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0014-4754/86/080884-10$1.50 + 0.20/0

Analytical chemistry

by S. Brun, J.C. Cabanis and J.P. Mestres

Laboratoire de Chimie Analytique, U.E.R. Sciences Alimentaires, Oenologie, Environnement, Faculté de Pharmacie, Université de Montpellier, Avenue Charles Flahault, F-34060 Montpellier (France)

Key words. Wine; analysis; component; testing; methods.

During the last twenty years, analytical chemistry has benefited considerably from the evolution of techniques and methods. This evolution – one could even call it a revolution – allows the detection and determination of some substances at ever lower concentrations. At the same time, our knowledge has been increased by the discovery of new substances in natural products and in our environment.

The grape, coming from a great variety of vines growing in different soils and climates, and the wine, marked by varied conditions of processing, preservation, and in some cases aging (and commercialization to varying extents), have benefited from this development. A better understanding or the final products and of the factors which affect them, beneficial and harmful, has favored the obtaining of quality products.

We shall briefly summarize the well-known origins of this important development, which has benefited every branch of enology.

In 1951, in his work 'Analyse des vins', P. Jaulmes[87] pointed out that 150 different components were present in wine; ten years later, J. Ribéreau-Gayon and E. Peynaud[139] took into account more than 200. The methods worked out since then have made it possible to go even farther; more than 1000 components are now considered to be present.

The last decades have also been marked by the development of research into food quality; the consumer has become harder to please, with quality playing a vital part in the international market. In order to define this quality, some precisely defined regulations were established and their analytical control organized. The same applies to wine, and both chemical and sensory analysis are used more and more to define the quality of wine with greater precision. We shall not discuss sensory analysis here, but we want to emphasize its supreme importance in the appraisal of wine quality and the efforts made to connect its results with those of chemical analysis.

The greater part of this report will be directed towards chemical analysis of wines during their processing in cellars and their aging in wine stores, with particular attention placed on official methods for the analysis of wines involved in commercial transactions. Our laboratory has participated actively in the work carried out in this field by the Office International de la Vigne et du Vin (OIV), under the auspices of the Sub-Committee on Methods for Wine Analysis and Evaluation, since its creation in 1951. Mme S. Brun has been a member of this committee since 1961.

1. Analytical methods and wine composition

Improvements in analysis are undeniably bound up with the development of chromatography.

Paper chromatography contributed to our early knowledge about the composition of coloring matter in grapes and wines[142]; it is still used routinely to reveal diglucoside anthocyanins, which are characteristic of American vines and of wines produced from hybrids, and it is used to provide evidence in disputes. For this reason paper chromatography is incluced in many official procedures; see for example 'Receuil des Méthodes Internationales des Vins' published by the OIV[127].

Thin layer chromatography (TLC) has supplanted paper chromatography as a means for separation and identification of components in a mixture, and is still in use. We cannot list its innumerable applications here. The advent of an instrument able to measure the intensity of the separated spots made quantitative determinations possible (QTLC). We should like to emphasize that the use of the chromatographic spectrophotometer makes it possible to carry out both qualitative and quantitative analysis in one operation; any method which makes this possible is of great interest. This elegant procedure is excellent for non-volatile compounds, and made it possible to clear up many analytical problems, while gas chromatography was better adapted for volatile compounds. Thin layer chromatography became less important with the advent of high pressure liquid chromatography (HPLC); we can nevertheless cite some applications of TLC in wine analysis, for example for the organic acids[34], sugars[49], and anthocyanins[33].

Gas chromatography (GC), since 1958, has been responsible for the most important advances in the analysis of wine, and for our improved understanding of its composition. Over the years, this procedure was progressively refined and perfected. The detection apparatus became more and more sensitive and was adapted to solve various problems. The number of liquid phases increased, together with our developing knowledge of the phenomena of partition and adsorption related to molecular polarity. The use of capillary columns, allowing the simultaneous separation of a hundred or more components, with rapid-response mass spectrometric or infrared spectrometric detectors, was decisive in the discovery of new components.

Automatic sampling devices allowed a proliferation of analyses; computerized monitoring made it possible for the instruments to operate continuously. The interpretation of the numerous results was much simplified by the availability of computers with appropriate software and hardware.

The formation of derivatives[21] such as trimethylsilyl[38, 59], acetyl[149] or methyl[2] allowed the use of GC for almost all groups of components, even non-volatile compounds. While a detailed description of this method is not given here, we refer the interested reader to some of the general reviews available[141, 178].

Knowledge of wine composition has benefited greatly from the advances in this technique. The publications treating the application of GC to wine analysis are innumerable. The bibliographies of the doctoral theses referred to here[21, 59, 63, 69, 143, 161] will supply readers with details of the essential literature.

The use of GC was expected, above all, to improve our understanding of grape and wine aromas. In 1970, R. Cordonnier[54] reviewed a wide variety of GC methods: from the direct injection of wine to prevent any loss of the volatile compounds, to methods entailing concentration (such as the headspace procedure and nitrogen entrainment followed by a cryogenic trap). The lowering of the limit of detection in the wine may help in finding trace levels of substances which could be specific for an aroma. Several reviews on this subject have been published[46, 54, 57, 77, 155]. The reader can also refer to the paper 'Flavor composition of wines: a review', by P. Schreier[155] for its tables listing hundreds of aroma components found and sometimes determined in wines. It is impossible to reproduce these tables here, for it would be difficult to justify the inclusion of certain substances at the expense of others. This review also lists the organic acids that have been found; their number reaches 135!

