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# Wine Analysis: **from 'Grape to Glass'**

An analytical testing digest of the  
wine manufacturing process

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Analytical testing solutions, involved in the production processes of wine, from the harvesting of grapes to the final bottled end product, encompass a number of varying chromatographic, as well as traditional, techniques. Critical parameters of importance to the wine producer are described, answering why, and how frequently these tests need to be completed. Throughout this digest useful links to external material are highlighted, enabling access to detailed information and supporting statements, cited in peer reviewed journal abstracts. Specifically, official methods of analysis for grapes, grape must, and wine, links to analytical instruments, methods and relevant EU and U.S. regulations covering wine production, additives, labeling and contaminants in wine have been included.



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Although the growing of grapes and production of wine has a history of several thousand years, it is only relatively recently that the process has become better understood and also better controlled. There is a unique complexity to wine production, depending both on factors that can be measured and therefore controlled and those that are essentially in the hands of nature. The quality of ripe grapes at the end of the annual vineyard cycle reflect the work of the viticulturist, but some events such as pest attack, diseases and the vagaries of climate are outside their control. The timing of harvest, pressing of grapes, chemical composition of grape juice and subtle compositional changes during fermentation need to be carefully monitored, and if necessary carefully adjusted. How well this done will ultimately be reflected in the quality and selling price of the wine. Processes that are allowed for wine production, as well as chemicals and other substances permitted to be used during these processes are tightly controlled throughout the world. There is some harmonization of wine standards through organizations such as the International Organization of Vine and Wine (OIV), that facilitates international wine trade, but there are also differences between what is permitted or what is not permitted in different geographical regions of the world for example, the European Union (EU), United States of America (USA) and Australia/New Zealand. However, at all stages in wine production, measurement is critical, knowing what to measure and when, and also having the skill and experience to appropriately use the information to make fine adjustments to the chemical composition of the grape must, which will ultimately impact on the quality of the finished wine. Continuous analysis needs to be made at all stages of wine production. This testing can vary from simple rapid checks for example, of sugar

content of grapes prior to harvest using a refractometer, continuous monitoring of sugars with a discrete analyzer (DA), acids by ion chromatography (IC), through to measurement of pesticide residue levels or stable oxygen isotopic ratios in the finished bottled wine, requiring sophisticated laboratory instrumentation. Thermo Fisher Scientific uniquely provides comprehensive support for all analytical measurements from the grape to the bottled wine. With an understanding of the importance of efficient and cost-effective testing, Thermo Fisher Scientific can provide the necessary instruments and consumables, tailoring advice to provide the appropriate analytical tools needed at all stages of the wine manufacturing process.



Thermo Fisher Scientific uniquely provides comprehensive support for all analytical measurements from the grape to the bottled wine.





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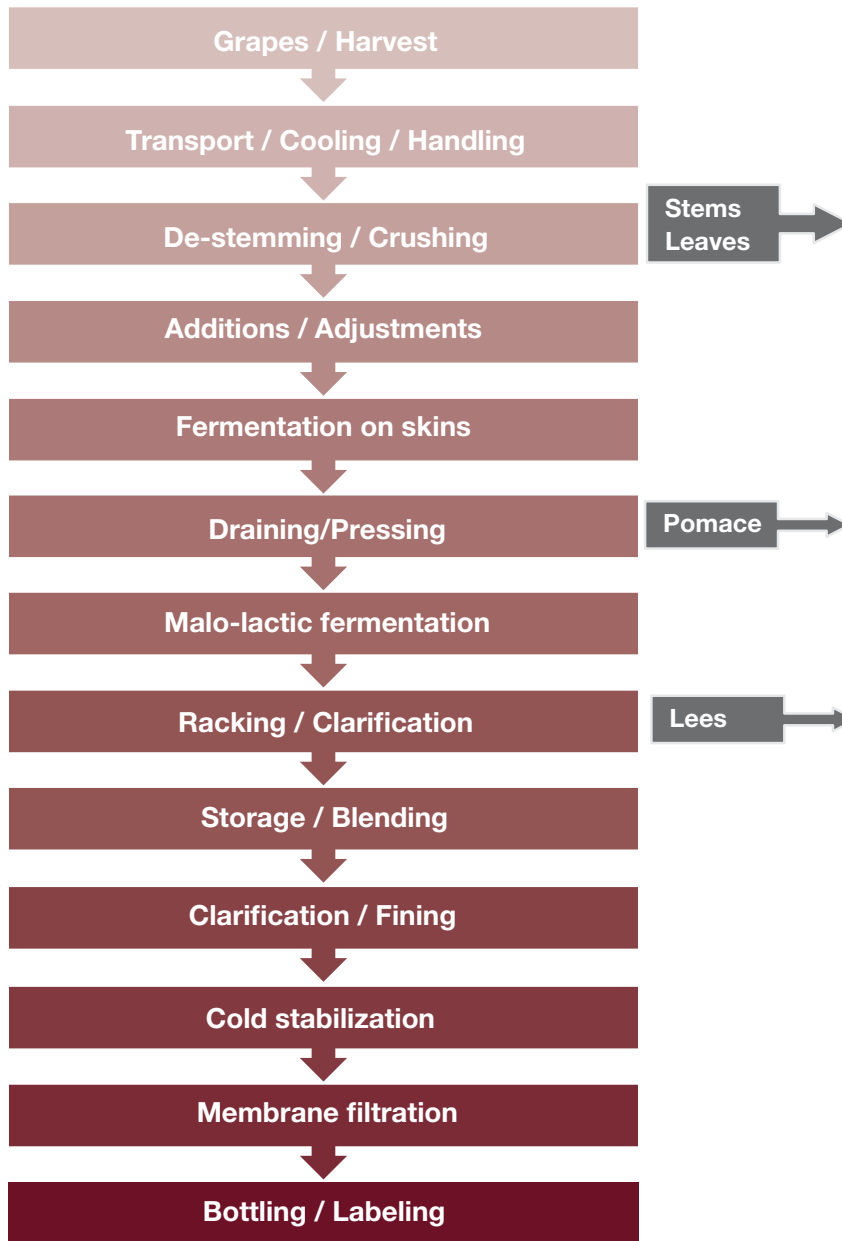
The wine production process, as shown, comprises a series of steps that need to be carefully managed and for which continuous checking and measurement is required.

**Sulfur dioxide**  
**Sugar**  
**Acids**

**Yeast**

Optional amounts of pressing added to wine

Sulfur dioxide



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The quality and 'style' of a wine depends not only on the composition of the juice obtained from healthy ripe grapes, but also other constituents that have roles of varying importance in determining the ultimate flavor and color of the end product. In general, grapes consist of clear juice (80%), skins (8%), seeds (4.5%), pulp (4.5%) and stems (3%). The skins, seeds, pulp and stems are collectively known as 'pomace'. As stalks contain tannins that add bitterness to wine, the grapes may be de-stemmed completely before they are crushed. The stalks or a small proportion of them, may be left on to increase the tannin content of red wine to give extra structure. Grape skins contain coloring substances, aroma compounds, flavor constituents and tannins, the extent of extraction which differs from red to white wine, impacts on the style of the wine. Tannins

can give a dry 'mouth-feel' to the palate, anthocyanins and flavones give grapes their color, while bioactive flavonoids impart claimed health-giving properties to wine. However, it is the flesh of the grapes that contains the water, sugars, fruit acids, proteins and minerals. The sugars are mainly fructose and glucose, and the most important acids in grapes are tartaric and malic acids.

The timing of picking of grapes is one of the most crucial decisions a grower will make during the vineyard year.

Ultimately, the taste and 'mouth-feel' sensations of a wine are due primarily to a few compounds that occur individually at concentrations above 0.1 g/L, such as ethanol, organic acids (malic, lactic and tartaric acids), sugars (glucose and fructose) and glycerol. Other compounds such as acetic and formic acids can positively contribute to flavor, but if in excessive amounts can have an adverse effect, or can alter the important 'balance' between acidity, sweetness and volatile notes in the wine aroma.



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There are a large number of authorized substances, which if necessary can be added to meet various needs before, during fermentation and post-fermentation. Sulfur dioxide, as an example, is used both as an antioxidant and disinfectant at many stages in winemaking, in particular, to prevent fermentation starting prematurely and inhibiting the action of wild yeasts and bacteria. If the pH of the wine must be too high, tartaric acid is usually added. Conversely, de-acidification may be necessary if the pH is too low using, for example, calcium or potassium carbonate, potassium bicarbonate, or a proprietary de-acidification agent. Yeast nutrients, such as B group vitamins (for example, thiamine),

may be added at the fermentation stage to increase yeast populations, and di-ammonium phosphate can help to ensure that all sugars are fermented out, to stop the undesirable formation of hydrogen sulfide.

After fermentation is complete, the coarse sediment is removed by racking or centrifugation. However, colloidal suspensions may also need to be removed or they will cause a wine haze and ultimately form a deposit. Fining agents such as egg white, gelatin, isinglass and sodium bentonite improve clarity and remove excess tannin and so improve the taste and appearance of the wine.

