8

Elements and Inorganic Anions in Winemaking: Analysis and Applications

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8.1. Introduction

8.1.1. Oenological Framework

The cationic and anionic mineral fraction making up wine ashes does not exceed 5000 mg/L (Eschnauer and Neeb, 1988) and is more frequently in the range of 2000–3000 mg/L. Of this, around 97% is essentially constituted of eight macroelements (K, Mg, Ca, Na, C, P, S, Cl), which are present with concentrations higher than 10 mg/L. The remaining 3% of the fraction, according to a classification proposed by Eschnauer *et al.* (2001), is represented by:

- micro- and trace elements with concentrations between 0.01 and 10 mg/L, including Si, Mn B, Rb, Zn, Sr, Fe, Cu, Al, F and I present in higher concentrations, and Sn, V, Ti, As, Ba, Pb, Br, Cr, Li, Ni, Co, Mo and Ag in lower concentrations;
- ultra-trace elements, with concentrations lower than 10 μg/L, which also includes rare earth and radioactive elements.

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Because of the role of the different elements in the chemical-physical stability of wine, mineral fraction, understood not just as an overall parameter expressed through the measurement of ashes and their alkalinity, but also as the quantification of individual elements, has been studied for decades. The main examples are precipitation in wine of tartaric acid salts with calcium and potassium, and precipitation caused by excessive content of iron (III) and phosphates ('iron casse'). This problem was remedied in Germany as early as 1923 by treatment of the wine with potassium ferrocyanide, and copper (I), for the elimination of which the use of sodium sulphide was proposed (Ribereau-Gayon, 1947). In addition to this, haze due to aluminium (Rankine, 1983), precipitation of calcium mucate in wines from botrytized grapes and of other elements (e.g. cadmium, manganese, mercury, lead, copper, tin and zinc) in the form of sulphides, may occur (e.g. Ribereau-Gayon, 1947; Garoglio, 1981; Ribéreau-Gayon *et al.*, 1980; Boulton *et al.*, 1995; Flanzy, 1998). Elements such as W, Zn, Cu, Co, Fe, Ni and Mn, in decreasing order, have the capacity to catalyze oxidative reactions. However, bearing in mind their concentrations, Fe and Zn primarily, and secondarily Cu and Mn, have a genuine catalytic role of technological significance. On the contrary, Al limits the oxygen consumption in wine (Vivas, 2002). It is likely that the capacity of many compounds to chelate Al^{3+} reduces their sensitivity to oxidation.

The elemental mineral composition of grapes, wines and derivates and by-products has been widely investigated, although the reasons for analyzing particular micro and trace elements have not always been completely clear, due to their limited technological significance (Ough and Amerine, 1988). A brief list of works integrating the publications cited in the commendable paper by Eschnauer and Neeb (1988), includes those by Margheri and Rigotti (1964), Ciusa and Giaccio (1972), Cerutti *et al.* (1981, 1984, 1990), Interesse *et al.* (1984, 1985) and Finoli *et al.* (1986). Eschnauer *et al.* (2001) estimated that at least 1000 papers were written between 1890 and 2000 on the content of trace and ultra-trace elements in wine, and around 350 regarding mineral elements. About 70 further studies focusing significantly on the elemental composition of grapes, wines and spirits and relative analytical methods, in addition to an equal number dealing with this topic in a marginal manner, have been published since 2000 according to the bibliographic research carried out using the Food Science and Technology Abstracts (FSTA) database.

Bringing together and summarizing the direct experience of the authors, deriving from everyday practice in the oenological laboratory, this chapter focuses on modern applications and the significance of the analysis of elements and inorganic anions in the oenological field. It updates data on grapes, wines and derivatives and the compositional effects of several winemaking treatments.

8.1.2. Elements

Soil is usually considered among the most important enrichment factors relating to the natural elemental content in grapes and wines, e.g. high contents of Na, and of B and V, in salty soils and in volcanic soils, respectively, are reported (Salaha-Moutsopoulou and Voudouri-Tsoukala, 1991; Savino *et al.*, 1994; Teissèdre *et al.*, 1996b; Conde *et al.*, 2002, Eschnauer, 1982).

Natural or artificial contamination often significantly increases the natural level of several elements in wine (Medina *et al.*, 1977; Seppi and Sperandio, 1978; Medina and Sudraud, 1980; Eschnauer, 1982; Pertoldi Marletta *et al.*, 1986; 1989; Prosperi and Piga, 1988; Lemperle and Lay, 1989a; Mc Kinnon *et al.*, 1992; Gulson *et al.*, 1992; Ough, 1993; Eschnauer and Scollary, 1995; Angelova *et al.*, 1999b; Médina *et al.*, 2000; Castiñeira Gómez *et al.*, 2004a) sometimes in relation to the addition of adulteration substances (e.g. preservatives, sweetening agents) as recalled by Eschnauer and Neeb (1988).

The potential toxicity of some elements – with particular reference to heavy metals and, among these, with particular attention being paid to lead – is one of the main reasons for investigating the content of such elements in grapes and wines (Castelli *et al.*, 1974; Basile and Tarallo, 1974; Crecelius, 1977; Stefani, 1978; Handson, 1984; Sherlock *et al.*, 1986; Gabrielli Favretto *et al.*, 1986; Elinder *et al.*, 1988; Smart *et al.*, 1990, Marini and Gallese, 1991; Teissèdre and Cabanis 1993; Teissèdre *et al.*, 1993; 1994; Ough, 1993; Šebecic *et al.*, 1998; Pinamonti *et al.*, 1999; Angelova *et al.*, 1999a). The contribution of wine with specific reference to the dietary uptake of some elements has been much more rarely investigated (e.g. Teissèdre *et al.*, 1996a; 1996b; Cabrera-Vique *et al.*, 2000; Terrés *et al.*, 2001), in particular in a context of positiveness. What is more, the real bioavailability of some elements, not only in wine but also along the whole soil-vine-grape-wine chain, has not been fully clarified. Tokalioglu *et al.* (2004) observed for 11 elements measured in 22 grape and corresponding polluted vineyard soils, a relationship between the content in soil and grapes for Cd, Pb, Zn, Mn and Fe. Soil-wine correlations in the content of several elements were also found by Almeida and Vasconcelos (2003). Karagiannidis and Nikolaou

(2000) observed that *arbuscular mycorrhizae* could reduce Pb and Cd uptake from soil, giving lower content of the two elements in grapes of Razaki cultivar grafted onto 110R rootstock. In wine, significant Pb, but also Sr and Ba, are linked to a dimer of rhamnogalacturonane II (Pellerin *et al.*, 1997; Green and Scollary, 2000), a pectic polysaccharide which can significantly condition their availability.

Depletion phenomena in the element content of wines are seen less frequently in everyday wine-making practice. In the case of heavy metals present in high concentrations, the only depletion interventions – without distinguishing between authorized and illegal procedures in the different countries – are chemical treatments with potassium ferrocyanide and Fessler's compound, and interventions with different chelate or ion exchange resins to mainly deplete Fe and Cu (Kern and Wucherpfennig, 1991; 1993; Fussnegger *et al.*, 1992; Mattivi *et al.*, 1994; Nicolini *et al.*, 2004c;), Pb (Mínguez *et al.* 1996; 1997), and various other elements in the preparation of rectified concentrated musts and Vermuts. A lowering in the content of some elements obtained biologically has been also observed with live and non-living biomass of *Saccharomyces cerevisiae* in laboratory conditions (Volesky and May-Phillips, 1995), and various micro-organisms have shown capacity for element accumulation (Norris and Kelly, 1977; Nakajima and Sakaguchi, 1986; 1993; White and Gadd, 1987; Volesky *et al.*, 1993; Blakwell *et al.*, 1995).

In relation to the health and technological aspects seen above, some cations – mainly Cu, Fe, Pb, Zn, K and Ca – are routinely monitored in quality control protocols and Hazard Analysis and Critical Control Points (HACCP) applied in wineries. Moreover, more and more restrictive 'technological' limits have been defined in wine transactions (e.g. Ni < 8–10 μg/L toward Germany) between private companies by buyers, particularly big bottlers or importers. Sometimes such 'technological' limits overlap with the existing legal or maximum acceptable limits which to date can be summarized as follows: As 0.2 mg/L, Cu 1 mg/L, Zn 5 mg/L, Pb 0.15 mg/L, B 80 mg/L (as boric acid), Cd 0.01 mg/L, F 1 mg/L (3 mg/L in the countries where criolite can be used as insecticide) and Br 1 mg/L. The International Organisation of Vine and Wine (O.I.V.) defined the limit of 60 mg/L for Na exceeding the equivalent chloride concentration, but contents higher than 60 mg/L (expressed exclusively as total Na), as well as higher than 60 μg/L for Li, can sometimes lead control agencies to carry out deeper analysis for suspected use of wines denatured by addition of 5–10 g/L LiCl. For grapes, limits of 20 and 50 mg/kg have been established for Cu and S respectively.

8.1.3. Inorganic Anions

The inorganic anionic fraction of grapes and wine is essentially constituted of sulphates, chlorides, phosphates and nitrates and has been investigated to a much lesser extent in recent years as compared to the cationic fraction. These anions are absorbed by plants through the roots from the soil, which is the main source, depending on geo-pedological origin and any fertilization, correction and soil improvement measures (Currle *et al.*, 1983).

