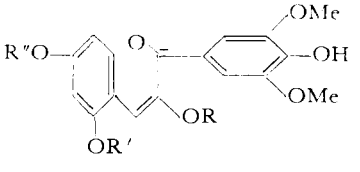
The first precise investigations of grape pigments were reported by Willstätter and Zollinger (*18, 19*), who characterized the anthocyan pigments of the "dark blue north Italian grape" (*V. vinifera*) as mainly "oenin" (malvidin-3-glucoside) with small amounts of the aglycone, malvidin, and malvidin diglucoside. These authors also presented evidence (*19*) that the skin pigments of *V. riparia* consisted mainly of the glucoside of a monomethyl derivative of delphinidin and a small amount of a dimethyl delphinidin derivative.




R = R′ = H, delphinidin
R′ = H, R = glucose, delphinidin-3-glucoside
R′ = R = glucose, delphinidin-3,5-diglucoside




R = R′ = H, petunidin
R′ = H, R = glucose, petunidin-3-glucoside
R = R′ = glucose, petunidin-3,5-diglucoside




R = R′ = R″ = H, malvidin
R = R′ = H, R″ = Me, hirsutidin
R′ = R″ = H, R = glucose, malvidin-3-glucoside (oenin)
R″ = H, R = R′ = glucose, malvidin-3,5-diglucoside (malvin)

[1] Present address: Department of Food Technology, Oregon State College, Corvallis, Ore.