As an example of the detection of characteristic compounds, terpenic compounds can be cited. An abundance of these is characteristic for the muscat grapes[14-16, 173, 174, 179], whereas 2-methoxy-3-isobutylpyrazine is characteristic of Cabernet Sauvignon grapes[55]. However, it is a well-established fact that aroma is the perception by our sense of smell of each individual volatile compound, and also of the whole volatile ensemble present. Our olfactory system is itself a detector with its own idiosyncracies and some of the substances that it perceives can only be weakly detected, or not at all, by the chromatographic methods; the opposite can also be true. A better understanding of aroma will have to be linked to a better knowledge of olfaction and its mechanisms. (Readers are referred to the March 1986 issue of Experientia for a multi-author review on Odor Biology.)

High pressure liquid chromatography (HPLC) is the lastborn among the chromatographic methods. The first commercial apparatus appeared in 1973. Since then, analysts have used this method with the same enthusiasm as they did earlier with GC, and it has been applied in the separation, characterization and determination of a large number of wine components or groups of components. The detection of the components separated in the column is easy, and the methods are sensitive if the substances absorb in the ultraviolet range. Substances which do not have this property can be characterized and determined after the formation of colored derivatives, or better fluorescent derivatives, in order to lower the limit of detection. These reactions can be carried out before or after passage through the column, but before the detection apparatus is reached. Other systems for detection have been developed for this technique: infrared, mass spectrometry, electrochemical detectors, interferential refractometry, etc.

As with GC, basic research on columns and choice of solvents for HPLC has been done in relation to the structure of the ions or molecules to be separated and their functional organic groupings. The classical normal phase columns (silica, alumina, etc.) have to be used with solvents which are absolutely anhydrous, but the use of reverse phase columns releases the analyst from these constraints, and makes it possible to use aqueous phases. The ion exchange columns are useful for amino acid separation. We have listed references to the separation of amino acids[47, 111, 145], anthocyanins[101, 181], acids[71], phenol acids[26, 137], biogenic amines[39] and sugars[70]. These are merely given as examples; the bibliography for the application of HPLC in wine and must analysis is very extensive. We think that for studies on the composition of wine it is important to emphasize the particular interest of HPLC for the study of phenolic compounds and especially of anthocyanins.

Atomic absorption photometry is the technique with which knowledge of the mineral composition of wine has made the greatest advances. Since 1970, with the flameless atomizer, trace elements at levels lower than 0.1 mg/l have been observed and measured. A comprehensive review was given by Eschnauer[66] in 1973 and brought up-to-date in 1982[67].

Atomic absorption spectrometry (AAS), with a flame atomizer, usually air-acetylene, was the first technique described. But it lacks sensitivity, owing to the poor yield of the atomizer. Meanwhile, it remains the routine procedure used for elements at a concentration higher than 0.1 mg/l.

Operating procedures differ[42bis] There can be direct vaporization of wine[156] for Li[41,119], Mg[29], Zn[32], Mn, Cu and Fe[73] after addition of a spectral buffer for Ca[28]. It could be possible to proceed after reduction to ash and dissolving in an acid solution for Li, Zn and Mn[86]; after wet digestion for Ca, Mg[81] and Cd[96], followed by chelation and extraction with dithizone for Cd[96], or even after a direct chelation extraction in the wine with APDC-MIBK for Cd[1] and Pb[115], in order to concentrate the elements to be titrated in the injected solvent. In addition to the classical flames, in recent years methods using electrical flames have appeared, with argon plasma being used as a source of excitation for emission. This technique has recently been applied to research into trace elements in Italian wines[86].

With the flameless atomic absorption technique, a few analysts proceed by direct injection of wine. For the most part the wine is acidified by various acid matrix modifiers, according to the element involved (Cd[117], Cr[18,118] and Ni[118]. For elements with a tendency to form carbides, the graphite tube must be treated as is done for Si[120]. Other elements are analyzed after sulfonitric mineralization, for example Cd[42], and As[121]. Lastly, the hydride technique (reduction with sodium borohydride) is also used. The method of addition of known levels has also been applied for Co[43] and Tl[68].

Other authors have used, besides these classical techniques, voltammetry by anodic redissolution for trace-element analysis[75,158], and X-ray fluorescence[183].

Enzymatic methods have also made great progress, and must be mentioned in connection with our improved knowledge of biochemical reactions and more efficient conditions for extraction and purification of proteins. These very specific methods are used in preference to any others in analysis when determining certain specific components of wines. We shall discuss these methods in more detail in Part 2.

Automatic analysis methods, with continuous flow and sequential methods, are an adaptation of classical methods to a quicker rhythm; working pace: they have altered the 'donkey work' of laboratories a great deal. In Part II, these methods will be treated at length.