Sulphates

As regards sulphates, Leske *et al.*, (1997) observed overall average values of 260 mg/L in Australian must, with a maximum of 1197 mg/L and marked differences among different areas of origin of grape, varying between 157 mg/L and 393 mg/L. Ough and Amerine (1988), reviewing previous works regarding wines from 15 countries, reported K_2SO_4 concentrations in wines from 70 mg/L to 4.39 g/L. Values of between 200–1300 mg/L were reported by Flak and Schaber (1989) in Austrian wines, with the highest frequency between 500 and 800 mg/L, and contents up to 2200 mg/L (as SO_4^{2-}) were reported by Lemperle and Lay (1989a, quoting Zee *et al.*, 1983), with German wines between 160 and 400 mg/L. Artificial increases of sulphates can come from the use of fermentation adjuvants, acidifying treatments with gypsum (an old practice only allowed for *Jerez* wines), and oxidation of SO_2, more intense in wood barrels. Increases in sulphates, as well as other inorganic anions, can derive from the fraudulent addition of the corresponding acids. At all events, the final content of sulphates in wine cannot be higher than 2 g/L, expressed as K_2SO_4, with the exception of *Marsala*, liqueur-like wines and 'mistella' where the limit is 5 g/L (Reg. Eu. Cons. No. 1493/1999; Italian G.U. L. 82/2006). The role of sulphates in the formation of protein haze in white wine was recently proven by Pocock *et al.* (2007).

Phosphates

The content of phosphates in wines usually varies between 50 and 1000 mg/L (as PO_4^{3-}) with a lower concentration in white wines as compared to the red ones (Garoglio, 1981; Zee *et al.*, 1983; Ough and Amerine, 1988). Flak and Schaber (1989) did not find significant differences in Austrian wine, neither as regards the type of soil in the different areas of production nor as regards the rootstock used. The location of phosphates in the grape berry has an important role in conditioning the final content in the wines, seeds contributing significantly (Wucherpfennig and Chen Hsueh-err, 1983). Further increases may derive from the use of common fermentation adjuvants, mainly diammonium phosphate.

Chlorides

Being mainly geogenic, the natural content of chlorides in wine is closely related to the grape growing area. The natural NaCl content in wines usually lies in the range between 20 and 200 mg/L, but values up to 1800 mg/L can be found in arid areas of Europe, such as Spain (Ough and Amerine, 1988) and the Greek islands, also in connection with grape withering (Salaha-Moutsopoulou and Voudouri-Tsoukala, 1991), and from Australia (Leske *et al.*, 1997). According to Wurzinger *et al.* (1992), up to 50–60 mg/L NaCl can be expected in genuine Austrian wines, with higher values for wines designated as 'mit Praedikat', namely from grapes which are riper and of better quality than those for table wines, and from 'burgenländischen Seewinkels' near Vienna because of the salty soil and groundwater. For chlorides, the water quality used for irrigation is particularly important, as Savino *et al.* (1994) stressed the apparent contradiction of wines produced in areas relatively close to the sea with lower contents than wines produced in inland but irrigated with brackish waters. National limits are sometimes defined for chloride, but a European harmonization is in progress and has defined the limit of 1 g/L NaCl for wines and 2 g/L for *Marsala*, fortified wines, mute musts by alcohol addition and 'mistella' (Reg. Eu. Cons. No. 1493/1999; Italian G.U. L. 82/2006).

Nitrates

As for nitrates, summarizing the data from several authors, Rapp (1989) reports contents in juices ranging from 1–2 mg/L up to decidedly rarer cases with 26 mg/L. Such values are lower than those reported by Cerutti (1999) for several marketed vegetables. For wines, Lemperle and Lay (1989b) report mean values ranging from about 8 to 15 mg/L and a maximum of 53.7 mg/L NO_3^-. Although a specific legal limit has not been defined for nitrates in wines, content higher than 20 mg/L is sometimes considered to suggest the fraudulent addition of well water or nitric acid and can lead control authorities to carry out closer analysis (Garcia Moruno *et al.*, 1994).

Phosphites and phosphonates

In contrast with the other inorganic anions, knowledge of phosphites and phosphonates in grapes and wine is less extensive and widespread among winemakers. The inorganic and organic salts of phosphorous acid, the latter being available in the form of alkyl compounds or esters of phosphorous acid, are widely used for the control of fungal diseases in plants. Phosphites and phosphonates have both a direct and an indirect effect on fungi and *oomycetes*. They can inhibit the oxidative phosphorylation process in the metabolism of *oomycetes* and have an indirect effect, stimulating the plant's natural defense response against pathogen attack. As phosphites, they are readily absorbed by the leaves and are highly mobile within the plant. None of the plant enzymes described by Ouimette and Coffey (1989; 1990), and Smillie *et al.* (1989) are able to oxidize phosphites into phosphate. This means that phosphorous acid cannot be a substitute for phosphorus fertilization (McDonald *et al.*, 2001). Fosetyl-Al, which releases phosphonate as a product of breakdown, is a systemic fungicide often used against root pathogens for its mobility in the plant (Cohen and Coffey, 1986). Different legal limits for phosphites have been set or are under discussion, waiting for a standardized European decision. For grape for example, to date, a level of 50 mg/kg of phosphonic acid has been set in Switzerland (RS 817.021.23; 10/10/2006) and in Italy (D.M. 27/08/2004, G.U. 292 14/02/2004). A limit for wine has not yet been defined, but may be derived from the limit for grape, taking into account the yield in wine.

8.2. Traceability of *Vitis Vinifera* Related Products on the Basis of Geographical Origin

As has happened for several other products, such as milk, cheese, meat, vegetables and fruit juices discussed by Crews (1998) and Kelly *et al.* (2005), in addition to the various elements, isotope ratios were also measured in wines and spirits, partially changing the more traditional reasons for elemental analysis and focusing on the possibility of differentiating or ascertaining the geographical origin of the products. Interesting literature on this topic as regards wines is given in a recent paper by Castiñeira Gómez *et al.* (2004b). This can be integrated with the pioneering works of Siegmund and Baechmann (1978) and Scarponi *et al.* (1982), as well as in the 1990s with the papers of Herrero-Latorre and Medina (1990), Day *et al.* (1994) on grape musts, Greenough *et al.* (1997), and Martin *et al.* (1999) on Bordeaux wines, and with the recent papers of Suhaj and Korenovska (2006) on Slovak wines and Almeida and Vasconcelos (2001, 2003, 2004) on the discriminative capability of Sr isotopes, previously investigated by Horn *et al.* (1993) in the rock-soil-vine-wine system. Coetzee *et al.* (2005) investigated the isotopic ratios of elements in South African wines from Stellenbosch, Robertson and Swartland in the Western Cape region. European Community (EC) projects have also paid attention to this topic, for example with the SMT4-CT95-7500 and G6RD-CT-2001-00646-WINE DB contracts, the latter to establish a specific database for analytical parameters for wines from non-European Union countries. In the light of the interfering factors presented above, it is quite surprising that geographical discrimination among wines can be attained. In any case, all these studies and research projects have also had important repercussions of a commercial nature, having multiple objectives, including the safeguarding of the product, the producer and the consumer and the repression of frauds, or at least of acting as a significant form of prevention and a deterrent. With regard to this, deterrence is a particularly important aspect, since chemical analysis combined with a statistical approach, such as discriminant analysis, can significantly contribute to 'moralizing' the market through consortia self-control measures, even when the certainty of results ascertaining geographical origin is less than 100%.

8.3. Elemental Analysis

The methods officially used in the wine trade transactions are summarized in Table 8.1. Generally, the OIV methods are officially adopted in the European Union without significant technical changes. The methods reported are mainly colorimetric, titrimetric, or use Atomic Emission Spectroscopy (AES, e.g. Flame Spectrophotometry), Atomic Absorption Spectroscopy (AAS), Hydride Generation-AAS (HG-AAS), Electrothermal-AAS (ET-AAS) and Vapour Atomic Flourescence Spectrophotometry (VAF).

The main pros and cons of the techniques more extensively used for elemental analysis in the oenological field are shown in Table 8.2.

Table 8.1 Comparison of the most relevant characteristics of the analytical methods provided by OIV and AOAC.

OIV methods	AOAC methods
Arsenic (MA-F-AS323-01-ARSENI, MA-F-AS323-01-ASSAA)	
Reference method: after mineralization and reduction using the $NaBH_4$ of wine, it is measured as H_3As at 520 nm by hydride generation – AAS.	
Usual method: after mineralization of the sample and reduction to H_3As, it is colorimetrically quantified at 520 nm.	
Boron (MA-F-AS323-03-BORE) is colorimetrically measured at 420 nm after dealcoholization, colour removal, and reaction with azometine H reagent.	
Cadmium and lead (MA-F-AS322-10-CADMIU, MA-F-AS322-11-PLOMB): they are directly dosed by ETAAS at 228.8 and 283.3 nm respectively.	
Calcium (MA-F-AS322-04-CALCIU) is analyzed by AAS at 422.7 nm after opportune dilution of the sample and addition of $LaCl_3$.	
Copper (MA-F-AS322-06-CUIVRE) is quantified in the diluted wine by AAS at 324.8 nm.	**Copper** (970.18). *Method I*: the diluted samples are analyzed by AAS at 324.7 nm using alcoholized standards. *Method II*: the copper content is colorimetrically measured after reaction with Zn dibenzyldithiocarbamate using a spectrophotometer at 438 nm.
Iron (MA-F-AS322-05-FER).	**Iron** (970.19).
Reference method: it is quantified by AAS in the diluted and dealcoholized wine at 248.3 nm.	*Method I*: a diluted solution of wine is analyzed by AAS at 248.3 nm and the concentration is quantified in comparison with alcoholized standards.
Usual method: it is colorimetrically measured on the H_2O_2 oxidized sample after reaction with an ortophenantroline reagent.	*Method II*: the content of iron is measured after reaction with 2,4,6-tripyridyl-s-triazine with a spectrophotometer at 593 nm.

Table 8.1 (Continued).

