

**SCHEME FOR THE SEPARATION OF THE EIGHT PERMITTED COAL-TAR DYES:**  
 Amaranth, Tartrazine, Erythrosin, Naphthol Yellow S, Light Green  
 S F Yellowish, Orange I, Indigo Disulfonic Acid and Ponceau 3 R

Rub a small quantity of the mixed dyes in an evaporating dish with supersaturated solution of  $(\text{NH}_4)_2\text{SO}_4$ , filter and wash with the supersaturated solution of  $(\text{NH}_4)_2\text{SO}_4$  until the washings are no longer red

**FILTRATE** contains *Amaranth*, *Tartrazine* and a small quantity of *Naphthol Yellow S*. Shake with acetic ether. Acetic ether removes *Naphthol Yellow S*. This solution is discarded.

**$(\text{NH}_4)_2\text{SO}_4$  SOLUTION** contains *Amaranth* and *Tartrazine*, which are removed by shaking with acetone. Dilute the acetone solution with  $\text{H}_2\text{O}$  and drive off the acetone. Supersaturate the aqueous solution containing *Amaranth* and *Tartrazine* with  $\text{NaCl}$ , filter, and wash with supersaturated  $\text{NaCl}$  solution until filtrate is no longer yellow

**FILTRATE** contains *Tartrazine*. Add excess of conc.  $\text{HCl}$  or glacial acetic acid and shake with acetone. Dilute the acetone solution with  $\text{H}_2\text{O}$ . Drive off the acetone and *Tartrazine* remains

**RESIDUE** contains *Amaranth*. Add excess of conc.  $\text{HCl}$  or glacial acetic acid and shake with acetone. Dilute the acetone solution with  $\text{H}_2\text{O}$ . Drive off the acetone and *Amaranth* remains

**RESIDUE** contains *Erythrosin*, *Naphthol Yellow S*, *Light Green*, *S F Yellowish*, *Orange I*, *Ponceau 3 R* and *Indigo disulfonic acid*. They are separated according to Price's scheme as given in *Circ. 180*, Bureau of Animal Industry, U. S. Dept. Agr.

The aqueous solution containing *Amaranth* and *Tartrazine* is supersaturated with sodium chloride, filtered, and residue and filter paper washed with the supersaturated solution of sodium chloride until washings are no longer colored yellow. The filtrate and washings contain *Tartrazine*, while the undissolved portion contains *Amaranth*. *Tartrazine*<sup>1</sup> is separated in the pure state from the sodium chloride solution by adding an excess of either glacial acetic acid or concentrated hydrochloric acid and extracting with acetone. The acetone is evaporated off on a steam bath, which leaves the *Tartrazine*<sup>2</sup> in a pure condition. *Amaranth* is obtained in a pure state by adding to the residue an excess of either glacial acetic acid or concentrated hydrochloric acid and extracting with acetone. The acetone is evaporated off on a steam bath, which leaves the *Amaranth* in a pure condition.

UNIVERSITY OF KANSAS  
 LAWRENCE, KANSAS

**SWEET WINES OF HIGH ALCOHOL CONTENT WITHOUT FORTIFICATION<sup>3</sup>**

By W. V. CRUESS, E. M. BROWN AND F. FLOSSFEDER  
 Received June 30, 1916

Port wine, as made in California, normally contains over 20 per cent alcohol by volume and California sherry over 18 per cent. Under ordinary conditions, the must, or crushed grapes, is allowed to ferment until 6 to 10 per cent alcohol is formed and the alcohol content is then increased to the desired point by the addition of 180 proof (90 per cent) grape brandy. Until the past season, a tax of 3 cents per proof gallon was paid on this fortifying brandy. Owing to the necessity for increased revenue, the U. S. Government in 1915 increased the tax from 3 cents per proof gallon to 55 cents per proof gallon.

If the fortified sweet wines were to be made in the usual way under this heavy tax, the cost of production would be prohibitive. Two methods were used by

<sup>1</sup> An extraction of about 98 per cent has been made.