Conclusion. It is from all these methods (and in particular the instrumental methods,) that our present detailed knowledge of grape and wine composition originates. Moreover their contribution is not limited to a simple enumeration of components. They facilitate, for example, the evaluation of a large number of samples with a rapidity which was never envisaged before (as for example, atomic absorption). Chromatographic methods, well adapted to the separation and determination of many components such as GC (more than one hundred components may be separated on a capillary column) or HPLC, offer the same possibilities. Thus, many parameters can be determined in a large number of samples in order to follow their evolution under various conditions. Those numerous results, which can be assimilated only with difficulty by the human mind, can then be interpreted by computers with methods such as multidimensional analysis. The most frequently used method is discriminant analysis. More and more, such methods are being applied to the analytical differentiation of wines[150,160,164], and in order to determine the influence of technological factors[143].

This statistical interpretation of results opens up new perspectives for the analyst, but he must exercise good sense and caution before drawing conclusions. In the following section, we shall talk about the development of these instrumental, enzymatic and automatic methods, with reference to the official rules and the practice in private laboratories.

2. Analytical methods and wine-testing

2.1. International and official methods for wine analysis

2.1.1. Introduction

In the beginning of this century, it was found necessary to keep a check on food products moving between different countries. The establishment of internationally accepted methods was desired in order to facilitate such trade.

In the case of wine, we had to wait until 1950 for the Office International de la Vigne et du Vin (OIV) to turn its attention to the standardization of the analytical methods used by all the wine-producing countries. In 1954, an international convention for the standardization of the analytical and evaluation methods for wines was signed by 12 producing countries (and has in the meantime been ratified by 20 countries). The convention laid down that a subcommittee for the standardization of the analytical and evaluation methods should be created; this body has since been meeting annually.

It was also anticipated that special analytical methods intended for the international wine market would be established, and that the contracting parties would adopt these in their national regulations. The 'Recueil des Méthodes Internationales d'Analyse des Vins' came into being in 1962. Methods for the determination of eleven products were described. Since then, many editions have been published, completing or bringing up to date the previous ones. The last, published in 1978[126], includes analytical methods for 45 parameters.

In France, official methods for wine analysis have existed since the beginning of the century. They were renewed in 1963 and harmonized with the methods outlined in the international manual published by the OIV. In 1971, EEC regulation No. 1539/71 obliged the different partners to follow these methods. This regulation was replaced in 1978 by regulation No. 2984/78, and then in 1982[53] by regulation No. 1108/82 with some modifications and extensions.

We should make it clear that the EEC regulation considered that it was useful to retain the OIV methods because they were known in all the wine-producing countries, and in fact the methods used by the EEC are, with but a few exceptions, almost identical to the OIV methods.

2.1.2. Procedure for the adoption of an analytical method

The standardization of an analytical method is the concern of the international organizations arbitrating the international exchanges involved. The OIV shares this concern, and has been represented in the consultations organized by the IUPAC (International Union of Pure and Applied Chemistry), the Association of Official Analytical Chemists (AOAC), the Food and Agricultural Organizaion (FAO), and the World Health Organization (WHO) during the last decade, on behalf of the international organizations responsible for perfecting the analytical methods, in order to describe procedures on the one hand, and on the other to organize their collaborative studies.

The choice of an analytical method requires previous determination of its repeatability and reproducibility, using collaborative studies; the norm ISO 5725 has been retained as a basis for the organization and for the statistical treatment of such studies.

During the international consultations, it was recommended that the different needs and means of each country be taken into account; hence, we can note some slowness in the adoption of enzymatic or complex instrumental methods requiring reagents or apparatus difficult to obtain in some countries.

International methods for analysis of wines and musts

	OIV References[126]		EEC References[53]	
Density at 20 °C	A 1		1	
– Pycnometer method		R		R
– Hydrometer method		U		U
– Hydrostatic Balance		U		U
Alcohol	A 2		2	
Distillation and				
– Pycnometer method		R		Q
– Hydrometer method		U		U
– Hydrostatic balance		U		U
– Refractometry		U		–
– Dichromate oxidation		S		
Total acidity	A 10		8	
– Potentiometric titration to pH 7		R		R
– Titration to pH 7 with an indicator		U		U
Volatile acidity	A 11		9	
– Steam distillation and volumetric titration		S		S
pH	A 31		14	
– Potentiometric measurement		S		S
Fixed acidity	A 11		10	
– Total acidity less volatile acidity		S		S
Extract	A 3		3	
– Vacuum distillation et 70 °C		R		–
– Calculation from the specific gravity of dealcoholized wine calculated with the Tabarie formula		U		S
Reducing sugars	A 4		4	
Luff-Schoorl method after clarification by:				
– neutral lead acetate				
with ion exchange		R		R
without ion exchange		U		U
– zinc ferrocyanide		U		U
Sucrose	A 5		5	
Qualitative detection				
– colorimetric		+		U
– thin-layer chromatography		+		R
Quantitative determination by reducing sugars before and after inversion		+		R
Tartaric acid	A 12		10	
– Precipitation and calcium racemate weighing		R		R
– Ion exchange separation and colorimetric determination		U		U
– Potassium monotartrate precipitation and acidimetric titration		Q		–
Malic acid	A 33			
Ion exchange separation and colorimetric determination		U		–
Lactic acid	A 27			
Ion exchange separation and colorimetric determination		U		U
Citric acid	A 29		12	
Barium citrate precipitation, oxidation and colorimetric determination		U		S
Sorbic acid				
Steam distillation and U.V. spectrophotometric determination	A 30	S	15	S
Sulfurous acid	A 17		13	
Air or nitrogen entrainment, oxidation in sulfuric acid and sulfuric acid titration		U		R
Iodometric titration		Q		U
Ash	A 6		6	
– Extract ashing at 500–550 °C		S		S
Alkalinity of ash	A 7		7	
Ash dissolution in a titrated acid and back-titration		S		S
Potassium	A 8		–	
– Tetraphenylborohydride precipitation and weighing		R		–
– Flame photometry		U		–
Sodium	A 25		17	
Flame photometry		S		S
Calcium and magnesium	A 26		–	
Ash dissolution and EDTA titration		U		–
Chlorides	A 15		–	
– Potentiometric titration		R		–
– Ion exchange separation and argentometric titration		U		–
Sulfates	A 14		–	
– Barium sulfate precipitation and weighing		R		–
– The same principle; more useful technic		U		–
Glycerol	A 21		–	
Oxidation in methanal and colorimetric titration		U		–
2,3 Butanediol	A 21		–	
Oxidation in ethanal and colorimetric titration		U		–