OIV methods	AOAC methods
Magnesium (MA-F-AS322-07-MAGNES) is quantified in the diluted wine by AAS at 285 nm.	
Mercury (MA-F-AS323-06-MERCUR) is measured by vapour atomic fluorescence detection at 254 nm.	
Phosphorus (MA-F-AS321-04-PHOTOT) after oxidation and ashing of the sample and reaction with a molybdovanadate reagent, it is colorimetrically measured.	**Phosphorus** (962.11) is colorimetrically quantified at 400 °C after reaction of the mineralized sample with a molybdovanadate reagent.
Potassium and sodium (MA-F-AS322-02-POTASS, MA-F-AS322-03-SODIUM).	**Potassium and sodium** (963.13) sample is analyzed by flame spectrophotometry after dilution and addition of $LiNO_3$.
Reference method: The opportunely diluted and CsCl added sample is analyzed by AAS at 769.9 and 589 nm respectively.	
Usual method: the diluted sample is analyzed using a flame spectrophotometer.	
Silver (MA-F-AS322-09-ARGENT) is measured by AAS at 328.1 following ashing and oxidation.	
Total brome (MA-F-AS321-01-BROTOT). After ashing at 525°C, it is colorimetrically quantified as tetrabromosulfonphtalein at 590 nm.	
Total nitrogen (A MA-F-AS323-02-AZOTOT) is quantified after mineralization, alkalinization and ammonia distillation by acid-base titration (Kjeldhal method).	**Total nitrogen** (920.70) is quantified using the Kjeldhal method.
(MA-F-AS323-02-AZOTDU) is dosed by universal detector following total combustion of the organic substances, reduction of the gases produced and removal of CO_2 and H_2O (Dumas method).	
Zinc (MA-F-AS322-08-ZINC) is quantified by AAS in the diluted and dealcoholized wine at 213.9 nm.	

Table 8.2 Main advantages and disadvantages of the more extensively used techniques for elemental analysis.

	Flame Atomic Absorption Spectrometry (FAAS)	**Electrothermal Atomic Absorption Spectrometry (ETAAS) e.g. Graphite Furnace Atomic Absorption Spectrometry (GFAAS)**	**Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)**	**Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**	**Anodic Stripping Voltammetry (ASV)**
Advantages	Inexpensive; Rapid for few selected elements; Limited use with organic solvents	Relatively inexpensive; Small sample volume; Excellent sensitivity	General-purpose technique; Good dynamic range; Accommodates organic solvents; Multi-elemental technique	Small amount of sample; Excellent dynamic range; Multi-elemental technique; Isotope determination; Semi-quant scanning; Excellent sensitivity; Limited interferences	Low cost of consumables and equipment; High sensitivity
Disadvantages	Poor sensitivity; Single element determination at-the-time; Large amount of sample; Narrow linear range	Single element determination at-the-time; High operating costs as consumables; Very narrow linear range; Time-consuming technique; Not suited for organic solvents	Medium cost of the instrument; Limited sensitivity; Sample volume requirements; Spectral interferences	High cost of the instrument	Few quantifiable elements (Cu, Pb, Cd, Zn)

Today, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) is the best compromise between costs and technical performance for winery quality control laboratories, while Inductively Coupled Plasma Mass Spectrometry (ICP-MS), because of its extreme reliability and productivity, is more widely used in research laboratories.

8.4. Architecture and Components of an Inductively Coupled Plasma Mass Spectrometer

ICP-MS appeared with the first prototypes in 1974, but diffusely it arrived on the international markets at the beginning of eighties. Nowadays, after several thousand instruments have been installed, it can be considered to be a mature and highly powerful technique. ICP-MS has the capability to analyze a wide range of elements in a variety of sample matrices. It is widely employed in Geological and Environmental Sciences, Semi-Conductor Industries, Material Science, Medicine, Agriculture, and Food and Beverages Sciences.

Figure 8.1 shows the vertical section of an Inductively Coupled Plasma Mass Spectrometer.

8.4.1. Sample Introduction System

The physical status of the samples determines the choice of the analytical approach. In particular, liquid samples can be processed by nebulization

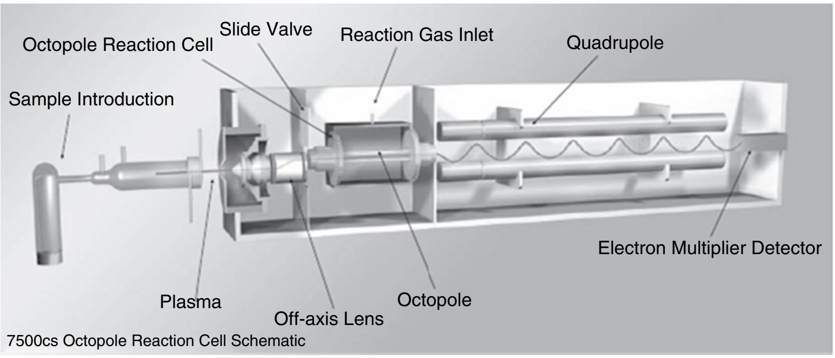

Figure 8.1 Vertical section of an Inductively Coupled Plasma Mass Spectrometer

or gaseous extraction. Solid samples can be analyzed after dissolution and/or mineralization, or by vaporization.

Liquid samples nebulization

The liquid sample introduction system most commonly used on an ICP-MS is very similar to that used on a flame Atomic Absorption Spectrometer or an ICP-OES. Liquid samples can be directly injected using a pneumatic nebulizer and a spray chamber.

Nebulizers

Conventional nebulizers

The ICP-OES nebulizers can aspirate up to 1–2% dissolved solids (a dry wine has 2–4% total extract). Some nebulizers, such as the Babington and cone-spray nebulizers, were studied to handle as much as 15–20% dissolved solids, but are not ideally conceived for use in ICP-MS. Pneumatic nebulizers are commonly used to generate an aerosol of samples with dissolved components below 0.2%. Pneumatic devices are made from glass or different kinds of polymers and use argon as nebulizer gas. The characteristics of some of the most popular pneumatic nebulizers are detailed below.

Concentric design, is indicated for clean solutions and can provide excellent sensitivity and stability, but the small orifices can be affected by occlusion problems, especially in the case of aspiration of large numbers of heavy matrix samples. In this device, the solution is aspirated through a capillary tube by the low-pressure condition produced by a high-speed gas flowing tangentially to the capillary axis. On the nebulizer tip the combination of low pressure and high gas flow breaks up the solution producing an aerosol.

Crossflow design. This nebulizer suffers from relatively reduced analytical sensitivity and precision if compared with the concentric one. It is devised for routine use and is probably the best choice for samples that contain a high concentration of dissolved solids or heterogeneous samples with small contents of undissolved matter. The aerosol is produced on the nebulizer tip where the drained sample collides with a perpendicular jet of argon gas.

Microflow design is based on the concentric principle. Typically it operates with higher gas pressure at less than 0.1 mL/min sample flow rates, while conventional nebulizers usually run at about 1 mL/min. This low flow rate is suitable in the case of applications with limited sample volume or with samples affected by memory effects, as is sometimes possible when analyzing wines with high extract.

Desolvating nebulizer
In this device the liquid sample is sprayed into a heated spray chamber, where the nebulizer gas transfers the aerosol through the membrane desolvator. An argon flow removes the solvent vapour from the exterior of the membrane. If compared to conventional pneumatic nebulizers, this system enhances analyte transport efficiency and limits solvent loading to the plasma. Oxide and hydride polyatomic ion interferences are significantly reduced, improving the detection limits by an order of magnitude.

Spray chambers

Because the plasma discharge is inefficient in dissociating droplets greater than ~10μm in diameter, the primary function of the spray chamber is to allow only the smallest droplets of about 5–10 μm to enter the plasma for dissociation, atomization, and finally ionization of the sample components. Its secondary aim is to reduce the effect of the peristaltic pump pulses on the nebulization process. Some ICP-MS spray chambers are also externally cooled (typically to 2–5 °C) to minimize the amount of solvent going into the plasma. Reduction of oxide species is the main benefit of this technical solution when volatile organic solvents are aspirated (e.g. ethanol in alcoholic beverages).

The *double pass spray chamber* (Scott-type) is the most common design, where the aerosol emerging from the nebulizer is directed into the central tube of the chamber. The larger droplets running the whole length of the tube emerge from it, and fall out by gravity through the drain exit. The fine droplets go back up passing between the chamber outer wall and the central tube and emerge from the spray chamber.

In the *cyclonic spray chamber* droplets are discriminated according to their size by means of a vortex produced by the tangential flow of the nebulizer. Smaller droplets are transferred with the sample aerosol into the ICP-MS, while the larger droplets collide with the walls and exit by gravity via a drain tube. If compared to the Scott design, the cyclonic spray chamber typically shows higher sensitivity.

Liquid samples gasouse extraction (Hydride/cold vapour generation system)

Hydride at ambient temperature (i.e. As, Bi, Ge, Pb, Sb, Se, Sn, Te) or cold vapour generation is a well established technique to enhance sensitivity by separating the analytes into gaseous form. This allows the increase of sample flow rates without loading plasma with water. Sensitivity can be enhanced by more than a factor of 100 and, additionally, some analytical interferences can be resolved. Hydride Generator systems operate with a gas-liquid separator which separates the gaseous phase from the liquid matrix. The modern designs use a porous membrane system in order to enhance gas/liquid separation and reduce noise/signal ratio. Often, the membrane systems can be plagued by clogging and ageing.

Solid sample introduction system

The direct analysis of homogenized solid material can be used if the sample of interest is hard to dissolve, or if the sample pre-treatment increases the risk of contamination or losses of the analyte. Solid samples (e.g. grape stones, skins) can be processed using lasers or heated cells to vaporize the sample. The vaporized sample can be directly introduced into the ICP-MS and measured. This technique is generally characterized by lower sample consumption and higher sensitivity. Solid sampling can be carried out for ICP-MS using electrothermal vaporization (ETV) or laser ablation (LA) sample introduction devices.

Electrothermal vaporization. With ETV/ICP-MS, a small amount of solid sample (~1 mg) is introduced into a graphite furnace and subjected to a multi-step temperature program for atomization. Additionally, a modifier can be used for thermal stabilization of the analytes or to improve the matrix mineralization. The vapours produced during the vaporization step are transferred into the plasma by argon carrier gas.

