C.S. OUGH
M.A. AMERINE

# METHODS FOR ANALYSIS OF MUSTS AND WINES

Second Edition

# Methods for Analysis of Musts and Wines

## SECOND EDITION

**C. S. Ough and M. A. Amerine**
University of California


**A Wiley-Interscience Publication**
**JOHN WILEY & SONS**
**New York · Chichester · Brisbane · Toronto · Singapore**

***Library of Congress Cataloging in Publication Data***

Ough, C. S.
Methods for analysis of musts and wines.

Rev. ed. of: Methods for analysis of musts and wines / M.A. Amerine and C.S. Ough. c1986.
"A Wiley-Interscience publication."
Includes index.
1. Wine and wine making—Analysis. I. Amerine, M. A. (Maynard Andrew), 1911- . II. Amerine, M. A. (Maynard Andrew), 1911- . Methods for analysis of musts and wines. III. Title.

TP548.5.A5085 1988 663'.2 87-28004
ISBN 0-471-62757-7

Printed in the United States of America
10 9 8 7 6 5 4 3 2 1

# Methods for Analysis of Musts and Wines

## SECOND EDITION

# PREFACE

The first edition of *Methods for Analysis of Musts and Wines* appeared in 1980. It is a fact that since then many of the procedures needed and being used in our laboratories have changed. Three examples are increased use of HPLC and GC, sometimes coupled with mass spectroscopy; automation of dispensing, recording, and calculation of results; and greater attention to statistical analysis of the results. These and other changes will no doubt continue. For this reason we have given an outline of some new procedures, since they will surely be modified or changed in the future. However, the experienced analyst should be able, from our text, to determine whether or not the procedure is of interest to his or her laboratory. We have also deleted methods that no longer seem appropriate or necessary.

One other significant change should be noted: the increased interest of regulatory agencies (and of the public) in the composition of wines. Some of this was no doubt stimulated by the European wine frauds involving diethylene glycol and methanol, but more so by the greater awareness and knowledge due to consumer concerns. There is still a continuing interest in the detection and measurement of minute amounts of pesticide degradation products or of the pesticides themselves. Also, trace amounts of toxic compounds or carcinogens produced naturally have become a more important concern. Whatever the source of the interest of the regulatory agencies, the wine analyst must be prepared to use the most sensitive procedures, no matter how time consuming and, alas, expensive they may be. One cannot, unhappily, predict that these analysts would discover and correct inappropriate production practices that result in undesirable residues in wines *before* government agencies discover them and require their regulation.

Finally, although we have cited hundreds of research papers on wine analysis, more are appearing. We strongly recommend that wine analysts regularly read, or consult via computers, the appropriate sections of *Chemical Abstracts* and *Food Science and Technology Abstracts.*

C. S. OUGH
M. A. AMERINE

*Davis, California*
*December 1987*

# Methods for Analysis of Musts and Wines

## SECOND EDITION

# CONTENTS

# INTRODUCTION

The physical, chemical, microbiological, and sensory analyses of musts and wines have become the cornerstones of quality control for the grape juice and wine industries. Every aspect of their production has to be controlled by microbiological, chemical, and sensory tests to reduce spoilage, determine the best and least expensive methods for handling the product, and ensure the highest quality. This extends to field tests during ripening, as well as to continuous controls during fermentation, processing, and aging.

Furthermore, legal controls on many of the constituents of wine, including ethanol, sulfur dioxide, and volatile acidity, require precise analytical results. The presence of prohibited compounds such as monochloracetic acid can be determined, and the presence of excessive amounts of a compound due to procedure or treatment (e.g., thujone in vermouth or sodium in cation-exchanged wines) may need to be established. Enological ratios have been established to prevent undue or illegal use of water, sugar, alcohol, or blending. A variety of ratios to detect sophistication based on analysis of 2000 wines is available (1). A number of others are found in the text. Often there is a need to determine rare constituents, sometimes present in only a few parts per billion. Time constraints and the small amounts to be determined may make these analyses expensive.

Modern wine making often involves blending. Here again analysis can be an important aid in achieving uniformity. The major types of commercial wine can and should be standardized by chemical analysis of critical components. Simultaneous sensory analyses are, of course, essential. As more and more of the components of wines are identified, the possibility of using analytical results to aid in predicting quality becomes difficult as the number of factors influencing "quality" increase. The whole concept of quality is so personal that precise definitions that have some meaning are difficult. This is not to say that within certain ranges and for major chemical differences some standards cannot be established. For example, if *brut* champagne is defined as a wine with less than 1.5% sugar, then that defines *brut* as far as sugar content is concerned. Recently, in order to protect certain appellations of origin, chemical proofs of their

origin using statistical methods have been used. Although the differences in concentration of lithium, and so on, in the wines of one district as compared to another district may establish origin, we would be happier if some statistically significant sensory differences were established.