Additives and processing aids	Rationale for use
Yeasts, nutrients, malolactic bacteria	Promote fermentation
Tartaric acid	Acidification
Potassium tartrate, potassium bicarbonate, calcium carbonate, calcium tartrate	De-acidification
Lysozyme, urease, pectinase, $\beta$ -glucanase	Juice extraction and clarification
Sulfur dioxide, ascorbic acid, carbon dioxide, nitrogen	Prevention of oxidation
Gelatin, egg albumin, casein, siliceous earths, isinglass, polyvinylpyrrolidone	Fining for clarification
Bentonite, tannin, gum arabic	Protein and color stability
Citric acid, gum arabic, ascorbic acid, sodium bentonite, potassium ferrocyanide	Prevention of metal hazes
Copper sulfate, charcoal/activated carbon, PVPP, casein	Removal of off-odors, flavors or color

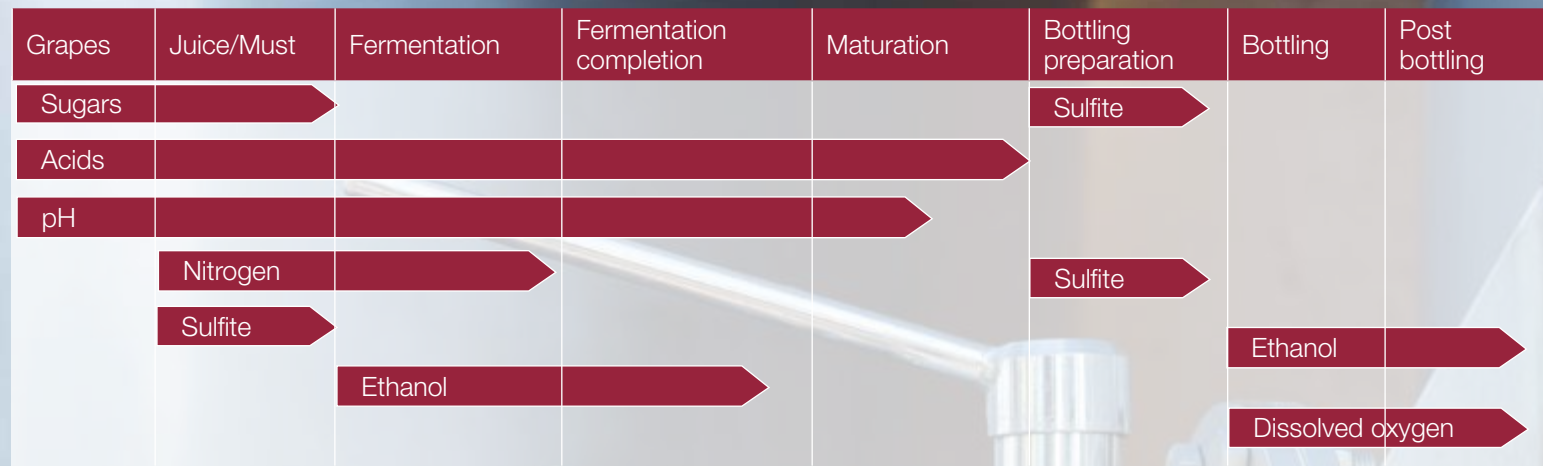
Examples of additives and processing aids that currently have widespread approval for use in different wine production areas of the world.

The table above lists some examples of additives and processing aids for which approval is common to the EU, USA, Australia/New Zealand and South Africa. In total there are a far larger number of additives available for use than those shown in this table, but there are also differences as to which geographical regions they are approved for use. Within a jurisdiction, there are rules concerning the additives that are permitted depending on the geographical area, where differences in climate might limit whether the grapes have sufficient sugars or the climate might cause high levels of malic acid.

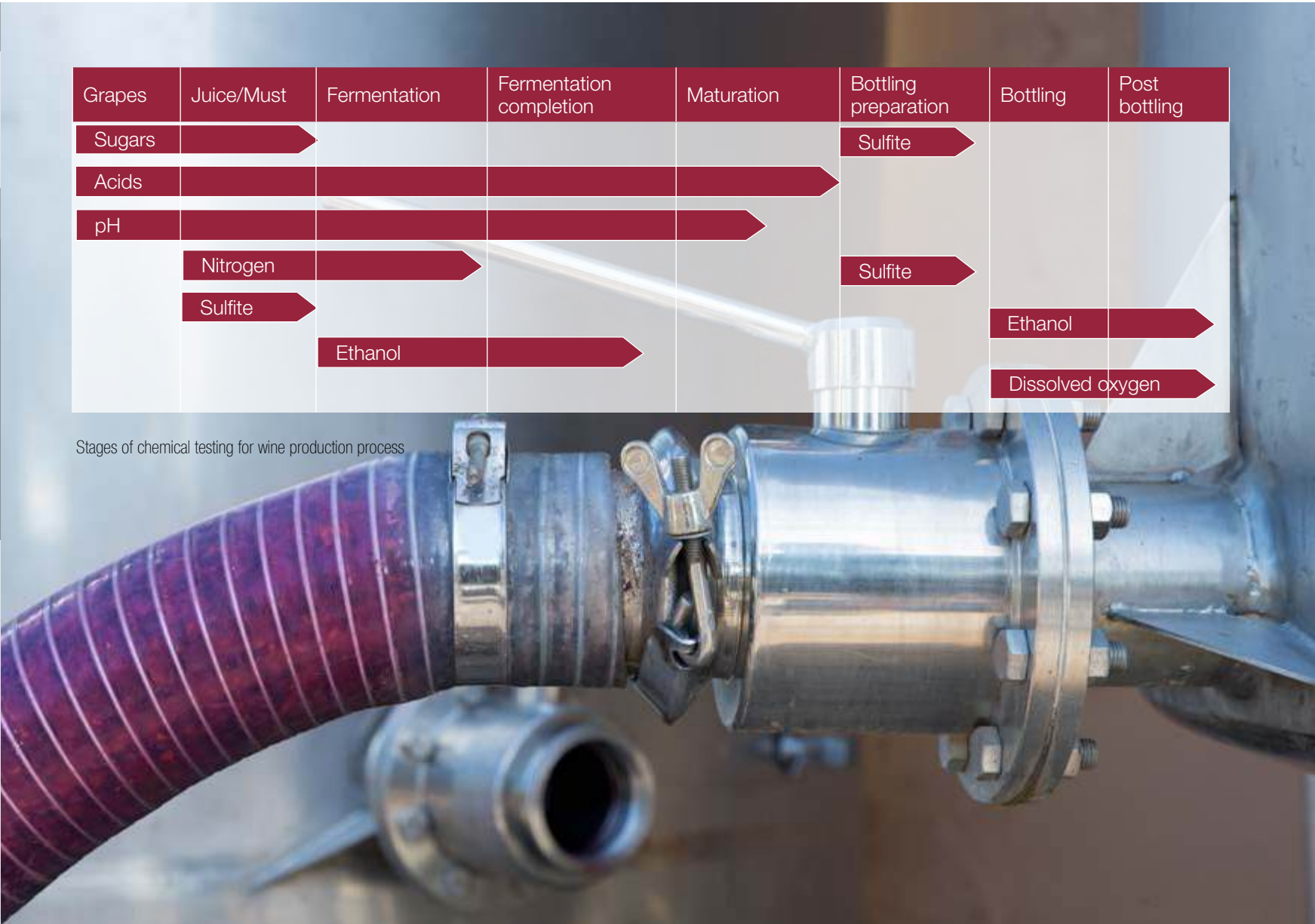
[The International Organization of Vine and Wine \(OIV\)](#) lists eighty-six substances used in oenology and provides specifications both in terms of their composition as well as permitted levels of additive impurities. An understanding of the complex restrictions and regulations for wine production is clearly important, but equally important is the ability to assess whether any substances need to be added and for this access to comprehensive measurement tools is essential.



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Stages of chemical testing for wine production process





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### Harvesting

As grapes ripen, the concentration of sugars and aroma compounds increases and the concentration of acids declines. The right time to pick the grapes at their optimum composition depends on the type of wine to be produced. For example, sparkling wine requires a higher acidity than still wine. In the weeks and days preceding harvest, grapes are regularly tested usually in the vineyard for sugar content, acidity and flavor.

### Crushing and de-stemming

Ripe bunches of grapes are fed into the crusher/de-stemmer. Stems are removed through a sieve system and grapes are then crushed by rollers. A 5–10% solution of sodium metabisulfite is added to the grapes to inhibit growth of wild micro-organisms and prevent oxidative browning of the juice. The level of sulfur dioxide is maintained at a minimum of 80 mg/L, if necessary more sulfite solution can be progressively added. The content of free sulfite and total sulfite in wine characterizes its quality and is therefore routinely determined (as individual analytical parameters) by wine producers and by official food control laboratories.

### Juice preparation

The free-run juice is separated from the crushed grapes, which are then pressed by gentle squeezing to obtain a high quality juice. The juice is allowed to settle overnight, or is centrifuged, to clarify it and if necessary enzymes to break down pectin are added to remove haze.

### Fermentation

Fermentation is initiated by inoculating the juice with specially selected wine yeast that converts glucose and fructose to ethanol. Fermentation is usually carried out under a blanket of carbon dioxide to exclude oxygen and maximize ethanol formation, which is affected by temperature, extent of agitation, sugar concentration, acidity, strain of yeast and yeast activity. Depending on the conditions, various intermediates in the fermentation process are converted to by-products that contribute to the characteristic flavor and aroma of the end product, although some by-products may be undesirable contaminants, or contribute to off-flavors.

Malolactic, or secondary fermentation, may follow yeast fermentation to soften any acidity by converting malic into lactic acid giving the wine more complexity and a slight 'buttery' note. Yeast is not involved in secondary fermentation which is carried out by *Lactobacillus*, *Leuconostoc* or *Pediococcus* bacteria.

Malolactic fermentation can be induced by warming the vats, or inoculating with these strains of lactic acid bacteria. Conversely, it can be prevented by treating the wine with sulfur dioxide and/or keeping the wine cool. Some white grape varieties undergo malolactic fermentation, whereas others may not be improved if they are valued for their crisp acidity.





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### Purification or Racking

After fermentation the wine is often clarified, by drawing off the wine into clean vats or barrels from the sediment (lees), in a process known as 'racking'. Additionally, fining can be initiated to precipitate out any proteins and tannins that are suspended in colloidal form in the wine. Gelatin might be used, or the suspension can be adsorbed onto the surface of substances such as bentonite. Wine is often also cold stabilized (left at 0 to -3 °C for 10–14 days), to crystallize out any potassium hydrogen tartrate.