Laser ablation. LA/ICP-MS is highly versatile and can analyze any kind of solid material. Compared with conventional ICP-MS, the main advantages of this technique are the fast preparation of solid samples without time-consuming dissolution processes, the certainty of a quantitative analysis, and the reduction of potential contaminations during sample preparation (Longerich *et al.*, 1993; Jarvis and Williams, 1993).

Since only dry gasses are introduced into the plasma a decreased polyatomic interference can be observed.

8.4.2. ICP Torch

The plasma is generated by passing argon through a series of concentric quartz tubes (the ICP torch) that are wrapped at one end by a radio frequency (RF) coil. Energy is supplied to the coil by the RF generator couples with the argon to produce the plasma. RF voltage induces rapid oscillation and collision of argon ions enhancing the temperature of gas to ~10,000 K.

The liquid droplets containing the elements to be determined are dried and heated to gas. As the atoms pass through the plasma, they absorb energy from the plasma and release one electron to form a singly charged ion. The ions then pass through the interface toward the ion lens. The advantages of argon gas are its relatively limited cost and easy availability at very high purity. Moreover, it has a first ionization potential of 15.75 electron volts (eV), higher than the first ionization potential of most other elements (helium, fluorine and neon excluded), and lower than the second ionization potential of most other elements (calcium, strontium and barium excluded).

8.4.3. Mass Spectrometer

Interface

The interface consists of two devices called cones: the sampler and the skimmer cone. They have an opening at the apex of approximately 1 mm in diameter in the sampler, and 0.4 mm in the skimmer cone. The first cone is located next to the plasma and in direct contact with it. The skimmer cone is located several millimetres behind the sampler cone and is in contact with the detector zone. The cones are funnel-like devices and are typically made of nickel or platinum. They are screwed into a water-cooled housing to prevent damage from the plasma heat. The beam of ions and neutrals produced by the plasma at atmospheric pressure is extracted through the sampler cone orifice and further through the skimmer cone orifice into the mass spectrometer region. The orifices must also be small enough to preserve a consistent vacuum behind the interface and large enough to prevent occlusion when samples with high dissolved solids are analyzed. The region between the two cones is evacuated to a pressure of a few Torr in order to remove all the molecules using a combination of mechanical roughing and turbo-molecular pumps.

Ion lens

The ion lens, positioned immediately behind the interface, is responsible for focusing and getting as many of the ions as possible into the mass filter. Since the ions generated in the plasma are nearly all positively charged and have a tendency to repel each other, the lens charged with the same sign of the ions acts as a focusing device, keeping the ion beam from diverging. A particular device placed next to the lens and named ‘Photon Stop’ or ‘Shadow Stop’ prevents the photons and non-charged species emitted from the plasma from reaching the detector. The common approach is to place a metal disc in the beam path deflecting ions off-axis.

Collision/Reaction Cell (CRC)

Collision and reaction cell techniques have been used for many years in the study of organic and biological mass spectrometry, but only in the last few years in ICP-MS. The development of collision and reaction cells extended the capability of the technique by allowing the selective attenuation or removal of problematic spectral interferences. Today a variety of collision/reaction cells using various gasses (H_2, He, CH_4, NH_3 . . .) are available, virtually able to eliminate the problems associated with polyatomic interferences for most elements in food and beverage matrices. However, the simultaneous multi-element capability and maximum productivity of ICP-MS is partially reduced by the different CRC tuning conditions required to eliminate a specific interference in a specific matrix.

Quadrupole

This is the device generally used as mass filter in routine analytical instrumentation. It is a tunable filter constituted of four rods approximately 20 cm in length and 1 cm in diameter. It works by allowing only a single mass to charge ratio (*m/z*) to pass through the detector at a given time. The quadrupole is set up to a combination of voltages and radio frequencies to guide the ions with the selected *m/z* through the quadrupole into the detector. Ions characterized by a different m/z are ejected through the spaces among the rods and pumped out of the quadrupole. By changing the voltage and frequency settings, the quadrupole can sweep across the whole mass range of the Periodic Table.

Detector

The detectors used in ICP-MS are not significantly different from the photomultiplier tube used in AA and ICP-OES. The ions passing through the mass filter strike the active surface of the detector, known as dinode, and generate a measurable electronic signal.

8.4.4. System Controller

It is a dedicated computerized data system that manages all aspects of instrumental control and converts the measured signal intensities into concentrations and generates a final report.

In a quantitative analysis, the concentration of an element is determined by comparing the counts measured for a selected isotope of that element to an external calibration curve. ICP-MS instruments can measure the signal of a single isotope as well as isotope ratios, the latter used in a variety of applications including geological dating of rocks, determining the source of a contaminant, and food traceability studies. Isotope dilution methods can also be applied, enriching the sample with one of the isotopes of the element of interest, that behaves both as a calibration standard and an internal standard. By coupling a chromatographic technique (i.e. HPLC, GC, capillary electrophoresis) to the ICP-MS it is possible to discriminate the chemical form of an element and perform elemental speciation (i.e. Cr(III)/Cr(VI)).

8.5. Analysis of Inorganic Anions

The reference analytical methods for anions in wines and by-products are summarized in Table 8.3. They use titrimetric, gravimetric approaches or selective ion electrodes, but today the most performed and extensively used approach for quality control analysis in oenology is ion chromatography.

Table 8.3 Reference analytical methods for anions in wine and relevant by-products.

OIV methods	AOAC methods
Chlorides (MA-F-SA321-02-CHLORU). Chlorides are quantified by titration with standard $AgNO_3$, using the Ag-AgCl electrode as indicating electrode.	**Chlorides** (966.10) *Method I*: a portion of alkalized wine is evaporated to dryness and ignited at $\leq 500\,°C$. Ashes are extracted with hot water. Chlorides are quantified in two ways. With the first $AgNO_3$ is added to the acidified solution. The precipitate is dried, ignited at 700 °–800 °C and weighed. The second is a volumetric method with the use of $FeNH_4(SO_4)_2$ as an indicator and $AgNO_3$ and KSCN as standard solutions. *Method II*: they are quantified using titration with a $AgNO_3$ standard solution, using an Ag-AgCl indicating electrode.
Fluoride (MA-F-AS321-03-FLUORU) The concentration of fluoride is measured using a fluoride selective ion electrode. The addition of a buffer provides a high constant ionic strength, complexes iron and aluminium and adjusts the pH to reduce $HF{\cdot}HF_2$ formation. Matrix effects are minimized by using standard addition.	**Fluoride** (2003.03) – the concentration of fluoride is measured with the use of a fluoride selective ion electrode.
Sulphates (MA-F-AS321-05-SULFAT). A $BaCl_2$ solution is added to a portion of acidified and boiling wine. The precipitate is washed, dried, ignited at 700–800 °C and weighed.	**Sulphates** (955.26). $BaCl_2$ solution is added to a portion of acidified and boiling wine. The precipitate is washed, dried, ignited at 700–800 °C and weighed.

8.5.1. Ion Chromatography

Ion chromatography (IC) was developed in the 1970s by Small *et al.* (1975). It is a powerful technique for the measurement of ionic species, such as fluoride, chloride, nitrite, nitrate, and sulphate and cations like lithium, sodium, ammonium, and potassium. It is very useful for analysis of anions for which there are no other rapid analytical methods, and it is also commonly used for organic species such as amino acids, amines, and proteins. The most commonly used detection method is conductivity, which can be applied to the detection of inorganic cations and anions in solution. Overcoming the problems relating to the high concentration of the ionic eluent, the conductimetric suppression technique led to a remarkable improvement in the sensitivity of the method. Ion chromatography is a form of liquid chromatography that uses ion exchange resins as a stationary phase to separate atomic or molecular ions. The stationary phase surface displays ionic functional groups that interact with ions of opposite charge. Silica-based stationary phases are unstable in the presence of high salt concentrations and at extreme pH, while the polystyrene divinyl benzene cross-linked polymers are very stable.

Chromatographic separation requires the mobile phase, buffered appropriately by adjusting the pH or ionic concentration, to cause the dissociation of analytes to ions, so that they are free to interact with the stationary phase.

Aqueous solutions like wine, juice and spirits can be directly analyzed after removing interferences, filtration, and dilution. Solid samples are generally homogenized and extracted with water or buffered solutions. Organic liquids can also be extracted, obtaining aqueous solutions of ions for analysis.

8.6. Applications for Grape, Wine and Derivatives

Examples of analytical and technological experiences obtained in everyday practice in an oenology-specialized laboratory are presented. Some compositional aspects of grapes and wines and related analytical problems are updated and discussed, as well as the effects of some winemaking procedures.

8.6.1. Mineral Elements

Composition of grapes

Basic composition and exogenous contributions

The elemental composition of 36 grape samples harvested at technological ripeness from 10 varieties is reported in Figure 8.2. Concentration ranges agree with the other data reported in the literature (Cabanis, 1998; Sponholz, 1989).