Other parameters: Ascorbic acid (A 28), cyanide (A 24), Succinic acid (A 13), Hydroxymethylfurfural (A 19), Ammonia (A 20), Carbon dioxide (A 39), Preservatives, Arsenic (A 34), Nitrogen (A 40), Boron (A 44), Bromine (A 23), Color (A 0), Color additives (A 43), Malvidine diglucoside (A 18), Ethanal (A 37), Iron (A 9), Fluorine (A 22), Manganese (A 42), Mannitol (A 21), Methanol (A 41), Phosphorus (A 16), Sorbitol (A 21), Lead (A 38), Zinc (A 45).

2.1.3. *Current international methods for analysis of wines*

Generally, the OIV manual describes two kinds of methods for each compound. One is a 'Reference Method' known to be the most accurate. It must be used if a dispute develops during a transaction. Then, there is what is known as the 'Usual Method', in which the techniques used are simpler, but results can be less accurate. Sometimes, also, a 'Quick Method' may be used.

In general, the EEC regulations select only one method from the manual in each case, and that is usually the reference method. The 'Usual Method' is used only if it is considered to be satisfactorily accurate.

In the table, we give a list of the parameters for which methods are described in the 1978 edition of the OIV manual. For the most important parameters we give briefly the principles of the methods described and indicate whether they are 'Usual Methods' (U), 'Reference Methods' (R), 'Quick Methods' (Q) or 'Unique Methods' (S). We also indicate those retained by the EEC. As can be seen from this table, essentially classical methods such as gravimetric, volumetric or spectrophotometric methods are described. Instrumental methods such as GC, HPLC, atomic absorption or enzymatic methods are not frequently used in deference to the difficulties encountered by some countries in performing these analyses. These problems have become less important over the years. For the last ten years, the OIV has been studying these analytical methods and submitting them to collaborative studies. The same has been done for the methods described in the manual and, if appropriate, the new methods are compared with these. We present here the results of these studies.

2.1.4. *International methods for wine analysis*

Classical methods. The collaborative studies confirmed the reference method using a pycnometer[89] and the usual hydrometer method for the determination of density and for the determination of the alcoholometric titre[100]. The same was done for the titration of total and volatile acidity[100].

For the titration of reducing sugars, the procedure of clarification was simplified; only the neutral lead acetate and the zinc ferrocyanide were retained. (In some exceptional cases, where their elimination is necessary, uronic acids are eliminated using an anion-exchange column). The only method retained was that described by Luff-Schoorl. This procedure, used for the analysis of sugars, was confirmed by the collaborative analysis[100]; the same was done for the estimation of tartaric acid, using the reference method with precipitation and weighing of the calcium racemate.

Concerning density and alcoholometric titre, we have to underline the value of two particular methods; the first one being the hydrostatic balance that yielded favorable results in our test in comparison with pycnometry[167], and the second being Paar's method[76, 151] based upon the measurement of the frequency of oscillation of a U-shaped tube, which behaves in the same way as a tuning fork. The tube is filled with the liquid to be studied. The accuracy of the method, depending on its standardization, can be excellent.

For sulfurous anhydride, the only method is that described by Paul[126], using entrainment followed by oxidation to sulfuric acid; this method is listed in the OIV manual. The procedure, after many studies, seems to be good, and the collaborative study being done at present will probably confirm its value.

Concerning fraud research, we cite the method for the titration of sodium azide[51], which is a illegal antiseptic used recently; this titration needs a double distillation followed by a colorimetric titration of the ferric complex of hydrogen azide.

Enzymatic methods. For the titration of malic, citric and lactic acids, the chemical methods were investigated in collaborative studies (for principles, see table) and were compared with enzymatic methods[131]. Their variability was too high and the reproducibility too low compared with those of enzymatic methods[17, 94, 113, 124, 132, 175] which were therefore retained.