### Maturation

Immediately after fermentation, a period of maturation is required, during which the tannins in the wine change through a series of complex interactions. Acidity levels fall, making wines more pleasant to drink, generally being softer to the palate. Maturation takes place in different types of vessels, including stainless steel vats and wooden barrels, whichever is chosen and the period of time for maturation, depends on the style of wine to be produced and cost factors. Inexpensive wines, intended for early drinking, need little or no maturation and are stored in stainless steel vessels providing an impermeable gas barrier. The wine is stored until required for bottling. White wine is generally stored in stainless steel or concrete vats until ready for bottling. Oxygen is excluded, and the vats are kept either completely full or blanketed with nitrogen or carbon dioxide.

In contrast, most high quality red wines undergo a period of barrel maturation for between 9 and 22 months. During this time, the wine undergoes controlled oxygenation and absorbs some oak-derived compounds, including wood tannins and vanillin. The smaller the barrel, the quicker the maturation time, the lower the temperature, the slower the maturation time.

### During maturation the wine is racked several times to aid clarification.

When barrels are new, they impart many oak derived substances to the wine, including vanillin, lignin and tannin, but as they are re-used the amount of these compounds imparted to the wine is progressively reduced. Over-oaked wines may well smell of burnt toast, fatty butter and marmalade and have a specific sweetness. The desirable fruity notes of the wine can be overwhelmed by those of the wood products. Achieving a fine balance of oak aromas and obtaining the right level of oak influence is yet another art of a skilled wine maker.

The high production cost of wine aging in wood barrels has led to the use of alternatives, such as the addition of wood-shaving chips during the wine aging process, approved by [EU Regulation 1507/2006](#).

Compared with traditional barrel aging, similar results can be obtained in a shorter contact time with the size of the wood-chips affecting the extraction rates. In order to understand how the choice of type of wood-shavings influences the wine aging process, the profiles of low molecular weight phenolic composition of Portuguese chestnut and French, American and Portuguese oak chips have been determined using a Thermo Scientific liquid chromatography – diode array detector / electrospray ionization - mass spectrometry ([LC-DAD/ESI-MS](#)) system.

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**Bottling**

Potassium or calcium tartrate crystals can sometimes form after the wine has been bottled and can appear on the cork or as sediment in the bottle. To inhibit tartrate precipitation in the bottle, the wine is chilled to  $-4\text{ }^{\circ}\text{C}$  and after approximately 8 days crystals will have formed, the cleared wine can then be bottled. A faster process of removal of tartrate involves reducing the temperature of the wine to around  $0\text{ }^{\circ}\text{C}$  and seeding it with finely ground tartrate crystals, followed by vigorous stirring. The seeds then attract further crystals to form and the entire process is completed in about 24 hours.

Sulfur dioxide that would have been used as an antioxidant and disinfectant during production is present in 'free' and 'bound' form in wine. Before bottling, the free sulfite levels are adjusted to between 25 and 35 mg/L, with higher levels necessary for sweet wines to inhibit further fermentation of the sugars. Wines with sulfite levels above 10 mg/L in the EU must be labeled because of potential allergenic reactions to consumers. A number of other treatments, including pasteurization or sterilization, may be carried out immediately prior to bottling to ensure final stability. Cold sterile filtration, through fine sheets or a membrane filter removes all yeast cells prior to aseptic bottling. Alternatively, thermotic bottling may be employed, when the wine is heated to  $54\text{ }^{\circ}\text{C}$  and bottled hot, or flash pasteurization, when the wine is heated to  $95\text{ }^{\circ}\text{C}$  for 1–2 min, then rapidly cooled and bottled cold. Another option is tunnel pasteurization, when wine is bottled cold and then passed through a heat tunnel, where the sealed bottles are sprayed with hot water to raise the temperature to  $82\text{ }^{\circ}\text{C}$  for 15 to 20 min.



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**Analytical testing through the production process**

Throughout the wine production procedure, analytical testing is essential to ensure that conditions are optimized for successful fermentation; if necessary any adjustments can be made by addition of appropriate substances. This testing is an on-going process and there are a variety of approaches and methods that can be employed for testing, depending on the scale of production, which determines the numbers of samples to be analyzed per day. Methods range from classical chemical analysis through to the use of modern analytical instrument techniques. The most important parameters that need to be measured in grapes, juice and must during fermentation, and in wine, are described below and the specific points in the wine making process where testing is performed are shown in the table on page 8.

**pH levels**

Typical pH levels in wine normally range from 2.9 to 3.9 and can be measured [using a pH meter](#). Care should be taken during pH measurement to ensure accurate results as there are various components in juice and wine that can affect the performance of the pH electrode; these include proteins, sulfides, tannins, and polyphenols.

**Acid levels or titratable acidity**

[Acid level](#) or [titratable acidity](#) is a measure of acid content in wine, juice, or must. It can be determined by classical titration with an indicator or potentiometric titration with sodium hydroxide and is usually expressed as g/L equivalent of tartaric acid. Alternatively when large numbers of wine samples need to be tested, the [Gallery](#) discrete photometric analyzer provides a dedicated automated system. Laboratories may choose to employ [ion chromatography](#) (IC), with suppressed conductivity detection, to separate a large variety of organic acids and inorganic anions and detect them with high sensitivity. This detection system overcomes the problem of poor ultraviolet (UV) absorption of several organic acids and interference from the sugars and wines and can be analyzed directly without extensive cleanup. Grapes contain significant quantities of organic acids that affect taste, color, and microbial stability of the juice, making measurement of acid levels one of the most important basic analyses conducted in a wine laboratory. Levels of tartaric acid can be as high as 15 g/L in unripe grapes, but even in ripe grapes levels range from 6 g/L in grapes from northerly vineyards to only 2–3 g/L in vineyards in the south. Similarly, levels of malic acid can be as high as 4–6.5 g/L in mature must in the north, but only 1–2 g/L in vineyards in the south. Citric acid levels average around 0.5–1 g/L, while acid levels average 1 g/L whilst other acids such as benzoic and cinnamic acids only occur in the mg/L range.

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Sugar levels are measured by refractive index (RI), using an Abbe refractometer 5 or Abbe refractometer 60, specific gravity or chemical reduction of copper salts. In the weeks and days preceding harvest, grapes are regularly tested for sugar content, to determine optimum harvest time. Conversely, to determine when fermentation is nearly finished, sugar levels are again monitored by specific gravity. Initially grape juice has a specific gravity greater than 1.000, due mainly to dissolved sugars, but when the specific gravity falls to 1.000, the wine is nearly ready. Some manufacturers also use "Clinitest" tablets (similar to those used in diabetic testing) or Fehling reaction for more precise monitoring as the color of the resulting solution indicates the amount of sugar remaining. A large amount of sugar results in complete loss of the blue copper (II) ions, leaving the red copper (I) oxide. With less sugar, some blue copper (II) ions remain and less red copper (I) oxide is formed. In the absence of sugar the solution remains blue. For routine high-throughput sugar analysis the Thermo Scientific Gallery™ discrete analyzer can be used to determine glucose and fructose levels. Sample pre-treatment is minimal; generally centrifugation or filtration is adequate to prepare the wine samples. Another popular analytical technique for sugar analysis is ion chromatography (IC), using the easy-to-use and reliable Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, with high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD).

Chaptalization is the process of adding sugar to unfermented grape must in order to increase the alcohol content after fermentation. In the EU, chaptalization is not permitted, except in designated wine growing zones in more northerly areas, where grapes might not ripen sufficiently to produce sufficient glucose and fructose. Control of chaptalization is fairly strict in many countries, although permitted in the in all states of the U.S., except for California. Illegal addition of sucrose during fermentation is generally detected by isotopic measurements using Isotope-ratio mass spectrometry (IRMS).



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Analysis of nitrogen (N), in the wine making process is recommended to ensure a good quality wine. Nitrogen is a key nutrient for yeast growth and therefore essential for successful fermentation of grape juice and must into wine. Nitrogen compounds in juice, must, and wine affect not only the fermentation process itself, but also the clarification process and final chemical composition of the wine, including its aroma.

The total nitrogen content of grape juice/must is highly variable ranging from as high as 1000 mg/L to as low as 50 mg/L. Only 'Yeast Assimilable Nitrogen' (YAN) is available for yeast metabolism, comprising mostly of ammonia (present as ammonium salts) and certain amino acids. Testing for nitrogen before and during the fermentation is desirable. If the YAN level is too low or too high this can have a negative impact on the wine making process and the wine itself. Decisions on how much and what types of nitrogen to add will

be informed by the results of the nitrogen testing. Low YAN can cause the fermentation to slow or get stuck, while high YAN can encourage spoilage bacteria, which can result in the undesirable formation of ethyl carbamate, histamine and phenyl ethylamine.

Ammonia nitrogen can be determined using an ammonia ion selective electrode, or a simple enzymatic method, with the determination using a UV/Visible spectrophotometer. Similarly amino nitrogen can be determined either using colorimetric reagents or enzymatically, again making the measurement with a spectrophotometer. There are also classical formaldehyde titration methods enabling YAN and titratable acidity to be determined. These procedures are all published as OIV official methods.

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## Sulfites or sulfur dioxide

A close-up photograph showing a hand holding a wine bottle, pouring a stream of dark red wine into a clear, rounded wine glass. The wine is captured mid-pour, creating a dynamic flow. The background is a plain, light-colored surface.

Sulfur dioxide ( $\text{SO}_2$ ) is widely used in wine production as a chemical antioxidant and inhibitor of microbial activity. There are a number of traditional methods for determining free and total  $\text{SO}_2$  in wine involving distillation or iodometric titration. Ripper titration can also be performed using a platinum, (Pt) and iodide electrode to signal the endpoint at a known mV value. Potentiometric titration improves the results, as the color and clarity of red wine does not interfere with the determination. Automated photometric analyzers are also available for the high-throughput analysis of sulfite in wine. Alternatively, total sulfite can be determined in wine using alkaline extraction followed by ion-exclusion chromatography with amperometric detection. Pulsed amperometric detection extends the lifetime of a working electrode or a disposable platinum working electrode can be used in place of a conventional electrode for the detection of sulfite.