<sup>2</sup> For the reactions which are used to identify the separated colors, see Allen's "Commercial Organic Analysis," Vol. V, 4th Ed.

<sup>3</sup> This interesting treatise is given in full with the exception of much of the tabulated matter which to our regret could not be included on account of lack of space. The comment on this tabulated matter makes the article sufficiently clear in itself. [EDITOR'S NOTE.]

California wine makers last season to avoid the tax entirely or to decrease the amount of brandy used in fortification. By one method, grapes of high sugar content were used. By employing selected pure yeast, potassium metabisulfite to eliminate wild yeasts or bacteria, and by keeping the temperature of the fermenting must down by artificial cooling to the optimum for fermentation, wines of approximately 15 per cent alcohol were produced. Where so much alcohol is formed by fermentation, only a small amount need be added in the form of fortifying brandy to produce wines of the required alcohol content. Where the wine is to be fortified, it must not contain more than 15 per cent alcohol by volume before fortification to comply with the U. S. Revenue Regulations.

By a second method, sweet wines of high alcohol content were made without fortification. The method consists in adding grape syrup to the must during fermentation. It is claimed that wines of over 18 per cent alcohol were produced in this way. To obtain data on this process, the following four experiments were carried out (tables omitted).

**EXPERIMENT 1789—SWEET WINE, SHERRY TYPE, NOT FORTIFIED, SYRUP ADDED DURING FERMENTATION**—Very ripe Semillon grapes of 27.3 Balling or Brix degree were used. The juice was allowed to settle 24 hrs. to clear partially and it was then inoculated with pure Burgundy wine yeast; 75 mg. of  $\text{SO}_2$  per liter were used before settling of the juice to check growth of wild yeasts during this period. The fermentation was carried out at a room temperature of 21–25° C. in a 5-gal. keg. Grape syrup of 60 per cent Balling was added near the end of fermentation. The wine was racked after fermentation and stored in glass demijohns.

**EXPERIMENT 1821—SWEET WINE, PORT TYPE, WITHOUT FORTIFICATION, SYRUP ADDED DURING FERMENTATION**—This lot was made in the laboratory in a 5-gal. barrel. Red grape juice from experimental juice made at Davis in 1914 was used. A syrup made by boiling down a mixture of red juice and raisin juice to 65 per cent Balling was added during fermentation. The wine was roughly filtered after fermentation and stored in a well filled and corked demijohn.

**EXPERIMENT 1966b—SWEET WINE, SHERRY TYPE, NOT FORTIFIED, RAISINS ADDED DURING FERMENTATION**—This experiment was carried out at the University Farm at Davis. Ripe Semillon grapes were crushed and pressed. The juice was given 75 mg.  $\text{SO}_2$  per liter and allowed to settle 24 hrs. It was racked and inoculated with pure yeast: 100 lbs. Semillon grapes were sun-dried to 63 lbs., crushed, and added to the fermenting must near the end of the fermentation. The mixture was later pressed and fermentation allowed to proceed at room temperature for a time and the final fermentation was carried out in a room kept at 23° C. The wine was stored several weeks at this temperature to subject it to extreme conditions favorable to spoilage.

**EXPERIMENT 1973—SWEET WINE WITHOUT FORTIFICATION, PORT TYPE, SYRUP ADDED DURING FERMENTATION**—This wine was made in practically the same way as Wine 1821. Grape syrup from Burger grapes and made by vacuum evaporation on a commercial scale was used to add during fermentation. Only one addition of syrup was made. The wine was racked clear after fermentation and stored in a well-filled 5-gal. demijohn.

The results reported below demonstrate that a sound sweet wine of over 18 per cent alcohol can be made by the addition of syrup during fermentation, provided the wine is filtered or racked clear after fermentation and stored in clean, well-filled containers.