Chemical analysis is also a useful tool in preventing losses in quality and quantity during operations and by-product recovery. Both microbiological and sensory procedures should, of course, also be used.

The selection of the proper method for analysis is not always easy. Should one use a simple, rapid, and insensitive procedure or a slower but more accurate one?

At least two principles govern the answers to these questions. The first concerns the degree of accuracy required. For many routine control purposes a relatively imprecise procedure is an adequate guide for winery practice. For example, the amount of free sulfur dioxide in a white table wine during aging need be known only approximately. On the other hand, at the time of bottling a more accurate determination is required. In research, even more precise procedures may be needed.

Second, choice of a method depends to a large extent on the number of analyses done in a laboratory. In a large laboratory doing hundreds of iron and copper analyses each year, automated atomic absorption spectrophotometry or another, similar, large-scale procedure is preferable. On the other hand, a small laboratory making only a few metal analyses each year could not justify such an outlay and would choose some other procedure, even though it required more time.

A laboratory that must make 10 or 20 alcohol determinations every working day may choose different procedures and equipment as compared to one where only a few determinations are done once a week, once a month, or only once or twice a year. It is quite possible that in very small wineries only a few essential chemical determinations are carried out. More complicated determinations may be done more rapidly and cheaply by commercial laboratories. In research work, very small differences between constituents may be important, particularly in rate reactions. Obviously the most accurate procedures should be employed here, but such methods are often inappropriate for routine winery analyses.

The more precise procedures are often called "reference" or "standard" as contrasted with "routine" or "ordinary" methods. Collaborative analyses of California wines by winery laboratories in 1965 and 1975 (2) showed considerable technician carelessness to be a factor in poor results from some laboratories. Improved results were obtained in 1976. The problem is not unique to California wineries. In collaborative studies of the American Association of Official Analytical Chemists (AOAC), aberrant (outlier) results are sometimes obtained, despite the use of "official" procedures. This was also true in recent

European collaborative studies (3–5). The importance of a chemical analysis should not be over- or underemphasized. The results should have some useful purpose in winery operation and should comply with winery or official standards. To avoid an important and necessary analysis simply because it is difficult is irrational.

As an example of the increasingly exotic procedures being applied to foods, it is now standard to determine the concentration of $^{14}C$. The normal $^{14}C$ content of the atmosphere decreases slowly with time. Thus fossil materials are very high in $^{14}C$. It was increased by the nuclear explosions in the atmosphere in the 1960s. Some countries have set minimum limits on $^{14}C$ to prevent use of ethanol produced from fossil fuels in alcoholic beverages. Lower limits may be needed for alcoholic beverages produced in the Southern Hemisphere, since few nuclear explosions occurred there (6, 7). A similiar problem occurs in detecting whether spirits are made from a forbidden raw material. Grapes and grains are $C_3$ plants, whereas sugar cane, corn, and sorghum are $C_4$ plants. $C_4$ plants are higher in $^{13}C$ than are $C_3$ plants. Thus whiskeys, which must be made of grains and which are high in $^{13}C$, are probably at least partially made from molasses or corn syrup (8).

There are a number of texts on wine analyses (9–22). Many indicate sources of error, especially Tanner (23).

German laws require considerable laboratory control not only of the finished wine but also of the musts. Practical and rapid methods were summarized (24). Jaulmes (25, 26) has reviewed the history of the official French methods for wine analysis. He emphasized the importance of accurate analyses to prevent sale of sophisticated or spoiled wines. A summary of the Receuil des Methodes International du Vin (OIV) and the European Economic Commission (EEC) methods for wine analyses now in place has recently been presented (27). The components considered and some of the types of analyses used is given in the following tabulation.