The content of some elements is affected by the more or less significant exogenous contributions due to vineyard treatments or powder, soil and

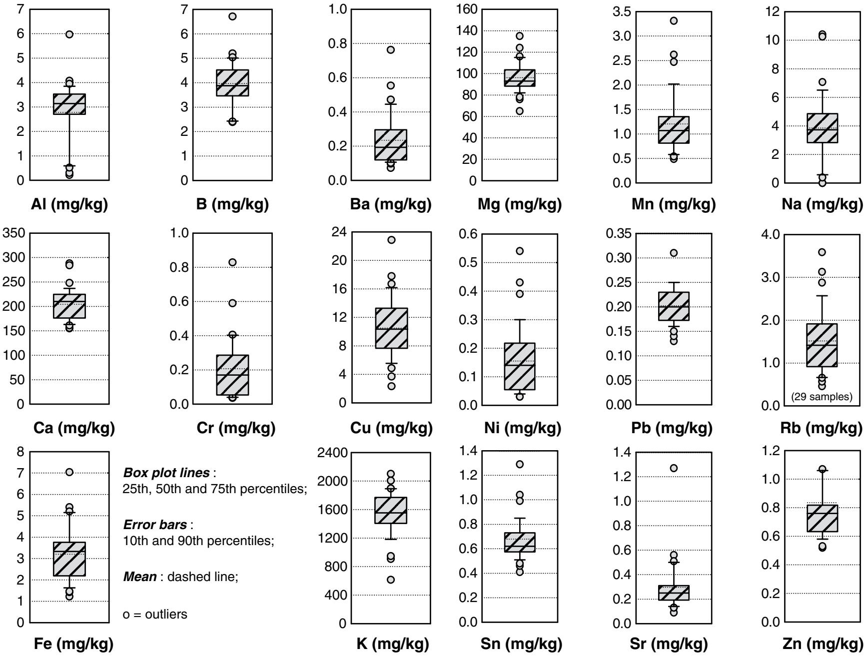

Figure 8.2 Box plots of the amounts of elements in 36 grape samples from 10 different varieties cultivated in northern Italy determined by ICP-MS

polluting deposits via air. This is evident from the data in Table 8.4 where unwashed control grapes were compared with the same grapes washed with a 2% ultra-pure nitric acid solution in high purity water before ICP-MS analysis. As confirmed by both parametric and non-parametric statistical tests, washing clusters significantly decreases the contents of six elements, Cu and Al to a more substantial extent. K, Mg, Pb and Sn also decreased, thus proving the exogenous contribution to their total content in grape.

Table 8.4 Effect of washing grape with 2% nitric acid high purity water solution. Median values are given (n.s. = not significant; $* = p < 0.05$; $*** = p < 0.001$).

element ($mg{\cdot}kg^{-1}$)	washed (n=24)	not washed (n=24)	sign.	element ($mg{\cdot}kg^{-1}$)	washed (n=24)	not washed (n=24)	sign.
Al	0.35	1.13	***	Ni	0.031	0.032	n.s.
Cu	1.44	11.19	***	Pb	0.028	0.054	*
Fe	2.14	2.37	n.s.	Rb	1.39	1.72	n.s.
K	2018	2356	*	Sn	0.004	0.083	*
Mg	74.7	90.4	*	Sr	0.199	0.246	n.s.
Mn	0.82	0.93	n.s.	Zn	0.526	0.558	n.s.
Na	1.36	1.74	n.s.				

Table 8.5 Cu content in wine grapes (mg/kg) and bottled wines (mg/L) from a single region in northern Italy.

	GRAPES						WINES (2002–2005)	
	1998	**1999**	**2000**	**2004**	**2005**	**2006**	**Red**	**White**
No. Samples	264	231	241	148	158	153	112	145
Min	1.6	1.5	1.9	1.6	1.2	1.1	0.01	0.01
25th percentile	5.8	4.1	5.7	5.9	4.7	3.4	0.07	0.08
Median	8.7	6.7	8.7	8.2	6.5	5.1	0.12	0.14
75th percentile	13.6	10.5	12.6	11.9	10.7	7.5	0.22	0.28
Max	41.8	29.9	32.6	28.7	25.1	19.7	1.34	1.65

Similar results were observed for washing treatments applied on an industrial scale to minimize pest residues, or carried out, for example by organic grape-growers, to reduce natural microbial contamination before crushing and deplete Cu, thus favouring the development of specific aroma compounds related to hyper-reductive winemaking techniques, e.g. 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate and 4-mercapto-4-methylpentan-2-one. The trend toward Cu reducing treatments in the vineyard, as required to minimize soil pollution and sterilization, is clearly shown in particular by the maximum 75th percentile and median values measured in grapes (Table 8.5) where data for extensive sampling carried out in the same region by a public control body are presented. As regards the wines, the measured values are lower than in grapes, as expected, indicating good winemaking technology and no difference was observed in the distribution parameters of reds and whites. Only two samples exceeded the OIV legal limit for wine (1 mg/L).

The problem of residual sulphur

Quantification of the residual sulphur in grapes as a possible precursor of compounds formed during and after fermentation and liable to cause off-flavours is of remarkable interest, permitting better planning of anti-oidium treatments and the establishment of an adequate winemaking strategy. As for any industrial transformation, quality and process control in winemaking requires analytical approaches providing rapid measurement. Several methods have been proposed in the last few decades, applying different analytical techniques such as colorimetry, ICP-OES, AAS and HPLC (Cassidy, 1976; Wenzel *et al.*, 1980; Wenzel, 1980; Thomas *et al.*, 1993; Gump *et al.*, 1996). A new HPLC-DAD method was recently proposed (Larcher *et al.*, 2007), its performance and rapidity adequately meeting the needs of winery laboratories. Two grams of grape previously destemmed, are crushed with a mixer and homogenated with Ultraturrax (10000 rpm + 3 g diatomite) and extracted twice with 50 mL of *n*-hexane. The hexane fractions are combined and concentrated to 2 mL under vacuum at 40 °C, dried under nitrogen flow and recovered with 2 mL of acetonitrile. The analytical conditions are reported in Table 8.6 and a HPLC chromatogram is shown in Figure 8.3.

Table 8.6 Instrumental setting for HPLC-DAD analysis of residual sulphur on grapes.

parameter	description
Elution condition:	isocratic
Column:	C_{18}, 250 × 3 mm (5 μm)
Mobile phase:	100% acetonitrile
Flow rate:	0.7 mL·min^{-1}
Column temperature:	30 °C
Injection volume:	50 μL
Run time:	10 min

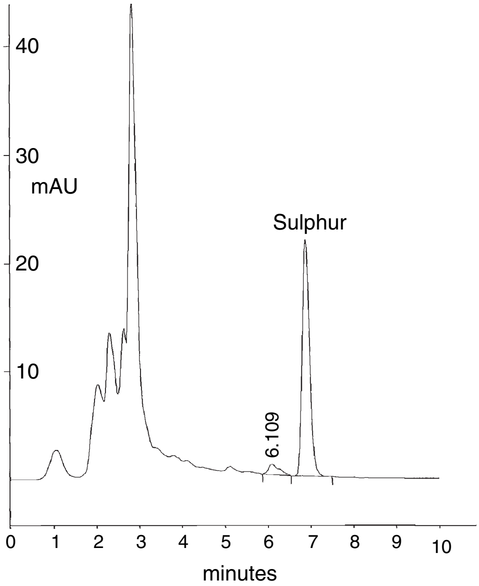

Figure 8.3 HPLC-DAD separation of sulphur in grape extract. The analytical conditions are reported in Table 8.6

The method is linear up to 10 mg/kg and the quantitation limit, assessed according Hubaux and Vos (1970), is 0.15 mg/kg.

Data from a survey of ripe grapes collected on delivery to the wineries for the 2005 vintage in Trentino are shown in Figure 8.4. Despite the seasonal climatic conditions having favoured dangerous *oidium* attacks and led to massive sulphide treatments, all the samples were far below the legal limit (50 mg/kg), showing the maximum value of 27 mg/kg. Only 16 samples (*ca.* 10%) resulted over 2 mg/kg, a level sometimes considered to be an indication of possible technological interference if not further depleted with juice settling.

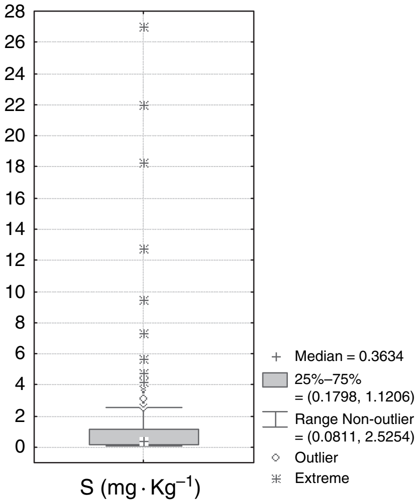

Figure 8.4 Distribution of the contents of residual sulphur in 159 grape samples of the 2005 vintage in Trentino (northern Italy)

Elemental composition of wine and technological interferences

A recent survey of commercially available wines (Larcher and Nicolini, 2001) substantially confirmed the typical ranges reported for each element by Eschnauer (1986), mostly showing values close to (Ba, Ca, Pb, Sn, Sr, Zn) or below (Ag, Cr, Li, Mn, Ni, V) the lower boundaries of the ranges reported by the German author. As compared to white wines, reds have significantly higher Ba, Mg and Ni (*ca.* +20–30%), B, Fe, K, Li, Pb and Sr (*ca.* +40–70%), and Rb (*ca.* +100%). Only Ca was lower (−13% *ca.*) in the reds, mainly as a result of the easier precipitation of salts at the higher pHs typical of such wines. Still whites have significantly lower content of Ba, B, Ca, Cr, K e Sr (*ca.* −10−30%) and Cu (*ca.* −60%) as compared to classic sparkling white wines (Nicolini *et al.*, 2003a), as a result of lower pomace pressing, accurate juice fractioning, higher stabilization and more intense precipitation and/or biosorption phenomena linked to two fermentations.

Over the last 50 years wine-making has seen a positive and significant decrease in the content of Ca, Mg, Fe, Pb and Zn in wines (Larcher and Nicolini, 2001), while there has been a trend towards an increase in K and related instability and pH problems, probably caused by changes in the levels of fertilization and irrigation, and in the management of green covering and canopy (Jackson, 1993; Fregoni, 1998). From here on we will focus on some winemaking practices in order to understand whether and how much they affect the elemental composition of wine and interfere with its 'geographical' characterization.