For sugars, both the enzymatic method and the chemical method give good results[97], and they will be conjointly adopted. Results obtained by the chemical methods are always higher that those obtained by the enzymatic one, because all the reducing substances present in clarified wine are titrated, not only glucose and fructose but also pentoses and uronic acids. The chemical method gives a measure of total reducing components. The enzymatic method allows the separate determination of glucose and fructose; it is then possible to establish their ratio in order to follow the evolution of fermentation.

For glycerol, an enzymatic method[161] has been described; this method has been applied directly to wine, in some cases after decoloration (red wines) by polyvinylpyrrolidone. This method has a satisfactory reproducibility[74] compared with the chemical method described in the OIV manual[91]; this latter method has consequently been modified[99, 128, 168].

Ethanol can be titrated by an enzymatic method[112]; later on, we shall see an application using an automated method. The same can be done for ethanol (acetaldehyde)[104] and succinic acid[105].

Gas chromatography. Many techniques using gas chromatography (GC) for the analysis of sugars and organic acids have been published. These substances must first be transformed into volatile derivatives. Formation of silyl derivatives was first proposed[20, 22], but the instability of these derivatives gave poor reproducibility, making it difficult to use this kind of derivative routinely. Instead, methyl derivatives[171] can be used, but this transformation cannot be done directly in the wine. The mixture must first to be simplified with the wine passing over an ion exchange column in order to separate sugars and polyols. The advantage in using this method is that it allows the simultaneous determination of the main organic acids. The determination of sugars using GC was also proposed using the formation of trimethylsilyl derivatives and the elimination by ion exchange of the acids which have the same retention time as the sugars[23, 59]. These methods for determining acids and sugars are time-consuming and delicate, and consequently they cannot be chosen as official methods. The use of HPLC looks more promising. GC also allows the determination of polyols, mainly glycerol. For glycerol, many techniques have been proposed, such as the formation of silyl derivatives directly in diluted wine[24, 59], and direct injection of wine using a column packed with Chromosorb 101[177], but this titration needs a column saturated with glycerol, and its reproducibility is not good enough. Another technique, entailing the direct injection of wine on a column packed with Tenax GC[45], was selected in 1981 as a 'Usual Method', after a collaborative study had been carried out. We have to underline that this technique allows the simultaneous determination of 2,3-butanediol.

With regard to the application of GC in wine analysis, higher alcohols[19] can be determined in the distillate, as well as ethanol, methanol, and ethyl acetate; this method is retained for these last two compounds in the manual of the OIV. GC has innumerable applications, and methods have been proposed for the determination of many other components or additives present in wine, for instance sorbitol[133] and sorbic acid[6, 25, 79]. Ethanol can also be determined in wine by GC[159, 180], giving the alcoholometric titre, but the necessary large-scale dilution of the wine is a source of error which is incompatible with the accuracy needed for official methods.

The last application is the use of GC to detect and determine the diethylene glycol fraudulently added to musts in order to improve the extract, and which is found in wines. A first publication[7] describes a method using a Carbowax 20 M capillary column and a temperature program, with direct injection of wine, or, for sweet wines, of the wine diluted with an equal volume of alcohol. Finally, mass spectrometry makes it possible to confirm the nature of the isolated peaks. Quantities of the order of 5 to 10 mg/l can be determined. Improvements have since been made by using a previous extraction of the diethylene glycol by a diethylether-acetone mixture in a carbonated medium[27].

High performance liquid chromatography. Recently, HPLC has contributed new techniques to wine-testing. For the organic acids, first proposals recommend the use of an anion exchange column and a solution of sodium formate, in order to separate galacturonic, succinic, tartaric and shikimic acids after their fixation on a resin pre-column, allowing the elimination of the other components. The detection is then done by refractometry. Exclusion and partition on a cation exchange column also give good results[135]; citric, tartaric, malic, lactic and succinic acids can be separated after direct injection of the musts and white wines and pretreatment of red wines with charcoal[5].

Reverse polarity phase chromatography, using an acid solvent, (phosphoric acid) in order to lower the ionization of acids, has also been applied, after isolation by ion exchange of the acids present in wine[152]. Three C 18 columns, 5 µm in diameter, 26 cm long, were attached together, in order to improve the separations; this gave good results[80] but had the disadvantage that the analysis is comparatively time-consuming. This technique allows the determination of citramalic acid after a five-fold concentration of the wine before injection. Indeed, the characterization of this acid has been retained to differentiate between products made of unfermented juice, blended with alcohol and rich in sugars, and sweet wines; the technique at present is thin layer chromatography[98, 144]. This acid can also be determined by both HPLC[5] and GC[171].

Glucides in wine have also been subject to numerous studies and many methods have been suggested to the subcommittee of the OIV[4, 5, 70, 78]. We shall discuss only the last reference[172] which gives a brief review of the previous proposals and describes a technique with direct injection of the centrifuged must or wine (from which gas has been removed if necessary) after their filtration through a membrane; this method utilizes an NH_2-loaded silica column, and refractometric detection for which the limits are in the range 100–150 mg/l for fructose and glucose. All these methods allow the simultaneous determination of the sugars and of glycerol.