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**Levels of fining agents**

After fermentation is complete, the amount of fining agent necessary to be added is determined by taking 100 mL samples of wine, treating them with a suspension of the fining agent and leaving them overnight. Very small amounts of fining agent are necessary, with 15 g of gelatin per 100 L of wine usually being sufficient. White wines are usually fined with bentonite, with the completeness of the fining tested by heating or with phosphomolybdic acid.

**Dissolved oxygen**

Dissolved oxygen in wine has a great effect on its quality, stability and longevity. Monitoring and controlling the oxygen incorporation at different stages of the wine making and bottling process is becoming a growing concern for wineries. Dissolved oxygen levels are a part of wines natural aging process, though adverse levels can cause discoloration to white wines and flavor degradation to both white and red varieties. Measuring the concentration of dissolved oxygen in wine after bottling can be carried out using a dissolved oxygen sensor and [portable meter](#). Silicone tubing is necessary to make a seal so that the bottle can be laid on its side, immersing the probe and temperature sensor into the sample while keeping all of the wine inside the bottle and the ambient oxygen out.



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It is important for the wine maker to know the ethanol level that could result during and upon completion of fermentation. Potential ethanol estimations are therefore conducted prior to harvest and approximately midway through fermentation. A number of different formulae can be used to estimate potential ethanol content based on the measurement of glucose and fructose. Midway through fermentation, potential ethanol estimates are made to give a more accurate picture of the ethanol content post-fermentation, taking into consideration the conversion rate of sugar to ethanol currently underway in the must. A sample of the must is obtained and immediately filtered to stop continued fermentation. The sample is then analyzed for ethanol content and glucose/fructose levels. Each wine maker uses their own particular interpretation of the results to estimate the final ethanol content of the wine. The most common method, used around the world, to measure alcohol content is ebulliometry which is based on boiling point depression, a simple technique measuring all alcohols (not only ethanol) with an accuracy of +0.5%. The [OIV Type I methods](#) determine ethanol after distillation using various end-measurements. Enzymatic analysis of wine can be employed to determine ethanol involving detection of nicotinamide–adenine dinucleotide (NADH) by [UV spectrophotometry](#). [Gallery](#) analyzers offer possibilities for automated ethanol determinations. Alternatively gas chromatography (GC), using the Thermo Scientific™ [TRACE™ GC system](#) also provides an accurate approach to measuring ethanol in wine providing a reliable automated system geared for a moderate to large winery.

The use of Fourier Transform Near Infrared spectroscopy ([FT-NIR](#)) offers the opportunity of fast determination of ethanol with no sample preparation required, making the technique ideal for on-site testing.





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The busiest testing period, during and immediately after harvest, more than a hundred samples per day may be sent to the laboratory to test for sugar, acetic acid, total acidity, and ethanol. After harvest, malic, lactic and tartaric acids are the required tests. Classical methods are appropriate for small-scale testing of one or two samples a day, for larger wine analysis laboratories, automated analyzers are increasingly being used. [The Gallery](#) is a fully automated bench-top discrete photometric analyzer that can be used to test for sugars (glucose, fructose), acids (L-lactic acid, L-malic acid, tartaric acid), sulfite and [pH in wine](#) samples. The Gallery system provides an integrated platform for two measurement techniques, photometric and electrochemical, that can be run in parallel. Discrete cell technology allows for measurement of several different tests for the same sample simultaneously without method changeover time. Individual reaction cells are isolated and temperature-stabilized. Ready-made system applications are offered for colorimetric, enzymatic and electrochemical tests. Sample pretreatment is minimal; generally centrifugation or filtration is adequate to prepare the samples. Results are ready within a few minutes. The Gallery system is able to achieve very low detection levels and its sophisticated dilution features help to manage a wide concentration range without user intervention. [Ion chromatography](#), using an autosampler, also provides an alternative approach to fast throughput analysis of wine for organic acids, sugars and sulfite requiring minimal sample preparation, and being a versatile technology available in most analytical laboratories.

There are defined terms that must be used for labeling to indicate the dryness or sweetness of wine, requiring knowledge of fructose and glucose levels and total acidity, making these measurements is critical for the bottled wine producers. For example a dry wine must contain <4 g/L of sugars or <9 g/L, provided that the total acidity expressed as g/L of tartaric acid is not more than 2 g below the residual sugar content. Medium and medium sweet wines must contain >12 g/L but <45 g/L sugar content, while sweet wine must contain >45 g/L residual sugar set out in [Regulation EC 607/2009 Annex XIV](#).

The Gallery system has tremendous flexibility and can be used as an automated method to measure free [SO<sub>2</sub> in wine](#) samples. The method is based on the classical colorimetric reaction between sulfur dioxide, p-rosaniline hydrochloride, and formaldehyde. The novel method has been shown to have a good correlation with flow-injection analysis for routine determination of SO<sub>2</sub> in red and white wine samples. Total sulfite can be determined in wine by addition of alkali and oxidation with hydrogen peroxide to convert sulfite to sulfate. After clean-up by solid phase extraction (SPE), the mixture is analyzed by ion chromatography using a [Thermo Scientific™ Dionex™ IC system](#) to measure levels of sulfate.

Rather more novel is the application of Fourier transform infrared spectroscopy ([FTIR](#)) for determinations to be made in grape juice, must, and wine. FTIR, very simply, analyzes a sample by making an infrared spectrum of the sample which is processed through Fourier transform calculations. FTIR does not directly measure the parameter of interest, but a calibration model identifies relevant parts of the spectrum, that can be used for measurement. When the FTIR is used in the near-infrared region, the long path lengths allow sampling through containers of glass and plastic making near it an ideal quality assurance and quality control (QA/QC), technique. Near-infrared also has the ability to monitor multiple parameters at the same time, using a single spectrum for identification or quantification of many crucial variables, important to determine during wine production. A recent publication has demonstrated the potential of attenuated total reflectance mid-infrared (ATR-MIR) spectroscopy, to monitor time-related changes during the fermentation of Chinese [rice wine](#).

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## Official methods of analysis



Official methods for the analysis of grape juice, must and wines are freely accessible in the International Organization of Vine and Wine (OIV) Compendium of International Methods of [Analysis of Wines and Musts](#), first published in 1962. The Compendium is an attempt to standardize methods of analysis to facilitate international trade in a similar way to Codex methods.

OIV has divided methods into four categories. Category I are 'defining methods' which are the only methods for establishing the accepted value of a specified parameter, for example, total acidity. Category II methods are designated as benchmark methods in cases where category I methods cannot be used. Such category II methods are recommended for use in cases of disputes and for calibration purposes. Category III methods meet all designated performance criteria and are used for monitoring, inspection and regulatory purposes. Category IV defines auxiliary methods that may use a recently implemented technique and the method performance has yet to be established.

OIV methods are divided into physical analysis (14 methods); chemical analysis: sugars (8 methods), alcohols (9 methods), acids (25 methods), gases (4 methods), other organic compounds (29 methods); non-organic compounds: anions (5 methods), cations (15 methods) and other non-organic compounds (14 methods). The methods cover parameters that are routinely measured during wine production (for example, sugars, acids, nitrogen), methods for approved additives and processing aids (for example, lysozyme, sulfur dioxide), methods for undesirable residues and contaminants (for example, pesticides, diethylene glycol, biogenic amines, ethyl carbamate, ochratoxin A) and methods that can be used to detect fraudulent practices such as illegal addition of sugar (isotope ratios, artificial sweeteners and colors). These methods range from classical techniques using colorimetric chemical reagents, distillation and titration through to modern methods to determine  $\alpha$ -dicarbonyl compounds by high performance liquid chromatography, ([HPLC](#)) [after derivatization](#), 3-methoxypropane-1,2-diol and cyclic diglycerols by gas chromatography mass spectrometry ([GC-MS](#)), eleven mineral elements by inductively coupled plasma atomic emission spectroscopy ([ICP-AES](#)) and  $^{13}\text{C}/^{12}\text{C}$  ratios of wine ethanol by [isotope ratio mass spectrometry](#) (IRMS).



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The International Organization of Vine and Wine ([OIV](#)) is the scientific and technical reference organization for the entire vine-to-wine production process, comprising of 46 Member States and has 10 non-governmental international organizations that participate as observers. The work of OIV covers vines, wine, wine-based beverages, table grapes, raisins and other vine-based products. OIV defines the characteristics of these products and their specifications, contributes to the promotion of good regulatory practices in order to ensure fair trading, as well as the integrity and sustainability of different viticultural products on the global market. OIV underwrites the harmonization and definition of new international standards in order to improve conditions for producing and marketing vitivinculural products. All OIV recommendations are adopted by a unanimous consensus of members and are frequently included in national and regional regulations (EU and Mercosur), or by Codex Alimentarius.

In some regions of the world, such as the EU, USA, Australia/New Zealand and South Africa, there are some common rules and equally there are some distinctly different restrictions that apply. In the EU, the European Commission Regulation ([EC](#)) No 606/2009 sets out detailed rules with regards to the categories of grapevine products, oenological practices and the applicable restrictions. EU legislation links approved use of additives to a specific function in the winemaking process. It specifically forbids any additive not authorized by the legislation. There are 43 oenological practices that are identified as approved with restrictions on condition of use. In a number of cases there are limits that are applied as maximum amounts that are allowed to be used in wine production. For example, the addition of lysozyme is permitted for fining of wine, but where added to both the must and the wine, the total overall quantity must not exceed 500 mg/L. Commission Regulation ([EC](#)) No 607/2009 lays down detailed rules concerning protected designations of origin (PDOs) and geographical indications, traditional terms, labeling and presentation of certain wine sector products.