| | OIV[a,b] | EEC[a] | | OIV[a,b] | EEC[a] |
|---|---|---|---|---|---|
| Density at 20°C | | | Malic acid | | |
| Pycnometer method | R | R | Ion exchange separation | | |
| Hydrometer method | U | U | and colorimetric | | |
| Hydrostatic balance | U | U | determination | U | — |
| Alcohol distillation and | | | Lactic acid | | |
| Pycnometer method | R | Q | Ion exchange separation | | |
| Hydrometer method | U | U | and colorimetric | | |
| Hydrostatic balance | U | U | determination | U | U |
| Refractometry | U | — | | | |
| Dichromate oxidation | S | | | | |

| | OIV[a,b] | EEC[a] |
|---|---|---|
| Total acidity | | |
| Potentiometric titration to pH 7 | R | R |
| Titration to pH 7 with an indicator | U | U |
| Volatile acidity | | |
| Steam distillation and volumetric titration | S | S |
| pH | | |
| Potentiometric measurement | S | S |
| Fixed acidity | | |
| Total acidity less volatile acidity | S | S |
| Extract | | |
| Vacuum distillation at 70°C | R | — |
| Calculation from the specific gravity of dealcoholized wine calculated with the Tabarie formula | U | S |
| Reducing sugars | | |
| Luff-Schoorl method after clarification by: | | |
| Neutral lead acetate | | |
| with ion exchange | R | R |
| without ion exchange | U | U |
| Zinc ferrocyanide | U | U |
| Sucrose | | |
| Qualitative detection | | |
| Colorimetric | — | U |
| Thin-layer chromatography | — | R |
| Quantitative determination by reducing sugars before and after inversion | — | R |
| Citric acid | | |
| Barium citrate precipitation, oxidation and colorimetric determination | U | S |
| Sorbic acid | | |
| Steam distillation and UV spectrophotometric determination | S | S |
| Sulfurous acid | | |
| Air or nitrogen entrainment, oxidation in sulfuric acid, and sulfuric acid titration | U | R |
| Iodometric titration | Q | U |
| Ash | | |
| Extract ashing at 500–550°C | S | S |
| Alkalinity of ash | | |
| Ash dissolution in a titrated acid and back-titration | S | S |
| Potassium | | |
| Tetraphenylborohydride precipitation and weighing | R | — |
| Flame photometry | U | — |
| Sodium | | |
| Flame photometry | S | S |
| Calcium and magnesium | | |
| Ash dissolution and EDTA titration | U | — |
| Chlorides | | |
| Potentiometric titration | R | — |
| Ion exchange separation and argentometric titration | U | — |

| | OIV[a,b] | EEC[a] | | OIV[a,b] | EEC[a] |
|---|---|---|---|---|---|
| Tartaric acid | | | Sulfates | | |
| Precipitation and calcium racemate weighing | R | R | Barium sulfate precipitation and weighing | R | — |
| Ion exchange separation and colorimetric determination | U | U | The same principle; more useful technique | U | — |
| Potassium monotartrate precipitation and acidimetric titration | Q | — | Glycerol | | |
| | | | Oxidation in methanal and colorimetric titration | U | — |
| | | | 2,3 Butanediol | | |
| | | | Oxidation in ethanal and colorimetric titration | U | — |

[a]R = reference method, U = usual method, Q = quick method, and S = only method.
[b]Other parameters (OIV): Ascorbic acid, cyanide, succinic acid, hydroxymethylfurfural, ammonia, carbon dioxide, preservatives, arsenic, nitrogen, boron, bromine, color, color additives, malvidine diglucoside, ethanal, iron, fluorine, manganese, mannitol, methanol, phosphorus, sorbitol, lead, zinc.

Enzymatic methods for organic acids are suggested as a definite improvement over the older methods shown above. In addition, in the case of fraudulent addition detection, the use of modern GC/MS techniques were recommended as the only plausible method available.

The paper also discusses at some length the philosophy of the OIV and EEC approaches to wine analyses. The references are almost entirely to European work. It briefly discusses gas chromatographic and HPLC methods and concludes that they may become useful in the future but the older methods are more appropriate and adequate at the present time.

There is a tendency for government agencies to adopt minimum and maximum legal limits for some constituents as quality standards. For example, for commercial grape juice, German law sets minimum limits of titratable acidity, alkalinity of the ash, and potassium and magnesium content. It also sets maximum limits on ethanol, volatile acidity, lactic acid, total sulfur dioxide, sodium, calcium, sulfate, nitrate, chloride, and free tartaric acid (28). Many countries set maximum limits for many elements. Future changes in limits are certainly expected. The present legal or suggested limits of some elements in musts and/or wines are listed in the tabulation on page 6 (29–38).