## ANALYSES OF EXPERIMENTAL SWEET WINES: After Storage the Wines were Analyzed and Tasted with the Following Results

Wine	Alcohol	Volatile Acid	Total Acid	Sugar	OBSERVATIONS
1789(a)	18.20	0.030	0.80	7.5	Excellent "sherry" flavor
1966b	19.9	0.406	1.20	2.7	"Tourne" bacteria. Spoiled
1821	18.40	0.076	1.10	11.40	Condition and flavor excellent
1973	18.30	0.060	1.00	14.40	Good "port" flavor

(a) At one time during fermentation Wine 1789 reached 20.1 per cent alcohol but the addition of syrup later reduced this somewhat.

Experiment 1966b shows that raisins may be used instead of grape syrup to increase the sugar in the fermenting must and to invigorate the yeast. It also indicates the danger of spoilage of such wine unless precautions are taken to get it clear after fermentation and store it in full containers. In spite of its alcohol content of 19.9 per cent, this wine was completely spoiled by the growth of "tourne" bacteria (*Bacterium manniopaeum*). It is a well-known fact that fortified wines of 19 per cent alcohol or over are not readily attacked by this organism although it is found living in wines of over 20 per cent alcohol. It is possible that alcohol formed by fermentation in the wine is less toxic than that distilled and added to the wine; that is to say, perhaps aldehydes or other compounds formed during distillation may increase the toxicity of the alcohol to the organisms of wine. Experiments made in 1915 on this point bear out the above statement.

The authors also have results of experiments showing the effect of a number of additions of syrup during fermentation: (a) one addition of syrup; (b) three additions of syrup; (c) four additions (tables omitted). These tests and data obtained from a number of similar experiments indicate that the number of additions of syrup does not materially affect the result if the additions are made before the yeast has become weakened.

## MAXIMUM ALCOHOL FORMED BY BURGUNDY WINE YEAST BY NORMAL FERMENTATIONS

In the following experiment, musts varying from 28.3 Balling to 43.9 Balling were made up by the addition of grape syrup to grape must. The different lots were inoculated and kept at room temperature until fermentation ceased. They were then analyzed for alcohol content.

RESULTS IN BALLING DEGREES				
Must	I	II	III	IV
Jan. 14.....	31.0	35.7	39.8	43.9
Jan. 17.....	11.0	35.5	38.5	41.6
Jan. 18.....	5.4	35.5	...	41.6
Jan. 19.....	4.0	34.5	37.5	41.6
Jan. 20.....	1.8	34.5	26.7	...
Jan. 24.....	...	25.5	26.7	...
Jan. 29.....	...	15.6	...	...
Jan. 31.....	...	...	...	...
Feb. 1.....	...	13.7	24.0	29.6
Feb. 9.....	...	13.0	22.8	27.4
Mar. 8.....	...	...	...	...
Apr. 5.....	...	13.0	...	...

## ANALYSIS OF WINES I-IV, APRIL 5, 1916

WINE	I	II	III	IV
Alcohol (per cent).....	17.2	15.2	11.4	11.3

From these results, it may be seen that musts of 35 Balling and above are not fermented to give very high yields of alcohol (see II, III and IV). The alcohol yield in I is remarkable and is about 0.6 per cent higher than is usually obtained with this yeast as a maximum by the usual "straight" fermentation of sweet must. It is probable that high concentrations,

e. g., 35 per cent Balling or over, interfere seriously with the growth of the yeast, giving a smaller crop of yeast or a weaker yeast than normally develops in musts of lower sugar concentration. The formation of 17.2 per cent alcohol by a single straight fermentation of must of 31 Balling has little practical value for unfortified sweet wine because the addition of syrup will be necessary after fermentation and would reduce the alcohol content so that the wine would be liable to spoilage. Therefore, the syruing method of fermentation is better because it gives high alcohol yields and also gives a wine with sufficient sugar for a sweet wine of Port or sherry type.