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The rules concerning protected designations of origin (PDOs) and geographical indications are particularly important as these can have a huge influence on the price that wine commands in the market. As part of a concerted effort to protect EU wines from labeling fraud, including illegal addition of sugars chaptalization from 1991 the EU established a [wine database](#) initially containing data on isotopic ratios of wines determined by nuclear magnetic resonance (NMR) and from 1997 this was extended to include other isotopic measurements by [Isotope ratio mass spectrometry \(IRMS\)](#). Samples of fresh grapes were taken from vineyards situated in specified wine growing areas with a clearly defined soil type, situation, vine training system, variety, age and cultural practices. The numbers of samples of wines collected for the database is linked to the volume of wines produced in the specific member states; it varies from 2 samples per annum from the UK to 400 samples per annum in France. This means that the database now contains at least 10,000 wine datasets from each of the larger wine producers, providing a valuable resource that can be used for enforcement purposes, to test whether a suspect wine fits the isotopic characteristics established over a 25-year period.

In the U.S., the regulations covering all aspects of wine production are prescribed in [Title 27 Alcohol, Tobacco Products and Firearms Part 24 Wine](#). These U.S. regulations cover all wines of every type and specifically refer to 'grape wines', whereas in other parts of the world 'wine' is by definition is only obtained from fermentation of fresh grapes. There are over 140 established American Viticultural Areas that are defined growing regions, distinguished by geographical and terroir features. Unlike EU regulations, the U.S. authorities only establish growing area boundaries and do not govern which varietals can be grown, or vineyard and winemaking practices. When a wine label in the U.S. carries a vintage, 95% of the grapes must be grown during the stated year, and when a wine label carries the name of a grape variety, the wine must be made from at least 75% of that grape variety.



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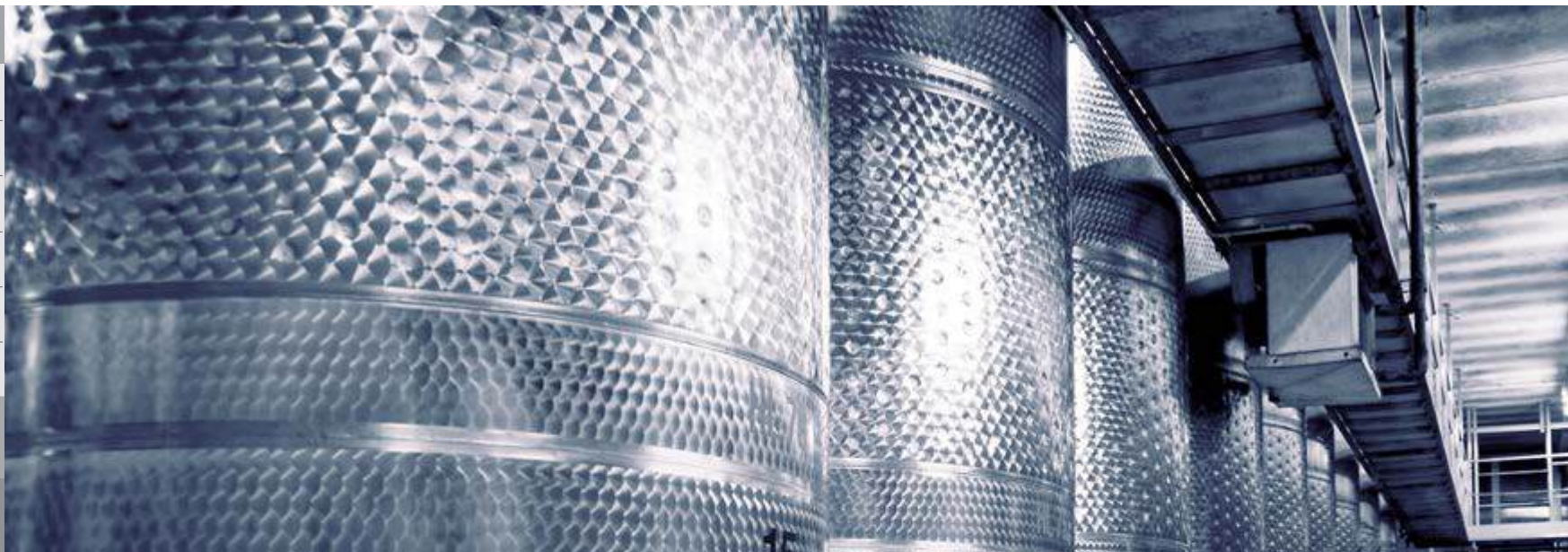
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In addition to protecting the wine industry against fraudulent authenticity practices, wine is included amongst other food and beverages where there are specific contaminant regulations. EU regulations stipulate a maximum limit of 0.2 mg/kg for lead in wine with the OIV recommended limit being 0.15 mg/kg. However, OIV also has recommendations for limits for other metals in wine such as arsenic (0.2 mg/kg), cadmium (0.01 mg/kg), copper (1.0 mg/kg), silver (0.1 mg/kg), sodium (80 mg/kg) and zinc (5 mg/kg) as well as limits for boron, bromine and fluoride.

The determination of trace and ultra-trace elements in wine is of great importance. On one hand, it allows detection of toxic elements and forms part of product quality control. On the other hand, elemental analysis of wine is also deployed within the context of provenance determination and the related detection of fraud or adulteration. OIV has a Type II inductively coupled plasma (ICP) ICP-MS method for simultaneous determination of 15

metals, boron and bromine in wine. The metals include aluminum, cadmium, cobalt, copper, strontium, iron, lithium, magnesium, manganese, nickel, lead, rubidium, sodium, vanadium and zinc. The wine sample is acid digested with indium and/or rhodium as an internal standard and analyzed directly by ICP-MS preferably with a gas collision/reaction cell. Various models of ICP-MS such as the Thermo Scientific™ iCAP™ RQ ICP-MS system provide total elemental analysis capability at sub parts per trillion (ppt), to parts per million (ppm) levels, which together with trace elemental analysis software, offer an intuitive, user-friendly platform designed to simplify workflows and maximize efficiency. The multi-element capabilities of collision cell technology (CCT) ICP-MS has been assessed for simultaneous determination of both spectrally interfered and non-interfered nuclides in French and Spanish wine samples using a single set of experimental conditions. Using a Thermo Scientific quadrupole-based X series 2 ICP-MS instrument equipped with nickel (Ni) cones and a hexapole collision/reaction cell, the ultra-trace determination of 55 elements was successfully conducted.



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If any fungal infection of grapes occurs, particularly after harvest, it is possible that the mycotoxin, ochratoxin A can be formed in the juice. It is sufficiently stable to survive fermentation and ultimately be present as a contaminant of wine. The EU has a maximum limit of 0.2 µg/kg for [ochratoxin A](#) in grape juice, wine, sparkling and aromatized wines. Ochratoxin A is particularly prevalent in red wine produced in the hotter southern parts of Europe. Official methods for ochratoxin A in wine have been published by the European Committee for Standardization ([CEN](#)) and by [OIV](#), both methods involving immunoaffinity column extraction and high performance liquid chromatography (HPLC) determination with fluorescence detection. Although levels of ochratoxin A in wine are generally low, it is still necessary to conduct routine analysis for which either the [Thermo Scientific™ Dionex™ UltiMate™ 3000](#) liquid chromatography (LC) system or the [Thermo Scientific™ Vanquish™](#) ultra-high-performance liquid chromatography (UHPLC) system with fluorescence detector are ideally suited.

Ethyl carbamate is a contaminant that can occur naturally in fermented beverages, such as spirits, wine and beer, forming during the fermentation process or during storage. Ethyl carbamate can be derived from various substances found within beverages, including hydrogen cyanide, urea, citrulline, and other N-carbamyl compounds. Cyanate is probably the ultimate precursor in most cases, reacting with ethanol to form the carbamate ester. Ethyl carbamate forms in stone fruit distillation, when exposed to light, from the natural precursors of fruit mash and ethyl alcohol. Hydrocyanic acid or the salts produced there from, the cyanides, are regarded as the most important precursors in the process. Hydrocyanic acid initially occurs in bound form in the stones of the fruits and is released through enzymes during the maturation process and after the harvest. Although ethyl carbamate does occur as a contaminant in wine, it is more prevalent in fortified and distilled spirits such as fruit brandy. In the EU, the only Member State with a limit for ethyl carbamate in wine is the Czech Republic which has set at a [maximum limit](#) of 30 µg/L. However, Canada has a similar limit for ethyl carbamate of 30 µg/L whilst the level

is set at 15 µg/L in the USA. Ethyl carbamate can be determined in wine by derivatization with 9-xanthidrol in an acidic medium, separation by HPLC and measurement by fluorescence detection. Alternatively gas chromatography-mass spectrometry ([GC-MS](#)) can be used employing selected ion monitoring using deuterated ethyl carbamate as an internal standard to improve quantification.

In grape must, the presence of organic bases (for example amines and amino acids), can facilitate sugar degradation which leads to the formation of a caramel flavor (5-hydroxymethylfurfural) and generation of a golden yellow coloration. The exact nature of the by-products depends on the sugars present, the pH, the temperature and the nature of any amine catalysts. Browning is a natural process that usually occurs in white wines, but also takes place in sparkling wines over time. It is a key quality indicator as consumers notice it and wineries make every effort to prevent it. The intensity of the color of wine using the [OIV method](#) is traditionally given by the sum of absorbencies (or optical densities) with a 1 cm optical path using for example an [Thermo Scientific™ Evolution™](#) or [Thermo Scientific™ GENESYS™ UV/Visible](#) spectrophotometer at wavelengths of 420, 520 and 620 nm. The shade of wine is expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm. There are also methods for direct measurement of 5-hydroxymethylfurfural as an indicator of browning either by a classical [OIV colorimetric method](#) or by an alternative [OIV HPLC method](#). The kinetics of 5-hydroxymethylfurfural formation has been recently studied in sparkling wines using a Thermo Fisher HPLC system with a photodiode array detector as well as liquid chromatography-mass spectrometry [LC-MS](#) using an ion trap detector. It was concluded that 5-hydroxymethylfurfural is a better time–temperature marker for wine than the absorbance at 420 nm, or measurement of total phenolics, because it shows higher linearity with time at all temperatures, is more sensitive to temperature changes, and has lower variability.