The effect of bentonites

Bentonite is one of the most important clarifying and fining agents in winemaking. Its use has been investigated frequently as regards the effects on the main elements, but few data are available for those in micro- and trace-quantities (Postel *et al.*, 1986; Enkelmann, 1988; McKinnon *et al.*, 1992; Leske *et al.*, 1995; Bauer *et al.*, 2001; Molina *et al.*, 2001). Bentonite fining (1 g/L) – carried out with nine different marketed products added to wine modified at 3 pH levels – resulted in statistically significant increases in most of the 44 elements measured and significantly lower contents of Cu, K, Rb and Zn. The increases were noteworthy (roughly 10-fold higher) for Ce, Gd, La, Nd, Pr and Y, and also very high for Be, Tl and U (4–6 fold higher); Sm, Eu, Dy, Ho, Er, Tm, Lu, Th, Yb and Hf contents reach measurable amounts only after bentonite treatment. In any case, significant differences among different bentonites were found for Al, Ba, Be, Ca, Co, Cr, Cs, Cu, Fe, Ga, Li, Mg, Mn, Pb, Rb, Sb, Sr, Tl, U, V and Zn, but no significant effect of pH between 3.0 and 4.0 was observed (Nicolini *et al.*, 2004a). An overall increase in rare earth elements (REE) caused by bentonite clarification is also reported by Rossano *et al.* (2007); on the other hand, these authors showed that filtration with cellulose powder only causes a small increase in the concentration of light REEs while filtration using silicate (SiO_2) releases in particular La, Ce, Pr, Nd, and Gd. As known and confirmed by the data in Table 8.7, redrawn by Nicolini *et al.* (2004a), the main oenological changes

Table 8.7 Changes observed in wine after addition of nine different bentonites (1g/L).

element	units	ICP	min	mean	max	element	units	ICP	min	mean	max
Be	μg·L^{-1}	MS	1.3	5.9	11.8	Ca	mg·L^{-1}	OES	2.6	18.3	26.3
Ce	μg·L^{-1}	MS	2.1	5.8	9.2	Co	μg · L^{-1}	OES	0.8	1.8	3.1
Cs	μg·L^{-1}	MS	−0.4	0.3	1.4	Cr	μg · L^{-1}	OES	−0.3	0.3	2.0
Ga	μg·L^{-1}	MS	0.3	5.9	17.3	Cu	mg·L^{-1}	OES	−0.17	−0.11	−0.08
Gd	μg·L^{-1}	MS	0.3	1.0	1.8	Fe	mg·L^{-1}	OES	0.17	0.45	0.67
La	μg·L^{-1}	MS	0.9	3.4	6.0	K	mg·L^{-1}	OES	−20.4	−8.6	2.6
Nd	μg·L^{-1}	MS	0.8	3.3	5.5	Li	μg · L^{-1}	OES	0.4	2.4	9.4
Pb	μg·L^{-1}	MS	−9.4	3.8	23.0	Mg	mg·L^{-1}	OES	−0.95	1.86	4.62
Pr	μg·L^{-1}	MS	0.2	0.8	1.3	Mn	mg·L^{-1}	OES	0.02	0.29	0.51
Sb	μg·L^{-1}	MS	−0.1	0.2	0.9	Na	mg·L^{-1}	OES	−12.0	12.7	29.4
TI	μg·L^{-1}	MS	0.1	0.3	0.4	Ni	μg · L^{-1}	OES	0.0	1.6	6.0
U	μg·L^{-1}	MS	0.3	1.7	4.2	Rb	mg·L^{-1}	OES	−0.24	−0.09	−0.03
Y	μg · L^{-1}	MS	1.3	3.7	6.4	Sn	μg · L^{-1}	OES	−5.1	1.6	5.9
Al	mg·L^{-1}	OES	0.58	1.37	2.43	Sr	mg·L^{-1}	OES	0.05	0.10	0.22
B	mg·L^{-1}	OES	−0.06	−0.01	0.04	Va	μg · L^{-1}	OES	−0.1	2.8	6.9
Ba	mg·L^{-1}	OES	0.00	0.07	0.20	Zn	mg·L^{-1}	OES	−0.03	−0.01	0.01

due to bentonite are in the content of Ca and Na. Speaking in terms of rate of oxygen consumption and potential catalytic activity in real wine (Vivas, 2002), the overall balance is not easily assessable, as Cu decrease and Al increase could counterbalance the increases of Mn and Fe, while the trend of Zn is not well defined.

The effect of yeast hulls

Biosorption is the sole mechanism for quick fixing of cations by non-living yeast cell walls (Blakwell *et al.*, 1995), a mechanism in which protein-polysaccharide complexes are involved. To understand the possible interference of such a mechanism with the mineral composition of wine, two amounts of yeast hulls (180 mg/L and 360 mg/L) were added to stabilized and sterile white and red wines (Nicolini *et al.*, 2004a). The yeast hulls used were provided by two different producers and triplicate experiments were carried out. Both untreated control wines and wines with yeast hulls were gently shaken for three hours, then centrifuged, and supernatant analyzed by ICP-MS. Al, B, Ba, Ca, Ce, Co, Cr, Cs, Cu, Fe, Ga, K, Y, La, Li, Mg, Mn, Na, Ni, Pb, Rb, Sb, Sn, Sr, U, V and Zn were over the relative DLs in all the samples, and the contents of Ce, Cu, Fe, La, Sb, U, V and Y were significantly depleted by the treatment, with a tendency for higher depletion with higher doses (Table 8.8).

Table 8.8 Average depletions (statistically significant, $p < 0.05$) in wine as a consequence of yeast hulls addition.

	Ce ($\mu g \cdot L^{-1}$)	**Cu** ($\mu g \cdot L^{-1}$)	**Fe** ($mg \cdot L^{-1}$)	**La** ($\mu g \cdot L^{-1}$)	**Sb** ($\mu g \cdot L^{-1}$)	**U** ($\mu g \cdot L^{-1}$)	**V** ($\mu g \cdot L^{-1}$)	**Y** ($\mu g \cdot L^{-1}$)
yeast hulls (180 mg·L^{-1}; n = 12)	0.08	15.1	0.30	0.06	0.13	0.10	1.81	0.03
yeast hulls (360 mg·L^{-1}; n = 12)	0.17	18.1	0.45	0.10	0.13	0.15	2.66	0.05

In percentage terms, the highest depletion was for U (24–36%), followed by Fe (17–25%), Ce (7–15%) and the other elements (<10%).

Tannin and gum arabic as winemaking additives

Oenological tannins are well known clarifying products originally authorized and used to precipitate proteins, thus minimizing the risk of protein haze, but in the last few years they have been added in wine in particular to take advantage of their usefulness as texturizers and colour stabilizers.

Gum arabic is an exudate made up of a mixture of polysaccharides and glycoproteins produced by the trees of different Acacia species growing in sub-Saharan Africa. It is an hydrocolloid traditionally widely used in oenology and the beverage industry as stabilizer, but more recently it has mainly been applied as texturizer. The elemental compositions of 21 tannins (Nicolini *et al*., 2004b) and 11 arabic gums available on the Italian market – the former from 1993 to 2001 and the latter in 2006 – were studied to assess the hypothetical maximum elemental increases in wine, assuming that such products, used for the new technological purposes mentioned, may remain in wine. The contribution of these additives to the mineral composition of wine is shown in Table 8.9. As regards tannins, and in comparison with the mean values for wine in the literature, the data show that significant increases in some elements could be caused by the addition of certain products in particular for Sn (*ca*. 20-fold higher), U (*ca*. 10-fold higher), Pd (*ca*., 6-fold higher), Pr and La (*ca*. two-fold higher). Among macroelements of technological or sensorial significance, 7 mg/L of Na could be given to wine by exogenous tannins. For all the other elements changes are insignificant, also in terms of healthiness (e.g. for Pb and Cd).

Gum arabic could significantly increase La, Pr and Th in wine (*ca*. 4–6 fold), also giving about a further 3 mg/L of Na.

Fermenting yeasts effect

While several factors increase the elemental content of wine, only a few among those not specifically directed to metal depletion, can contribute towards reducing the amounts of some elements. As regards this, a biosorption effect of heavy metals by *Saccharomyces cerevisiae* was observed by Volesky and May-Phillips (1995) on a laboratory scale. Changes caused by the yeast strain in the mineral composition of wines achieved under the same real winery conditions on semi-industrial scale in 11 white juices fermented with 4 different commercial strains, have been studied (Nicolini and Larcher, 2003). In particular, of the 20 elements measured, yeast strains were shown to affect the final content of Co, Cu, Mg, Na, Pb, Sr and Zn. It is reasonable to suppose this is related to the different genetic capabilities of the strains to produce H_2S as well as different metabolic needs, e.g. for Zn and Co, beside biosorption on cell walls. Technologically speaking, the most noteworthy average differences among strains were for Cu (0.08 mg/L) and

Table 8.9 Elemental content in arabic gums and tannins and maximum hypothetical increases in wine due to gum (500 mg/L dry weight) and tannin (200 mg/L dry weight) addition assuming absence of precipitations.