HPLC has been applied to the solution of various analytical problems in wines and musts. An example is sorbic acid. The currently described official method entails ultraviolet spectrophotometry after steam distillation[108]. It is not sufficiently precise, however, because of interference of phenolic acids with detection. Over the last ten years, gas chromatographic methods have been published[6, 25]; these have a high sensitivity, but necessitate a prior extraction. Reverse phase liquid chromatography[79] allows the same sensitivity to be achieved with direct injection of the wines diluted (red) or not (white); this method also permits the determination of salicylic and benzoic acid.

Another matter is the determination of biogenic amines like histamine. The 'malaise' suffered by some wine consumers has been attributed to an excess of histamine, though no proof of this has ever been demonstrated. This suspicion has led to many studies on histamine and other biogenic amines. The reports of the subcommittee of the OIV on analytical methods for the years 1983[129], 1984[130] and 1985[131], contain abstracts of work done on this subject. They specify the quantities of biogenic amines present, their determination, and the toxicity of histamine and its origin.

The proposed methods differ in the means of separation used; the extraction can be done by treatment with ion exchangers[52, 103, 134] or by extraction with n-butanol[48, 169, 170, 182].

Some methods call for an identification and a semi-quantitative evaluation after a mono-[48, 169] or bidimensional[169] thin layer chromatography, using ninhydrin[48] or the reagent of Pauly[48, 169] as staining reagents. Other methods for histamine involve spectrofluorimetry after the formation of a fluorophor by its condensation with ortho-phthalaldehyde[103, 134, 170].

HPLC has also been recommended, using a reverse phase on n-alkyl-loaded silica with a moderately polar eluant, acetonitrile. After chromatography, the histamine condensed with fluorescamine is titrated by spectrofluorimetry[52]. In another procedure, dansyl derivatives are prepared; these are extracted with ethyl acetate and separated using a normal column[72]. Lastly, Mayer and Pause recommend treating the wine with PVP in order to eliminate most of the amino acids; after condensation with ortho-phthalaldehyde, amines are separated on a reverse phase column and then determined by spectrofluorimetry[58, 114]. The Subcommittee for Analytical Methods of the OIV determined that all these differents methods were adequate for the assay of biogenic amines, including histamine, and did not find it necessary to single out a specific one.

Atomic absorption spectrometry. In connection with the main cations (potassium, sodium, calcium and magnesium), the conclusions of the OIV collaborative studies can usefully be discussed. The methods of the OIV analytical manual (estimation of sodium and potassium by flame photometry, and of calcium and magnesium by complexometry) were tested in comparison with atomic absorption spectrophotometry. Both methods, flame photometry and atomic absorption spectrophotometry, gave satisfactory values for repeatability and reproducibility for sodium and potassium[131]. On the other hand, the collaborative study gave such large discrepancies for the analysis of calcium and magnesium by complexometry that statistical evaluation could not be carried out; this method was therefore removed from the manual and replaced by atomic absorption spectrometry, which gave satisfactory repeatability and reproducibility[131].

It has to be specified that the tested atomic absorption spectrometry method is carried out using a standard curve and not after the addition of standards[83].

For a number of oligo elements, arsenic, lead and zinc, the OIV has fied acceptable limits. The EEC also works with such limits and analytical methods are therefore necessary to enforce them. For the first oligoelements considered by the OIV, the classical colorimetric methods were retained. The method for arsenic uses a sulfonitric mineralization, forming arsenic hydride, which can be estimated after reaction with silver diethyldithiocarbamate, giving a colored product[88]. This method was adopted by the AFNOR and the ISO. It may be soon be replaced by atomic absorption spectrometry[121]. Two techniques can be used in this method: in the first, called 'hydride generation', the mineralized compound is reduced to arsenious hydride with which the flame is fed. The second method also requires a premineralization and concentration of the arsenic by complexation and solvent extraction before its introduction into the graphite furnace. In this case, the advantages of atomic absorption spectrometry over the classical method are not clear, and the choice of a method which is not of a type routinely carried out will depend upon the appropriate instrumentation being available.

For lead, the assay method under consideration is mineralization with perhydrol, followed by the colorimetric determination of a lead dithizone complex[92, 165]. This determination is difficult, as all the reagents used need to be previously purified in order to eliminate any traces of lead that they may contain, whereas the atomic absorption method, which can be used when a graphite furnace is available, involves the direct injection of acidified wine into the furnace. Medina[116] uses the previously proposed phosphoric acid[84, 85]. Perchloric acid is also recommended[72] to remove interfering compounds. Differential pulse anodic stripping voltammetry[176] gives the same results.

If a graphite furnace is not available, and if one has to work with flame atomization, direct injection cannot be used because of the low sensitivity of this method (about 100 to 1000 times lower, depending on the element involved). In order to concentrate the element, a complex is formed and then extracted by solvent; this solvent is then pulverized in the flame. As an example, we can cite the use of ammonium pyrrolidine dithiocarbamate (APDC) as a complexing agent and methylisobutylketone as a solvent[115].