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As with any other plants, grape vines are susceptible to many types of pests and diseases, usually attacking during the growing season. Whilst spraying with fungicides, herbicides and pesticides remains essential, the move to integrated pest management programs aims to use agrochemicals in a targeted way and reduce overall pesticide usage. Spraying at optimum times in the growing season is carried out to minimize or prevent disease and control weeds. The first spraying, perhaps using lime–sulfur (contact), may start in the spring as the buds swell and soften. In early summer, foliage spraying is often done to prevent fungal infection and mildews forming. If good agricultural practice is followed, pesticide residues in grapes and consequently in juice should be below maximum residue levels (MRLs). After fermentation, pesticide residue levels in wine are always lower than those in the grapes and in the must, except for those pesticides that do not have a preferential partition between liquid and solid phase (azoxystrobin, dimethoate, and pyrimethanil) and can be present in wine at the same concentration as in the grapes. Clarifying substances such as bentonite, activated carbon, diatomaceous earth, gelatin, polyvinylpyrrolidone, potassium caseinate, and colloidal silicon dioxide can reduce or eliminate most pesticides. No MRLs have been established for pesticide residues specifically in wine, but the MRLs set for the raw commodity (wine grapes) are generally applied to wine. These MRLs are set out in an EU Directive and can be accessed through the EU Pesticide MRL [database](#).

The widely employed QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), method for extraction and clean-up of food and beverages prior to chromatographic analysis has also been employed by OIV as a Type II method for the determination of [pesticides in wine](#) prior to GC-MS or LC-MS/MS. The QuEChERS procedure involves cleanup by dispersive solid-phase extraction (dSPE) using primary secondary amine (PSA) sorbent, which effectively retains organic acids, sugars, and phenolic pigments. For red wine, a higher quantity of PSA than normally used in the dSPE step is required to sufficiently remove all co-extracted phenolic compounds. [For 24 pesticides spiked at different levels into various wines](#) QuEChERS cleanup followed by LC-MS/MS was carried out using a Dionex UltiMate 3000 LC coupled to a Thermo Scientific™ TSQ Vantage™ tandem mass spectrometer. Pesticide recoveries averaged 83–91% with RSDs of 3.3 to 12.5%. Fourteen commercially available bottles of red wine from various geographical regions around the world were tested in duplicate using the developed method. Of the fourteen wines tested, six samples were found to contain one or more of carbendazim, pyrimethanil, bifentazate, tebuconazole, and cyprodinil. The concentrations of these five pesticides detected ranged from 2.2 to 13 ng/mL which is approximately 100 to 1000 times lower than the MRLs set for wine grapes by the EU.



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All materials whether plastics, metal or even wood used in the production of wine must comply with [EU regulations](#) in terms of their suitability. Only approved substances can be used in manufacture of plastics for contact with wine and any transfer from plastics materials and articles into wine must be below stipulated migration limits. These regulations cover all plastics used in the winery during production (for example, plastic tubing) and in particular materials used for wine storage that might be glass reinforced plastics, or possibly polymeric coatings on metal storage or transport containers. The grape juice was formally fermented in wax-lined concrete or plastic vats, but now stainless steel is used for all wines except for certain high-quality types that are fermented in wood. Wooden barrels are the container of choice for chardonnay, sauvignon blanc and pinot noir and the wood is smoked during processing, forming additional flavor compounds (particularly tannins) which are leached into the wine, giving it further complexity.

Although glass wine bottles are unlikely to leach contaminants into the wine even after many years' of storage, corks are increasingly being replaced by synthetic materials and have to be compliant with materials and articles regulations and consequent migration

limits. Plastics wine bottles and closures which are sometimes used for cheaper wines must be fully compliant with [EU regulations](#), particularly as ethanol in wine is a fairly aggressive medium that can promote migration.

A 'corked wine' is one that has an unpleasant off-flavor caused by molds and is the commonest of the undesirable flavors found in wine. Generally, the cork is responsible for tainting wine with 2,4,6-trichloroanisole. However, barreled wine may also be contaminated with 2,3,4,6-tetrachloroanisole, which can be present in the damp atmosphere of wine cellars in the presence of woods treated by polychlorophenols. Wines often have this defect and it can affect up to 5% of bottles sealed with conventional corks. An [OIV method for 2,4,6-trichloroanisole](#) migration, tests the corks by maceration in either wine or a aqueous-alcoholic solution, until a balance is obtained. Trichloroanisole in the headspace is sampled from above the macerate by solid-phase micro-extraction (SPME), then analyzed by GC-MS or GC electron-capture detection (ECD). The Thermo Scientific™ [TRACE™](#) Ultra Gas Chromatograph and Thermo Scientific™ [TriPlus RSH™ Autosampler](#) can be used to automate the complete SPME cycle, from fiber conditioning to desorption prior to GC-ECD or GC-MS, for the analysis of 2,4,6-trichloroanisole in migration test solutions, or in wine itself.





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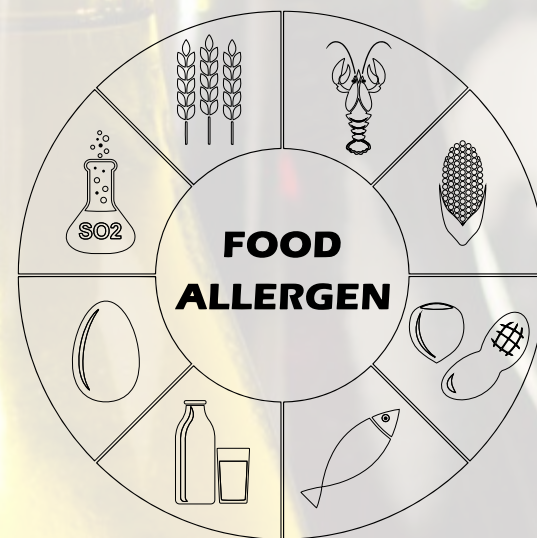
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Wine is also covered by [EU labeling regulations](#) requiring the labeling of foods and beverages known to contain certain specified allergens. For wines, labeling is required if sulfite residues exceed 10 mg/L. In addition to allergen labeling requirements for sulfite, the [total SO<sub>2</sub> content of wines](#), other than sparkling wines, may not exceed 150 mg/L for red wines and 200 mg/L for white and rosé wines. However, this limit of SO<sub>2</sub> for wines with the sum of glucose and fructose content of not less than 5 g/L, is raised to 200 mg/L for red wines, 250 mg/L for white and rosé wines and 300–400 mg/L for certain designated wines. Apart from [classical methods](#) to determine [sulfite in wine](#) the Gallery system provides a fully automated system to measure free [SO<sub>2</sub> in wine samples](#), or one might choose to measure [total sulfite by ion chromatography](#) after conversion to sulfate.

Labeling of wine is also required if milk (caseins) or egg residues (lysozyme) used in clarification treatments are present in wine. Although fish is a specified allergen, fish gelatin or Isinglass, used as fining agents, in wine are excluded from these allergen labeling requirements. Different analytical methods are being used for quantitative analysis of allergens in wine, based on either immunoassay or mass spectrometry. Immunoassays such as the enzyme-linked immunosorbent assay (ELISA), have unique advantages of simplicity and speed of analysis, but they are usually performed on a single target and can suffer cross-reactivity reactions and poor accuracy. In contrast mass spectrometry has the advantage of high sensitivity and specificity, and can screen for a number of allergens in a single analysis, but the sample preparation can be complex. An [LC-MS/MS method](#) has been published for the simultaneous determination of ovalbumin,  $\alpha$ - and  $\beta$ -casein in red wine, involving an easy protein cut-off concentration protocol combined with size-exclusion-based purification followed by tryptic digestion. Detection of target peptides is

by LC-MS/MS using a HPLC system coupled online with a Linear Trap Quadrupole (LTQ), ion trap. Good results in terms of sensitivity were obtained, with limits of detection (LoD), and limits of quantitation (LoQ) ranging from 0.01 to 0.8 mg protein/mL wine and from 0.03 to 2 mg protein/mL, respectively. A similar approach using the [Thermo Scientific™ Orbitrap™](#) HRAM technology, has been used for peptide measurement prior to a combination of ultrafiltration of wine, tryptic digestion of the dialyzed wine extracts and detection of peptides, providing the most intense electrospray ionization response. Based on eight peptide markers the method was capable of detecting and quantifying simultaneously, some of the proteins derived from fining agents, that is, caseins, ovalbumin and lysozyme, with LoDs found to be in the range between 0.4 and 1.1  $\mu\text{g/mL}$  with the [Orbitrap system](#) operated at a resolving power of 50,000 providing high specificity.



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It is also worth noting that other additives used in wine production have maximum limits, for example sorbic acid, used as a preservative in dealcoholized wine has a limit of 200 mg/L. Benzoic acid, dimethyl dicarbonate and natamycin all have specific approved uses and associated limits, for which official methods of analysis are published. There are a number of food additives, including colors that are used in some fortified, aromatized, alcohol-free wines and aromatized wine-based drinks. These additives and maximum permitted levels are all specified in [EU Regulation 1129/2011](#). From this brief description of the regulations at EU level that apply to the wine production process through to the finished product, it is clear that while additives can be carefully controlled, ensuring levels in bottled wine are compliant, there is a significant role for official laboratories to carry out routine analytical checks.