Element (μg·kg^{-1} dry weight)	Average contents in wine (μg·L^{-1}) according to literature	bibl. ref.	GUM ARABIC (n = 11)					TANNIN (n = 21)		
			Powder form (n = 7)		Liquid form (n = 4)		Max.increase (μg·L^{-1} wine) caused by gum arabic (500 mg·L^{-1})	median	max	Max. increase (μg·L^{-1} wine) caused by tannin addition (200 mg·L^{-1})
			min	max	min	max				
Ag	13	f	0.6	4.6	0.3	0.8	0.002			
Al (mg·kg^{-1})	0.66 mg·L^{-1}	a	2.48	32.74	1.04	4.56	0.016 mg·L^{-1}	17.1	126	0.025 mg·L^{-1}
As	80	c	0.9	49.9	0.9	19.3	0.025			
B (mg·kg^{-1})	4.70 mg·L^{-1}	a	0.405	0.634	0.114	0.172	0.0003 mg·L^{-1}	22	118	0.024 mg·L^{-1}
Ba (mg·kg^{-1})	0.08 mg·L^{-1}	a	1.418	85.90	0.378	3.79	0.043 mg·L^{-1}	1.0	69	0.014 mg·L^{-1}
Be	0.08	b	0.8	2.5	0.4	0.8	0.001	4	67	0.013
Bi	0.15	b	1.3	2.6	0.7	0.8	0.001			
Ca (mg·kg^{-1})	76.0 mg·L^{-1}	a	4592	7714	1981	4200	3.857 mg·L^{-1}	881	4000	0.800 mg·L^{-1}
Cd	1	b	0.2	0.7	0.1	0.2	0.000	20	146	0.029
Ce	0.02	b	18.5	89.7	5.4	20.5	0.045	11	78	0.016
Co	2.50	a	27.2	72.9	8.3	11.0	0.036	124	442	0.088
Cr	22.1	a	6.9	405	8.4	130	0.203	173	1148	0.230
Cs	2.7	b	5.2	36.3	2.3	18.4	0.018	26.2	506	0.101
Cu (mg·kg^{-1})	0.26 mg·L^{-1}	a	0.83	1.33	0.15	0.52	0.001 mg·L^{-1}	0.8	3.0	0.001 mg·L^{-1}
Dy	0.02	b	0.6	6.7	0.3	1.5	0.003	0	6	0.001
Er	0.01	f	0.3	3.6	0.2	0.8	0.002			
Eu	0.005	b	2.1	8.3	0.3	0.6	0.004	0	3	0.001
Fe (mg·kg^{-1})	1.93 mg·L^{-1}	a	3.16	42.76	2.74	11.55	0.021 mg·L^{-1}	12.4	163	0.033 mg·L^{-1}
Ga	3	f						84	4754	0.951
Gd	0.02	b	1.3	8.5	0.5	1.8	0.004	1	9	0.002
Ge	0.1	f	1.2	18.8	3.1	7.7	0.009			
Hf	0.7	b	0.5	2.2	0.3	0.6	0.001	16	731	0.146
Hg	0.05	f	0.0	11.5	0.0	0.8	0.006			
Ho	0.004	f	0.1	1.3	0.0	0.3	0.001			
K (g·kg^{-1})	1.05 g·L^{-1}	a	2.49	32.31	2.40	10.03	0.016 g·L^{-1}	4.2	9.6	0.002 g·L^{-1}
La	0.006	b	18.3	47.3	3.4	8.7	0.024	5.9	50	0.010
Li	6.74	a	14.5	43.0	6.3	10.5	0.021	198	3443	0.689
Mg (mg·kg^{-1})	78.9 mg·L^{-1}	a	1423	2164	401	700	1.082 mg·L^{-1}	369	1386	0.277 mg·L^{-1}
Mn (mg·kg^{-1})	0.96 mg·L^{-1}	a	1.50	7.71	0.62	1.69	0.004 mg·L^{-1}	11.4	323	0.065 mg·L^{-1}

Table 8.9 (continued).

Element ($\mu g \cdot kg^{-1}$ dry weight)	Average contents in wine ($\mu g \cdot L^{-1}$) according to literature	bibl. ref.	GUM ARABIC (n = 11)					TANNIN (n = 21)		
			Powder form (n = 7)		Liquid form (n = 4)		Max.increase ($\mu g \cdot L^{-1}$ wine) caused by gum arabic (500 $mg \cdot L^{-1}$)	median	max	Max. increase ($\mu g \cdot L^{-1}$ wine) caused by tannin addition (200 $mg \cdot L^{-1}$)
			min	max	min	max				
Mo	6.00	f	2.4	211	4.9	82.5	0.106			
Na ($mg \cdot kg^{-1}$)	13.4 $mg \cdot L^{-1}$	a	113	5620	20	1893	2.810 $mg \cdot L^{-1}$	573	36230	7.246 $mg \cdot L^{-1}$
Nd	0.04	b	8.4	45.5	2.9	10.5	0.023	5	40	0.008
Ni	24.4	a	56.8	337	15.4	76	0.168	931	3449	0.690
P ($mg \cdot kg^{-1}$)	180 $mg \cdot L^{-1}$	b	4.29	24.93	1.30	8.22	0.012 $mg \cdot L^{-1}$			
Pb	41.9	a	7.1	41.6	1.6	3.5	0.021	59	779	0.156
Pd	0.03	b	0.1	0.9	0.1	0.5	0.000	0	963	0.193
Pr	0.001	b	2.2	10.7	0.7	2.5	0.005	1	10	0.002
Rb ($mg \cdot kg^{-1}$)	2.41 $mg \cdot L^{-1}$	a	2.88	10.52	1.21	3.17	0.005 $mg \cdot L^{-1}$	3	102	0.020 $mg \cdot L^{-1}$
Sb	6	b	0.2	1.8	0.1	1.1	0.001	14	487	0.097
Se	4.6	e	6.0	31.1	6.7	16.3	0.016			
Sm	0.02	b	1.3	9.1	0.5	2.0	0.005	0	8	0.002
Sn	20.9	a	1.5	84.5	2.0	26.2	0.042	0	2436	0.487
Sr	277	a	42526	80049	13173	25038	40.0	1163	6501	1.300
Te	n.a.	f	0.1	0.3	0.0	0.1	0.000	0	29	0.006
Th	0.001	f	2.5	11.8	1.3	3.1	0.006			
Ti	70	**d**	102	1177	39	263	0.588			
TI	0.1	**b**	0.6	2.4	0.2	0.9	0.001	9	108	0.022
U	0.003	**f**	0.3	6.9	0.9	2.5	0.003	3	145	0.029
V	46.8	**a**	5.3	1187	21.3	478	0.593	0	216	0.043
Y	0.06	**b**	4.8	33.4	2.1	7.3	0.017	4.2	25	0.005
Yb	0.04	**f**	0.1	3.0	0.1	0.6	0.001			
Zn ($mg \cdot kg^{-1}$)	0.51 $mg \cdot L^{-1}$	**a**	0.32	1.50	0.17	0.55	0.001 $mg \cdot L^{-1}$	1.89	12.4	0.003 $mg \cdot L^{-1}$

(**a** = Larcher & Nicolini 2001, *Ital. J. Food Sci.*, **13/2**, 237–245;
b = Eschnauer & Neeb 1988, in *Wine Analysis*, Linskens & Jackson (Eds.), Springer-Verlag, Berlin, 67–91;
c = Interesse *et al.*, 1985, Z. Lebens. Unters. Forsch. 181:470–474;
d = Interesse *et al.*, 1984, Z. Lebens. Unters. Forsch. 178: 272–278;
e = Bellanger *et al.*, 1992, *J. Int. Sci. Vigne Vin*, **26(4)** 287–293;
f = Eschnauer *et al.*, 2001, Bull. Erbsloeh;
n.a. = not available).

Zn (0.14 mg/L), as well as for Co (1.7 µg/L) if considered with respect to a very low 'normal' content in wine. In any case, it is interesting to note that even the winemaker's simple choice of the yeast strain to be used for fermentation may affect the final composition of wine elements.

The effects of a specific metal depletor

A vinylimidazole/vinylpyrrolidone co-polymer called Divergan HM rapidly and significantly depleted Cu, Fe, Zn, Al, Cr, B and Ag (Mattivi *et al.*, 2000; Eder *et al.*, 2001; Nicolini *et al.*, 2004c), being more effective when applied in juice than in wine. The highest depletion capabilities are for Cu and Fe (45–90%), but a significant reduction can also be obtained for Zn and Al (25–60%), in particular if the polymer is present in the wine during fermentation. Due to its characteristics, it can be considered an effective alternative to potassium ferrocyanide treatments, particularly against high levels of Cu in juice.

Grappa

This is the typical Italian grape-based pomace distillate. Produced and kept with a higher alcohol content, before sale it is diluted with water to a content of between 38% and 60% alcohol by volume (usually 42–43%) and filtered. As a consequence of water dilution, haze and turbidity of the product may occur. The elemental composition of grappa is related in particular to the stability of the product – mainly caused by Cu, Fe and related colour changes, and Ca and Mg precipitations with sulphates – and the presence of heavy metals eventually leached by distillation apparatus, tanks and pumps, wooden casks, filter pads and glass bottles. A survey of limpid and apparently stabile 58 grappa samples produced in northern Italy, some processed by very small distilleries, is shown in Table 8.10. The data show that the maximum values of several elements are clearly due to the cited leaching phenomena.

8.6.2. Inorganic Anions

Main inorganic anions

A recent investigation performed by ionic chromatography updated information both on the main inorganic anions in grape juice (obtained by crushing and pressing at 2.5 bar) and industrially produced wines

Table 8.10 Elemental contents in 58 grappa samples produced in northern Italy determined by ICP-MS. Elements in concentration above the detection limits in at least 10% of samples are reported.

	Al (μg·L^{-1})	**As** (μg·L^{-1})	**B** (μg·L^{-1})	**Ba** (μg·L^{-1})	**Ca** (mg·L^{-1})	**Co** (μg·L^{-1})	**Cr** (μg·L^{-1})	**Cu** (mg·L^{-1})	**Fe** (μg·L^{-1})	**K** (μg·L^{-1})	**Li** (μg·L^{-1})
Isotope	27	51	11	137	40	59	52	63	56	39	7
N_0 samples > DL	38	29	41	50	33	12	57	58	25	36	41
25th percentile	<9	<0.3	<10	<0.3	<0.06	<0.3	5.1	3.88	<10	<0.03	<0.3
Median	11.3	0.16	21.0	0.17	0.26	<0.3	6.7	6.24	<10	1.30	0.55
75th percentile	46.3	1.51	55.5	3.18	1.67	<0.3	9.0	9.53	33.9	10.7	0.95
Max	271	21.7	245	11.7	12.4	3.98	25.5	47.4	178	32.9	4.20

	Mg (mg·L^{-1})	**Mn** (μg·L^{-1})	**Na** (mg·L^{-1})	**Ni** (μg·L^{-1})	**Pb** (μg·L^{-1})	**Rb** (μg·L^{-1})	**Se** (μg·L^{-1})	**Sn** (μg·L^{-1})	**Sr** (μg·L^{-1})	**V** (μg·L^{-1})	**Zn** (μg·L^{-1})
Isotope	26	55	23	60	208	85	78	118	88	51	66
N_0 samples > DL	57	47	53	39	10	36	37	21	47	21	53
25th percentile	0.1	1.2	1.18	<0.3	<0.3	<0.3	<0.3	<0.3	0.5	<0.3	7.9
Median	0.39	9.4	3.18	1.0	<0.3	1.7	0.4	<0.3	2.4	<0.3	26.0
75th percentile	1.06	58.0	24.4	1.9	<0.3	10.4	0.6	0.48	12.0	0.78	45.7
Max	6.19	272	58.3	2052	199.5	37.4	2.4	1.38	45.4	15.6	1902

from the Trentino-Alto Adige region (Nicolini *et al.*, 2002). Results are reported in Table 8.11. The data show slightly higher values of nitrate in juice in comparison with the contents 1.83 ± 0.32 mg/L and 2.16 ± 0.32 mg/L observed in white and red grapes from Sicily, respectively. It was observed the vintage year also influences the anionic composition of juice. Compared with white wines, reds had roughly double the content of chloride and about 40–50% more sulphate and phosphate.