More recently, cadmium has been studied. Because the amount of cadmium in wines is about 20 times lower than that of lead, its determination can only be done with sufficient accuracy by atomic absorption spectrometry; the method described by Medina[116] for lead can be applied, and, if a graphite furnace is not available, one can use complex formation and extraction[96, 125]. Out of more than 500 wines analyzed in the Federal Republic of Germany, South Africa, Greece, Hungary, France and Luxembourg, only four samples contained an amount of cadmium between 0.010 and 0.015 mg/l. All the others contained less than 0.010 mg/l, the limit which had been set as permissible.

For lead and cadmium the technique using direct atomization in a graphite furnace was approved by the OIV, but it requires expensive apparatus, and thus it seems necessary to authorize a method using flame atomization as well.

Zinc is another element for which a maximum acceptable limit has been fixed (5 mg/l). In 1974 the OIV outlined in its manual a colorimetric method with dithizone, using extraction of the complex formed directly in the wine[31, 126]. At that time, flame atomic absorption on diluted wine was not retained because it was only rarely used.

Two other mineral components, iron and copper, are usually determined in wines because of their great importance for the qualities of the final product. Many sensitive chemical methods exist for their titration. The OIV manual[126] describes a reference method for iron in which, after liquid mineralization, iron is determined by colorimetry using orthophenanthroline. In many laboratories, this excellent method has given place to flame atomic absorption[31, 59], in which wine is directly pulverized in the flame after proper dilution. As for copper, the OIV did not find it necessary to describe a method in its manual, but many techniques are available in the 'Feuillets Verts'. On the other hand, the EEC[59] gives a technique using flame atomic absorption on wine which is diluted if necessary.

We shall now discuss three metalloid elements for which maximum limits have been fixed: fluorine (0.5 mg/l), boron (80 mg/l of boric acid) and bromine (1 mg/l, rarely exceeded except in vineyards with briny subsoil).

Fluorine is a beneficial element if it is ingested in small quantitities; however, its ingestion rapidly becomes injurious, and the limit of toxicity is only very little above the useful dosage. The OIV manual[126] recommends a classical chemical method for fluorine determination[87], involving ashing, formation and then steam distillation of fluorosilicic acid, volumetric titration of the fluoride with thorium nitrate. The disadvantage of this fine method is that it is a lengthy one. The specific ionic electrode, which can be used directly on the wine, offers a rapid method for this determination, and this method was recommended by the OIV. Nevertheless, it is not used very often, so the chemical method, which is easily applied in any laboratory, keeps its place in the analytical manual.

The determination of boron became necessary because of the antitartar properties of boric acid and its derivatives. This illegal treatment must be checked by determination of boron, and by comparison with the natural admissible limit. This determination is made by chemical methods after wine mineralization. Reagents employed until now (acetylquinalizarine or 1, 1′-dianthrimide[126] have the disadvantage that they must be used in highly concentrated sulfuric acid. The use of azomethine II, in an aqueous medium, has recently been proposed. This method involves only alcohol elimination by concentration and a previous passage over Polyclar A.T. This procedure, which has been studied by many laboratories, could serve as a substitute for the present method in the manual. The use of a fluoroborate electrode allows a precise and simple potentiometric titration[56].

The titration of bromine became necessary because brominated derivatives of acetic acid are forbidden antiseptics but are sometimes fraudulently used. Their characterization as organic bromine derivatives is difficult because of the hydrolysis which occurs after a certain time. The only way is to characterize them by demonstrating an abnormal increase in the total mineral bromine; this is done after ashing, by a colorimetric titration[40]. Although this is an old method, there has not yet been any controversy about it.

Isotopic analysis. Isotopic analysis is applied in fraud research in wine. We shall cite two examples.

The use of synthetic alcohol in order to enrich some kinds of wine is forbidden. The only authorized alcohol is that made from natural products, and mainly of viticultural origin. The presence of synthetic alcohol is shown by the estimation of ^{14}C in a wine distillate with an alcoholic titre above 90 vol.%[82, 137, 138]. The ^{14}C radioactivity is measured using a scintillation counter, expressed in disintegrations per second, and then compared to the average natural radioactivity.

'Chaptalization', which is the addition of sugar to the must, is strictly regulated by the EEC. It is forbidden in some areas, but authorized in others within certain limits. The photosynthesis of sugars is accompanied by the splitting of chemical bonds between oxygen atoms and those of carbon and hydrogen. The isotopes of hydrogen are of particular interest because the natural relative variation of deuterium can be very significant. This principle is applied in a method[64, 109, 110] for the detection of added sugar. Its acceptance by the EEC and IOV should permit the clearing up of the difficulties resulting from fraudulent use of 'chaptalization'.

2.2. *Automatic analysis methods*

The first work done in order to automatize analytical methods for wines was carried out in 1969 by the Dijon station of INRA. A continuous flow technique was adjusted to the usual chemical manual methods for the titration of alcohol[146], volatile acidity[147], sulfurous acid[123, 147, 148] and total polyphenols[3].

The first chains of analysis appeared in 1974. Since then, the methods first described have been improved[61, 62], particularly in rate, accuracy, and the solution of problems caused by the dirtying of circuits. New continuous flow methods have appeared, which are based on chemical methods, such as those for tartaric acid[9], higher alcohols[107], and methanol[106]; these two last methods have been particularly adapted to the testing of spirits and alcohols.