## USE OF SAKÉ YEAST

Saké yeast is used in Japan for the fermentation of rice mash in the manufacture of Japanese saké beer. It is accredited with the ability to form 22 per cent alcohol in rice mash. Wine yeast is described in the literature as being able to form in must not more than 16.5 per cent alcohol by volume.

The saké yeast has grown in grape must several generations to accustom it more or less to the new medium. Straight fermentation and fermentations to which syrup was added were tried with this yeast but 15.2 per cent alcohol was the maximum formed.

## SAKÉ YEAST ALCOHOL YIELDS

	Per cent
Alcohol by "straight" fermentation.....	13.3
Alcohol by "syrued" fermentation, one addition of syrup.....	15.2
Alcohol by "syrued" fermentation, two additions of syrup.....	14.6

Apparently the saké yeast does not become easily acclimated to grape must.

## TESTS TO DETERMINE NATURE OF THE SUBSTANCE IN GRAPE SYRUP WHICH STIMULATES YEAST TO HIGH ALCOHOL FORMATION

Invert sugar syrup, made by hydrolyzing cane sugar with HCl and neutralizing with KOH, was added to must during fermentation but gave no increased alcohol yield. Ammonium salts gave no definite effect. The dealcoholized extract from fermented must did not give the desired result. Evidently growth of yeast and fermentation use up the invigorating substance. The ash of must gave no definite result. Phosphates seemed to increase the alcohol yield, but not markedly so. The experiments on this point are so far more or less negative and indefinite.

## SUMMARY AND CONCLUSIONS

I—In four small scale fermentations representing five or more gallons each, wines of over 18 per cent alcohol were made by fermentation of grape must by "Burgundy" wine yeast and the addition of grape syrup during fermentation. One of these wines reached 19.9 per cent alcohol.

II—The maximum alcohol obtained by a "straight" fermentation with the same yeast was 17.2 per cent; in most cases, the maximum was 16.6 per cent or less by this method. The fact that the syruing method in practically all cases gave wines of 18 per cent alcohol or over shows that the exceptionally high yields are due to the addition of the syrup.

III—Tests made to throw light on the character of the invigorating compound of the syrup showed

that it was not the sugar of the syrup, but did not give any definite evidence as to what the compounds causing the increased activity of the yeast might be.

IV—Partially dried grapes may be used instead of syrup during fermentation to increase the sugar content and invigorate the yeast. Therefore, no expensive evaporating system is necessary in the application of the new method.

V—Where the wine made by the new method was not filtered and carefully handled, it was spoiled by the growth of "tourne" bacteria. Where it was racked clear or filtered after fermentation and kept in well-filled packages, it kept well and developed an agreeable "rancio" or "sherry" flavor.

Whether the method will become important commercially remains to be seen. It was applied on a large scale during the past season by several large companies in California, but the results of this work are not available for publication.

VITICULTURE DIVISION  
UNIVERSITY OF CALIFORNIA, BERKELEY

### TOTAL CARBON IN SOIL BY WET COMBUSTION

By C. J. SCHOLLENBERGER  
Received August 23, 1916

The determination of total carbon in soil is necessary in certain soil investigations. Of the several methods used, that of combustion in a furnace, absorption and weighing of the carbon dioxide obtained is probably the most accurate, provided the necessary precautions are observed. The latter include not only the complicated purifying and drying train common to all gravimetric carbon determinations, but a second combustion tube with copper oxide or some similar arrangement to insure complete oxidation of volatile carbon compounds which may be distilled off before a temperature sufficiently high for complete combustion has been attained. A further precaution necessary in the case of soils high in carbonate carbon is a determination of carbonate in the residue after the ignition.