Legal or quality control limits for other constituents are suggested in the text.

| Element | Range of limits (mg/L) | Reference |
|---|---|---|
| Aluminum | 8 | 29, 30 |
| Antimony | 0.15–0.2 | 31, 32 |
| Arsenic | 0.1–1.0 | 14, 30, 31 |
| Boron[a] | 10–100 | 14, 30, 31, 33 |
| Bromine[b] | 0.5–1.0 | 31, 33 |
| Cadmium | 0.1–1.0 | 29, 31, 32, 34, 35 |
| Chromium | 0.1 | 29, 31 |
| Copper | 0.1–5.0 | 31, 34–36 |
| Fluorine | 0.5–5.0 | 14, 31, 33, 35 |
| Iron | 5 | 28 |
| Lead | 0.3–1.0 | 14, 29–31, 34, 36 |
| Lithium | 16.4 | 37 |
| Mercury | 0.02 (?) | 31 |
| Nickel | 0.1–0.3 | 31 |
| Selenium | 0.1–2.0 | 31, 32 |
| Sodium[c] | 60 | 14 |
| Tin | 0.5–5.0 | 6, 31, 35, 38 |
| Zinc | 5.0–40.0 | 14, 30, 34, 37 |

[a] As boronic acid.
[b] Local limits to 2.5 allowed.
[c] Local limits for certain vineyards allowed.

Some of the procedures for the enzymatic determination of a number of compounds in beer (39, 40) are also applicable to wines. Continuous automatic analysis of wines for ethanol and sulfur dioxide has been proposed (41, 42). Not only are continuous methods of analysis practical for laboratories handling a large number of analyses, but computers can be programmed to make the necessary calculations and to record the results. Meyer (43) gives an example of how pycnometer values can be rapidly and accurately converted to percentage of ethanol. Automated and enzymatic procedures were compared with conventional ones for repeatability and reproducibility (44). In 21 laboratories the results for specific gravity, ethanol, total extract, fermentable sugar, total acid, and free and total $SO_2$ were similar, with reproducibility being slightly better for the automated procedures. The European Common Market has not approved automated procedures for ethanol, total sugar, and total and free $SO_2$ (45).

One of the advantages of high-performance liquid chromatography (HPLC) is that many constituents can be quantitatively determined in one run, thus saving much time. We cite only two examples of many: Ethanol, glycerol, glucose, fructose, tartrate, malate, lactate, succinate, acetate, and citrate were determined in one run in 25 min (46). Acetaldehyde, methanol, four higher alcohols,

ethyl acetate, ethyl lactate, acetoin, 2,3-butanediol, and glycerol were measured in one run (47).

Micromethods for ethanol, reducing sugars, and $SO_2$ have been used to give rapid results (39, 48).

The sensory examination of wines falls outside the scope of this book. For a discussion of this subject, see References 9, 10, 49, and 50. For statistical analyses of the sensory and analytical data, see References 50 and 51.

For sensory data the chi-square distribution is used for determining the significance of differences obtained by paired, duo–trio, and triangular tests. When testing for the significance of the differences between means of scores, the *t*-distribution may be used. For more general testing the analyses of variance is employed. One can determine not only the significance of the difference between wines but also the relative reliability of the results of different judges. In sensory tests, care must be exercised in analyzing results of two-tailed and one-tailed tests. This problem does not arise with analyses of data from different analytical procedures.

For analytical data the procedures of Youden and Steiner (52) are standard and useful. In general, one can determine the mean, the standard deviation, the coefficient of variation, confidence limits, and the difference between population means; one can also apply analysis of variance in testing the significance of various factors (methods, analyses, etc.). The larger the standard deviation, the greater the error in determination (random error). Statistical procedures applied to analytical data obtained from two different methods provide a measure of precision in estimating differences between the two means.

Two kinds of error are present in the results of analyses: systematic errors and random errors. Systematic errors arise from differences in the skill or technique of the analysts, since one may have consistently high values and another may have consistently low values (i.e., bias). Essentially this is the difference between an observed mean and a true or target value. The method used must be specific and adequately calibrated. Tests with a standard procedure and the test procedure are called for. The *t*-distribution may be used to determine confidence limits for the two results. Lacking a standard method, one may compare results on normal and abnormal material between analysts or laboratories using the same method or using some reference method, analyze special samples with stated values, add pure materials and determine recovery, or add possible interfering substances. The precision of an estimator is a measure of its repeatability. Precision can be expressed in terms of the variance of an estimator, with a large variance signifying lack of precision and a sizable error of the determination (random error).

The way the individual results cluster around the population mean is usually measured by calculating the standard deviation $\sigma$, which is defined as the square root of the average of the squared differences from the mean. Since the true