At the same time, chains of analysis based upon enzymatic principles have appeared[12, 13], for the determination of organic acids, malic acid[8, 12], lactic acid[8, 12], citric acid[12], succinic acid[11, 12] and glycerol[10].

The automatic determination of alcoholic titre was the first concern of the analyses, because of its importance in wine analysis. The proposed method[146], using sample dilution, distillation and then oxidation of the alcohol with sulfochromic reagent, did not give satisfatory results. The analysts then looked for a method which could be used directly on wine. A first autoanalyzer, working without previous separation of the alcohol, was proposed[65, 166]. Its principle was based upon the measurement of the variation in enthalpy brought about by the mixing of the wine and a solution of sodium perchlorate. It was abandoned for another method, also working directly on wine, based upon near infrared detection, which has been applied to the checking of numerous food products. Comparison between this technique[44] and the reference method (pycnometry) showed that the maximum observed difference did not exceed 0.1 vol.% if the standardization was carefully done. The most recent of the automatic methods for alcohol titration uses enzymatic treatment of diluted wine[13, 35, 90]. This method, which is very accurate, seems to be a promising one for the future.

Scholten et al.[153, 154] recently described a continuous flow automat allowing the titration of 12 parameters (glycerol, total SO_2, L-malic acid, total acidity, L-lactic acid, total sugars, glucose, glucose and fructose, tartaric acid, citric acid, relative density, alcohol). This analyzer is computerized, so that the computer takes into account the 12 parameters and uses those results in order to determine 5 more parameters (original density of the must, total alcohol, total extract, non-sugared extract, residual extract). The methods used include enzymatic ones (glycerol, malic, lactic and citric acids, glucose, glucose and fructose), colorimetric ones (p-rosaline for SO_2, bromocresol purple for total acidity, neocuprin for total sugars, metavanadate for tartaric acid) and finally refractometry for the alcohol. The same methods were carried out manually, and for all parameters the accuracy of the continuous flow determination was better than that of the corresponding manual analysis.

Automatic methods need frequent standardization; this can only be done using the reference methods in order to verify the standards. This checking has to be done rigorously to avoid systematic errors. With the reduction of human intervention, automatic methods reach a level of reproducibility which is altogether satisfactory. The use of standards furnished by an official body could be an appropriate solution.

Because of the working rhythm achieved, the application of automation makes it possible to carry out statistical studies more easily and on a larger scale, and, in regional laboratories, permits better quality-control. Automated methods will certainly develop further in the future, and their classification as official methods will have to be considered.

3. *Conclusion*

As in all fields of applied science, enology has benefited greatly from the extraordinary development of analytical methods. Such methods, using the specific properties of components, like partition, adsorption, solubility, have made great progress in wine analysis possible. The development of gas chromatography, and of appropriate mass spectrometric or infrared detectors, has given us an immense amount of information about wine composition; it is usual to obtain up to one hundred spectra for only one injection in a gas capillary column. Computers are essential in the interpretation of these, and can store information (up to about one hundred thousand mass or infrared spectra on one hard disk).

With such perfected (although – needless to say – highly expensive) means at our disposal it is clear why our knowledge of wine composition has increased by leaps and bounds during these last twenty years.

The results from the use of HPLC are less spectacular, but this technique has not yet achieved its full potential, which will involve the improvement of detectors.

We have studied the problems encountered in the choice of official methods using all the new technologies. The first step is to use them in parallel with classical methods. Wine quality control must take advantage of these technological advances, but must also carefully take into account their price and the possibilities of each country concerned.

Furthermore, the methods chosen must be ones which can be used over a reasonable period of time so that they can be profitable. It will not be feasible to abandon them, except in exceptional cases, every time a new or improved method appears in the international scientific literature.

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Applied microbiology

by S. Lafon-Lafourcade

Institut d'Oenologie, Université de Bordeaux II, and INRA, 351 Cours de la Libération, F-33405 Talence Cedex (France)

Key words. Fermentation, alcoholic, yeast ghosts; fermentation, malolactic; spoilage; lactic acid bacteria; acetic acid bacteria.

1. Introduction

Wine fermentation may be seen as an ancient biotechnology, even older than that of bread-making. However, the quality of wines has not ceased to improve and diversify, attesting to the progress arising from an increasing knowledge of microbiology.

Traditionally the transformation of must into wine remains a 'spontaneous phenomenon'. Two principal fermentations participate successively in the production of wines. First, yeasts transform sugar into ethanol, then lactic acid bacteria transform malic acid into lactic acid; the malolactic fermentation is general for red wines but occasional for white. Acetic acid bacteria, which are responsible for the vinegary spoilage of wines, must be inhibited.

During the vinification the microbiological selection that occurs is dictated by the composition of the media[55,56]. The high concentration of sugar and acid pH of the must affect the growth of yeasts[33]. In such conditions the yeasts follow a specific cycle of growth and metabolism[38]. Fermentation activity is progressively inhibited by the products of sugar metabolism. The fermentation may be modified by the presence and secretions of other microor-