In this connection it may be well to call attention to the fact that the wet combustion-volumetric method described by Ames and Gaither<sup>1</sup> is capable, with slight changes, of affording results which compare very favorably with those obtained from a furnace combustion and gravimetric determination. The changes referred to consist in the use of a mixture of phosphoric and sulfuric acids, instead of sulfuric acid alone, with chromic anhydride as the oxidizing agent, in the substitution of barium hydroxide for sodium hydroxide as the alkaline absorbent for carbon dioxide, and in the replacement of the modified Camp absorption tube described in the original article by Meyer's absorption apparatus or Truog's bead tower,<sup>2</sup> together with changes in procedure made necessary by the changes in reagents employed.

The apparatus used and the method of operation are practically the same as those described in the article first cited, with the changes as noted; the absorption apparatus being filled with the alkaline absorbent, a gentle suction is turned on to facilitate the

addition of the reagents. To the sample (1 to 3 g.) in the boiling flask is added 10 cc. of the oxidizing mixture made as follows: chromic anhydride 85 g. dissolved in 100 cc. water and diluted to 250 cc. with 85 per cent phosphoric acid. The 10 cc. portion of oxidizing mixture is followed by 25 cc. 85 per cent phosphoric acid and by 25 cc. concentrated sulfuric acid. The mixture is heated rapidly at first, but with a lower flame when the reaction is well started; after the chromic acid is largely decomposed, a more intense heat may be used, but very hard boiling is unnecessary; the heating should be continued about 30 min. after boiling begins. A current of purified air is aspirated through the apparatus in volume just sufficient to prevent the hot acid mixture being forced up the stem of the separatory funnel for addition of reagents. The apparatus should be so arranged that the condensed water will run down the outside of the stem of this funnel and not drop directly into the hot acid; this simple precaution will prevent much trouble and breakage of apparatus.

The partial substitution of phosphoric for sulfuric acid reduces to a negligible quantity the fuming noticed when sulfuric acid alone is used; heavy fumes of sulfuric acid are objectionable, not only for the reason that they cause a consumption of the alkaline absorbent, but because they prevent complete absorption of carbon dioxide. Phosphoric acid alone was tried, but invariably gave results too low; a certain amount of sulfuric acid is absolutely necessary to insure complete oxidation. The determination is completed by titrating the barium carbonate as described by Cain<sup>1</sup> or by titrating the excess of barium hydroxide as described by Truog.<sup>2</sup>

The latter method, while not absolutely accurate under all conditions, as was pointed out in a former article<sup>3</sup> is sufficiently so for most purposes and is very much more rapid and convenient; with either method, the solutions used should be standardized against a sample of known carbon content. Blanks should be run for carbon in reagents, etc.

PER CENT TOTAL CARBON IN SOIL BY SEVERAL METHODS					
Sample	1	2	3	4	5
Furnace combustion, Gravimetric, . . . .	0.94	2.69	1.13	0.88	2.95
New wet combustion, Volumetric, . . . .					
Ba(OH) <sub>2</sub> , . . . . .	0.94	2.66	1.13	0.87	2.84
Old wet combustion, Volumetric, . . . .					
NaOH, . . . . .	0.90	2.62	1.10	0.80	2.72
Parr method, . . . . .	0.83	2.66	1.10	0.97	..

In the table are presented results obtained on five samples of soil by the gravimetric method (combustion with copper oxide in combustion tube, absorption and weighing of carbon dioxide), by the wet combustion-volumetric method as modified, titrating excess barium hydroxide without removal of carbonate, by the wet combustion-volumetric method as originally described, and by the Parr method of combustion in bomb with sodium peroxide and measurement of carbon dioxide in gas burette. The results tabulated are from the averages of at least two closely agreeing determinations.

OHIO AGRICULTURAL EXPERIMENT STATION  
WOOSTER, OHIO

<sup>1</sup> THIS JOURNAL, 6 (1914), 465.

<sup>2</sup> *Ibid.*, 7 (1915), 1045.

<sup>3</sup> *Ibid.*, 8 (1916), 427.

<sup>1</sup> THIS JOURNAL, 6 (1914), 561.

<sup>2</sup> *Ibid.*, 7 (1915), 1045.