EU legislation is detailed in the processes it allows: heat treatment, centrifuging, filtration, removal of sulfur dioxide and electro dialysis, but it does not specifically include racking, cold stabilization or the use of barrels. In contrast the USA legislation lists some of the more novel treatments only and omits more traditional processes such as racking. In a similar way to the EU, in the U.S. the Bureau of Alcohol, Tobacco Products and Firearms stipulates the materials authorized for the treatment of wine and juice, for which there are many similarities, but also big differences with the EU. For example, in the U.S. the addition of fumaric acid is permitted to correct natural acid deficiencies in grape wine subject to the restriction that the fumaric acid content of the finished wine shall not exceed 3 g/L, whereas in the EU fumaric acid is not permitted and only the use of L-tartaric acid, L-malic acid, DL malic acid, or lactic acid is approved for acidification purposes. In the

EU the addition of tannin is permitted for clarification purposes whereas in the USA it is permitted both for clarification and to adjust tannin content, but the residual amount of tannin, calculated in gallic acid equivalents, shall not exceed 0.8 g/L in white wine and 3.0 g/L in red wine. Total tannin shall not be increased by more than 150 mg/L by the addition of tannic acid (poly-galloylglucose). Within the scope of this document, it is not possible to provide a detailed critique of similarities and differences in wine regulations. However, the examples given clearly illustrate the importance of being able to accurately measure specific parameters from the perspective not only of the wine producer, but also the authorities.



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The focus of this publication is the analytical measurement and testing requirements during wine production, from the harvesting of grapes to final bottling and maturation of wine. It involves targeted analysis of small molecules, and the role in fermentation and subsequent stages of wine production, this is largely understood. However, there is a fascinating complexity to the overall chemical composition of wine, that not only affects its color and taste, but also the presence of 'bioactive' compounds, underlying the intriguing question of whether there are beneficial health effects of wine consumption. It is this complexity that differentiates one wine from another and leads to 'vintage' wines of extraordinary quality and premium prices. In recent years, the developments in sophisticated instrumental techniques for isolation and identification of volatile and non-volatile components, particularly using LC-high resolution mass spectrometry have aided in research to begin to unravel the complexity of wine.

It is impossible in a few paragraphs to definitively describe the complex composition of wine as there are a vast array of compounds which contribute to color, flavor and bioactivity.

Phenolic compounds are very influential constituents of grapes and wine, affecting organoleptic properties through their contribution to astringency, bitterness and color. Phenolic compounds of which anthocyanins (anthocyanidin-glycosides) are amongst the most important, play a significant role in the ageing of wines, as well as in grape browning. It is also the phenolic content of wine that has been ascribed to several important health benefits associated with modest consumption especially of red wine. The distribution and concentration of grape anthocyanins depends on the cultivar, maturity, climatic conditions, production area and fruit yield. In general, malvidin is the major anthocyanidin with between 50–90% occurring in different red grape varieties. Levels of acylated anthocyanins are largely influenced by the grape variety but they could be completely absent from some varieties. Flavanols, flavonols, and dihydroflavonols, like the anthocyanins belong to the flavonoid family. Within each flavonoid class, there is also a huge diversity in chemical structures derived from modifications of the three ring skeleton, including hydroxylation, methylation of some of the phenolic hydroxyls, glycosylation, acylation of the alcoholic hydroxyl groups and polymerization, giving rise to hundreds of possibilities. The composition and diversity of flavanols in wine is linked to taste (especially astringency and bitterness) and in the development of oxidative browning, haze and precipitates.



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The huge complexity of the anthocyanins is illustrated by a [study of red wine grape pomace](#). After extraction and fractionation, the anthocyanin and anthocyanin-derived compound composition of each fraction was evaluated by LC-DAD/MS. Using a Thermo Scientific Vanquish UHPLC system, monitoring with photodiode array (PDA) detector and mass detection using an [LTQ Orbitrap XL](#) mass spectrometer more than 50 different anthocyanin and anthocyanin-derived compounds were found. They were identified as mainly pyranoanthocyanins including A- and B-type vitisins and methylpyranoanthocyanins as well as oligomeric malvidin-3-O-coumaroylglucoside-based anthocyanins.

In contrast to methods used to detect and identify individual phenolic compounds, Fourier transform infrared spectroscopy (FTIR) using attenuated total reflectance (ATR) has been applied for the determination of total phenolic and flavonoid contents and [antioxidant capacity](#) of dessert wines. Classical methods were used to measure total phenolic content, total flavonoid content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). Statistical techniques were used for calibration and validation of the spectra obtained using a [Nicolet FTIR](#) to provide a rapid screening technique for total phenolic and flavonoid contents in Moscatel dessert wines and rough estimates for DPPH and FRAP antioxidant capacities.

The non-flavonoid phenolic constituents in wine are divided into hydroxybenzoic acids and hydroxycinnamic acids, volatile phenols, stilbenes and miscellaneous compounds (for example, lignans and coumarins). Although, the non-flavonoid constituents are non-colored they are known to enhance and stabilize the color of red wines by intra- and intermolecular reactions. They furthermore contribute to wine flavor (volatile phenolic acids) and some of them (for example, resveratrol) exhibit potent biological activities.

Polyphenols from red wine have been reported to exert potent antioxidant effects that prevent low-density lipoprotein (LDL) oxidation and despite some reports of the absence of an association, they are serious candidates to explain the protective effects of vegetable and fruit consumption against cancer and cardiovascular diseases. The beneficial effects of wine may not be attributable to a single polyphenol, but rather to a complex mixture of polyphenols. There is evidence that antioxidant properties underlie most of the effects of wine, but at the same time, certain effects cannot be mimicked by common dietary antioxidants. The balance of evidence is that polyphenols present in red grapes and derived products appear to exert beneficial effects on health.



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Unravelling the Complexity of Wine

Non-Volatiles - Color, Taste and Bioactivity

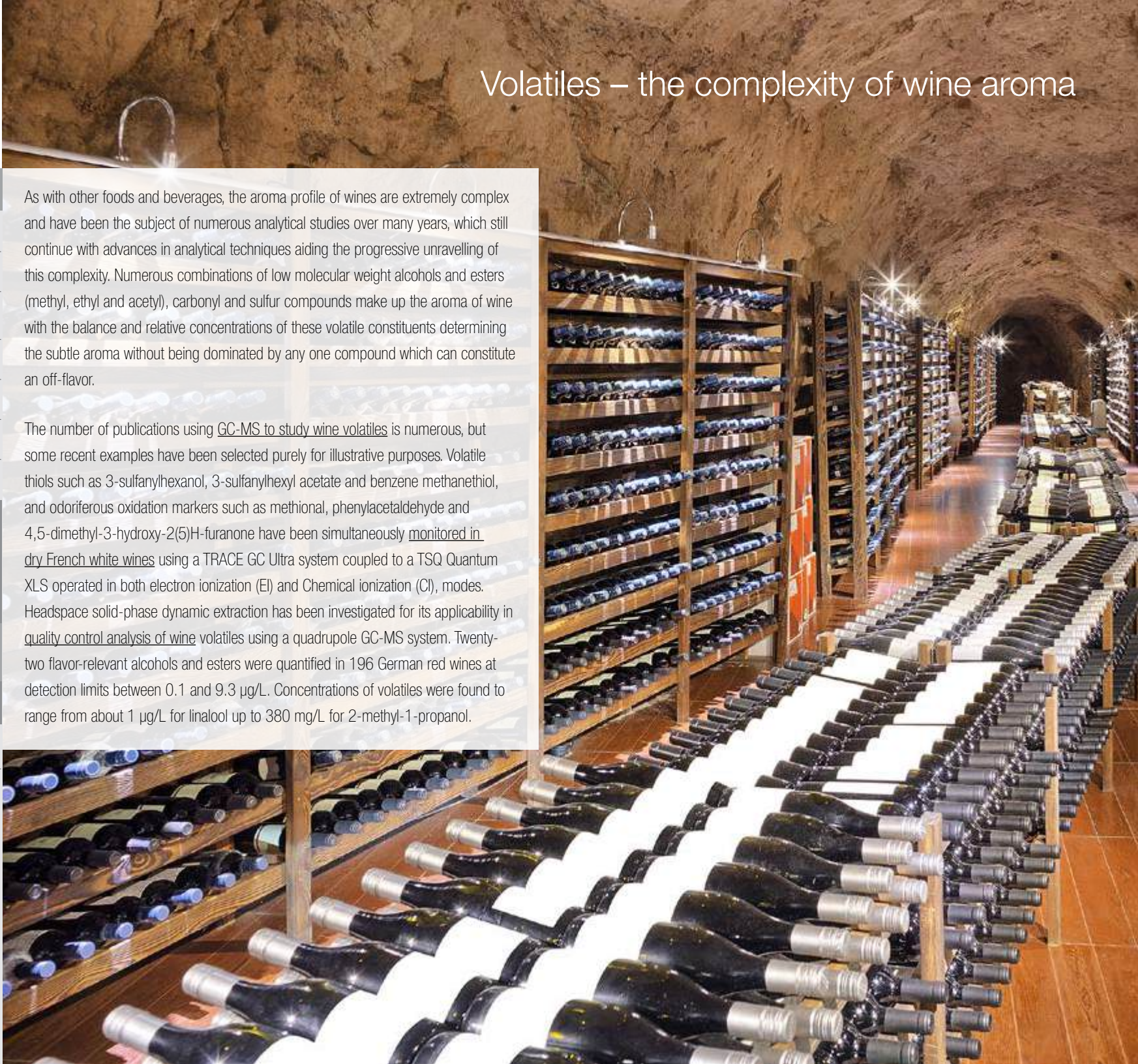
Volatiles – The Complexity of Wine Aroma

Future Wine Testing

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As with other foods and beverages, the aroma profile of wines are extremely complex and have been the subject of numerous analytical studies over many years, which still continue with advances in analytical techniques aiding the progressive unravelling of this complexity. Numerous combinations of low molecular weight alcohols and esters (methyl, ethyl and acetyl), carbonyl and sulfur compounds make up the aroma of wine with the balance and relative concentrations of these volatile constituents determining the subtle aroma without being dominated by any one compound which can constitute an off-flavor.

The number of publications using GC-MS to study wine volatiles is numerous, but some recent examples have been selected purely for illustrative purposes. Volatile thiols such as 3-sulfanylhexanol, 3-sulfanylhexyl acetate and benzene methanethiol, and odoriferous oxidation markers such as methional, phenylacetaldehyde and 4,5-dimethyl-3-hydroxy-2(5)H-furanone have been simultaneously monitored in dry French white wines using a TRACE GC Ultra system coupled to a TSQ Quantum XLS operated in both electron ionization (EI) and Chemical ionization (CI), modes. Headspace solid-phase dynamic extraction has been investigated for its applicability in quality control analysis of wine volatiles using a quadrupole GC-MS system. Twenty-two flavor-relevant alcohols and esters were quantified in 196 German red wines at detection limits between 0.1 and 9.3 µg/L. Concentrations of volatiles were found to range from about 1 µg/L for linalool up to 380 mg/L for 2-methyl-1-propanol.



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Grape and wine analysis is one of the few areas where classical 'tried and tested' methods based on simple inexpensive techniques, still exist alongside new automated technologies and extend to some of the most sophisticated analytical techniques available today. Thermo Fisher Scientific uniquely provides equipment and instrumentation to meet the most basic as well as the most sophisticated of needs, operating at the forefront of analytical chemistry. Wine testing cannot replace the skills of the wine-maker, but providing real-time data indicating critical compositional parameters before, during and after fermentation can provide insights which allow the wine-maker to optimize production and produce wines of a consistently high quality. For the wine control authority, analysis provides the means to ensure compliance with rules of wine production and the means to guard against wine fraud, whether it be illegal addition of sugar or mislabeling the geographical origin of wine. For the future, hand-held devices based on spectroscopic techniques such as FTIR will play an increasingly important role in rapid and routine wine testing, whilst sophisticated separation and identification tools will continue to develop.



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**Peer Reviewed Journals:  
GC and GC-MS Methods**

Title	Authors	Publication
<a href="#">Chemical and sensory characterisation of Sangiovese red wines: Comparison between biodynamic and organic management</a>	Giuseppina Paola Parpinello, Adamo Domenico Rombolà, Marco Simoni, Andrea Versari,	Food Chemistry 167 (2015) 145–152
<a href="#">Volatile phenols depletion in red wine using molecular imprinted polymers</a>	Rafaela Teixeira, Sonia Dopico-García, Paula B. Andrade, Patrícia Valentão, José M. López-Vilariño, Victoria González-Rodríguez, Concepción Cela-Pérez, Luís R. Silva	J Food Sci Technol (2015) 52(12):7735–7746
<a href="#">Identification of gum Arabic in white wine based on colloid content, colloid composition and multi-element stable isotope analysis</a>	Sabrina Sprenger, Klaus Meylahn, Anne Zaar, Helmut Dietrich, Frank Will	Eur Food Res Technol (2015) 240:909–921
<a href="#">Improved sample preparation for GC–MS–SIM analysis of ethyl carbamate in wine</a>	Ian C.C. Nóbrega, Giuliano E. Pereira, Marleide Silva, Elaine V.S. Pereira, Marcelo M. Medeiros, Danuza L. Telles, Eden C. Albuquerque Jr. c, Juliane B. Oliveira, Dirk W. Lachenmeier	Food Chemistry 177 (2015) 23–28
<a href="#">Dissipation of Fungicide Residues during Winemaking and Their Effects on Fermentation and the Volatile Composition of Wines</a>	Raquel Noguero-Pato, Tania Fernández-Cruz, Thais Sleiro-Sampedro, Carmen González-Barreiro, Beatriz Cancho-Grande, Diego-Augusto Cilla-García, Mariá García-Pastor, Mariá -Teresa Martínez-Soria, Jesús Sanz-Asensio, and Jesús Simal-Gándara	J. Agric. Food Chem. 2016, 64, 1344–1354
<a href="#">Comparison of electron and chemical ionization modes for the quantification of thiols and oxidative compounds in white wines by gas chromatography–tandem mass spectrometry</a>	Cécile Thibon, Alexandre Pons, Nadia Mouakka, Pascaline Redon, Raphaël Méreau, Philippe Darriet	Journal of Chromatography A, 1415 (2015) 123–133

**Peer Reviewed Journals:  
ICP-MS Methods**

Title	Authors	Publication
<a href="#">Classification of Croatian wine varieties using multivariate analysis of data obtained by high resolution ICP-MS analysis</a>	Dasa Kruzlicova, Željka Fiket, Goran Kniewald	Food Research International 54 (2013) 621–626
<a href="#">Geographical traceability based on 87Sr/86Sr indicator: A first approach for PDO Lambrusco wines from Modena</a>	Caterina Durante, Carlo Baschieri, Lucia Bertacchini, Marina Cocchi, Simona Sighinolfi, Michele Silvestri	Food Chemistry 141 (2013) 2779–2787
<a href="#">Evaluation of the multi-element capabilities of collision/reaction cell inductively coupled plasma–mass spectrometry in wine analysis</a>	Guillermo Grindlay, JuanMora, Margaretha T.C.de Loos-Vollebregt, Frank Vanhaecke	Talanta (2014), 128, 379–385



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**Peer Reviewed Journals:****IRMS Methods**

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<a href="#">The effect of stopping alcoholic fermentation on the variability of H, C and O stable isotope ratios of ethanol</a>	M. Perini, R. Guzzon, M. Simoni, M. Malacarne, R. Larcher, F. Camin	Food Control 40 (2014) 368-373
<a href="#">O–H–C isotope ratio determination in wine in order to be used as a fingerprint of its regional origin</a>	B. Raco, E. Dotsika, D. Poutoukis, R. Battaglini, P. Chantzi	Food Chemistry 168 (2015) 588–594
<a href="#">Mass Spectrometric Analysis of the <sup>13</sup>C/<sup>12</sup>C Abundance Ratios in Vine Plants and Wines Depending on Regional Climate Factors</a>	A. M. Zyakun, L. A. Oganesyants, A. L. Panasyuk, E. I. Kuz'mina, A. A. Shilkin, B. P. Baskunov, V. N. Zakharchenko, and V. P. Peshenko	Journal of Analytical Chemistry, (2013) 68, 13, 1136–1141
<a href="#">H, C, and O Stable Isotope Ratios of Passito Wine</a>	Matteo Perini, Luca Rolle, Pietro Franceschi, Marco Simoni, Fabrizio Torchio, Vincenzo Di Martino, Rosa Maria Marianella, Vincenzo Gerbi, and Federica Camin	J. Agric. Food Chem. 2015, 63, 5851–5857

**Peer Reviewed Journals:****Ion Chromatography Methods**

Title	Authors	Publication
<a href="#">Determination of Free and Total Sulfite in Red Globe Grape by Ion Chromatography</a>	Jiangli Lin, Yajuan Zhu, Wenhui Cheng, Jinxia Wang, Bin Wu, Jide Wang	Food Science and Technology Research Vol. 20 (2014) 1079-1085
<a href="#">Verifying the botanical authenticity of commercial tannins through sugars and simple phenols profiles</a>	Mario Malacarne, , Tiziana Nardin, Daniela Bertoldi, Giorgio Nicolini, Roberto Larcher	Food Chemistry, Volume 206, (2016), Pages 274–283

**Peer Reviewed Journals:****Photometric Analyzer Methods**

Title	Authors	Publication
<a href="#">Effect of headspace volume, ascorbic acid and sulphur dioxide on oxidative status and sensory profile of Riesling wine</a>	Ksenia Morozova, Oliver Schmidt, Wolfgang Schwack	Eur Food Res Technol (2015) 240:205–221





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**Peer Reviewed Journals:  
LC and LC-MS Methods**

Title	Authors	Publication
<a href="#">Identification and Organoleptic Contribution of Vanillylthiol in Wines</a>	Morgan Floch, Svitlana Shinkaruk, Philippe Darriet, and Alexandre Pons	J. Agric. Food Chem. 2016, 64, 1318–1325
<a href="#">Identification of phenolic compounds in red wine extract samples and zebrafish embryos by HPLC-ESI-LTQ-Orbitrap-MS</a>	Anna Vallverdú-Queralt, Nuria Boix, Ester Piqué, Jesús Gómez-Catalan, Alexander Medina-Rejon, Gemma Sasot, Mercè Mercader-Martí, Juan M. Llobet, Rosa M. Lamuela-Raventos	Food Chemistry 181 (2015) 146–151
<a href="#">Determination of ethyl carbamate in fermented liquids by ultra high performance liquid chromatography coupled with a Q Exactive hybrid quadrupole-orbitrap mass spectrometer</a>	Xirong Zhao, Changxing Jiang	Food Chemistry 177 (2015) 66–71
<a href="#">High-throughput method based on quick, easy, cheap, effective, rugged and safe followed by liquid chromatography-multi-wavelength detection for the quantification of multiclass polyphenols in wines</a>	Ariel R. Fontana, Rubén Bottini	Journal of Chromatography A, 1342 (2014) 44–53
<a href="#">Determination of 4-ethylcatechol in wine by high-performance liquid chromatography-coulometric electrochemical array detection</a>	Larcher R, Nicolini G, Bertoldi D, Nardin T.	Anal. Chim. Acta 609 (2), 235 –240
<a href="#">Identification and quantification of 56 targeted phenols in wines, spirits, and vinegars by online solid-phase extraction-ultrahigh-performance liquid chromatography-quadrupole-Orbitrap mass spectrometry</a>	C. Barnaba, E. Dellacassa, G. Nicolini, T. Nardin, M. Malacarne, R. Larcher	Journal of Chromatography A, 1423 (2015) 124–135





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