Table 8.11 Inorganic anion content in ripe grape juices and in white (W) and red (R) wines produced in Trentino-Alto Adige region (northern Italy). Analyses were performed by ionic chromatography.

Matrix	Anion ($mg \cdot L^{-1}$)	Wine colour	N°	Min	25° percentile	Median	75° percentile	Max
JUICE	Chloride (NaCl)		321	4.1	13.4	19.0	24.5	55.8
	Phosphate (PO_4^{3-})		309	42	190	260	326	598
	Sulphate (K_2SO_4)		321	92	198	236	278	547
	Nitrate (NO_3^-)		493	<0.2	2.36	3.61	5.23	19.5
WINE	Chloride (NaCl)	W	438	3.8	11.5	18.9	25.1	152
		R	183	8.1	27.3	39.1	52.0	146
		W + R	*621*		*14.7*	*22.4*	*35.4*	
	Phosphate (PO_4^{3-})	W	425	116	253	297	345	1110
		R	170	180	402	444	501	1021
		W + R	*595*		*270*	*327*	*426*	
	Sulphate (K_2SO_4)	W	438	176	345	420	502	855
		R	183	317	522	643	748	1003
		W + R	*621*		*377*	*462*	*590*	

Phosphites and phosphonates

A method of ion chromatography with conductivity detection of ions after eluent suppression for analysis of phosphites (phosphonate ion), was developed (Glenn *et al.*, 1990; Ouimette and Coffe, 1988). It is rapid and relatively simple and shows recoveries are higher than 95% and a detection limit in grapes of 0.5–1.0 ppm is reported.

A survey carried out between 2002 and 2006 on the phosphite contents in grapes and wines from Trentino (Italy) gave the results displayed in Figures 8.5 and 8.6, respectively. In both products, roughly 80% of samples had contents lower than 6 mg/L.

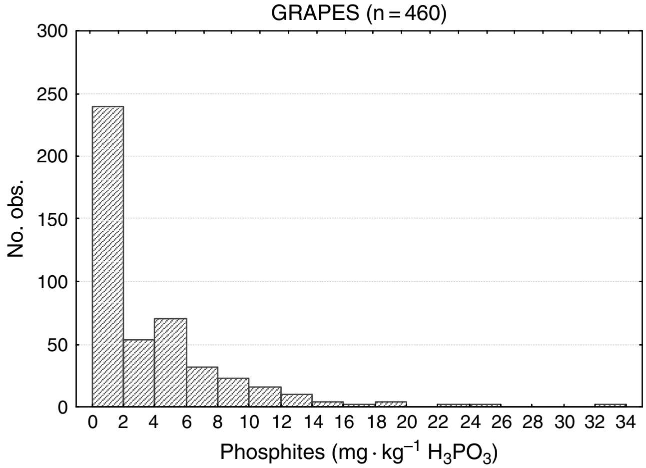

Figure 8.5 Distribution of the content of phosphites in grapes from Trentino (Italy)

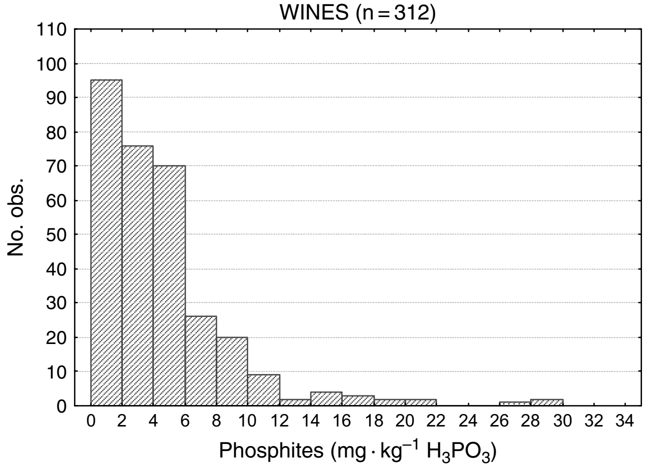

Figure 8.6 Distribution of the content of phosphites in wines from Trentino (Italy)

Data in Table 8.12 relative to phosphite in wine do not evidence differences in the distribution parameters of reds and whites.

Table 8.12 Phosphites in red and white wines from Trentino (Italy).

Phosphites ($mg{\cdot}L^{-1}$ as H_3PO_3)	**No. of Samples**	**25th percentile**	**Median**	**75th percentile**	**90th percentile**	**Max**
Red	129	<DL	3.60	5.50	9.13	20.0
White	183	<DL	3.88	5.81	9.70	29.8

8.6.3. Geographical Origin of Wines/Grapes

Element-based differentiation

Examples of geographical differentiation are also given for Italian sparkling wines processed according to the classical method in Trentino, Franciacorta and Oltrepò Pavese (Nicolini *et al.*, 2003b). Using parametric and non parametric statistical tests, the content of 15 elements in wines proved to be significantly related to the geographic provenance. In particular, Oltrepò sparking wines proved richer in almost all the measured elements (Figure 8.7), and Li, B, Sr and Rb mostly contributed to discriminate and re-attribute to the origin zones. Similarly, three grape-growing areas in Trentino were distinguished on the basis of the contents of Ba, Co, Li, Mn and Na in 67 commercially available and experimental *Müller-Thurgau* wines of various vintages (data not shown).

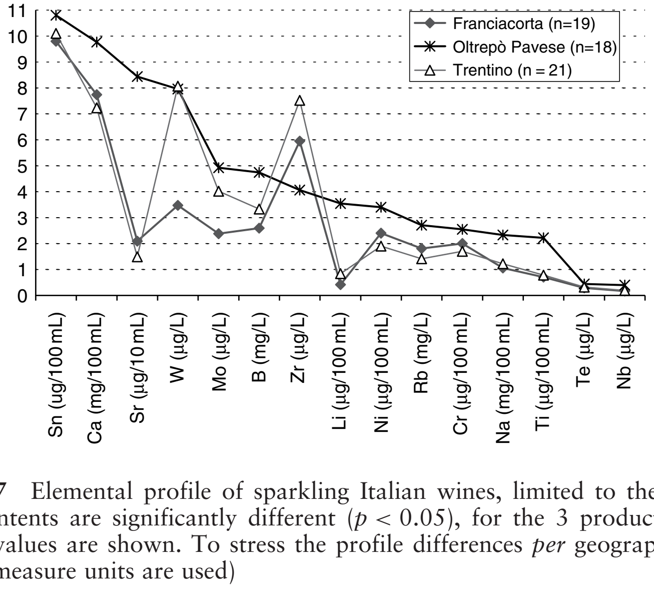

Figure 8.7 Elemental profile of sparkling Italian wines, limited to the elements whose contents are significantly different ($p < 0.05$), for the 3 production areas (Median values are shown. To stress the profile differences *per* geographic origin different measure units are used)

Lead isotope ratios (IRs) have also been used to distinguish wines from different geographical areas (Larcher *et al.*, 2003 and literature cited). However, in the case of genuine Italian wines, lead IRs proved not to be a very sensitive and effective tool for authenticating the origin of wines from different regions, although $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ could differentiate some macro-regions. In particular, high $^{208}Pb/^{206}Pb$ and low $^{206}Pb/^{207}Pb$ ratios characterized the wines from north-western Italy. The complexity of viticultural orography, population distribution, pollution sources and origin of Pb with different isotope compositions used by the petroleum companies can account for this partial differentiation.

Joint use of several parameters

Data on mineral elements, the stable isotope ratios of light bioelements and inorganic anions can be jointly used for distinguishing or ascertaining geographical origin, to improve discriminant performance. An example was given by Depentori *et al.* (1998) that using the isotope ratios (IRs) of hydrogen on methyl site of ethanol $(D/H)_I$ by Site-specific Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR), oxygen ($^{18}O/^{16}O$), carbonium ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) by Isotope Ratio Mass Spectrometry (IRMS), as well as the content of Na, Mg and chloride in about 1000 genuine Italian wines, differentiated their regional origin. Figure 8.8 shows the improvement in terms of correct reclassification percentage of samples to their geographical area achieved by this joint approach. The average correct reclassification increased from 43.5%, only using isotope measures, to 67.8%, when the mineral elements are also used. Similar results were observed by Day *et al.* (1994) for musts from different French regions, respectively. By determining Ca, Mg, Sr and Zn by AA they improved the overall classification of musts from 78% (with isotope data only) to 89% also considering the stable IRs measured by SNIF-NMR. In wines from the principal production zones in France, Day *et al.* (1995) achieved nearly 100% of correct classification using stable IRs measured by SNIF-NMR and Ba, Sr, Rb and Li by AA and ICP-OES.

Joint use of IRs (D/H; the isotopic ratio $\delta^{13}C‰ = 1000 \times (R_{sample} - R_{reference})/R_{reference}$, calculated on the reference international standard), some elements (Na, Sr, V, Rb) and anions (nitrate, chloride) gave positive results also for distinguishing mute and concentrated musts used for balsamic vinegar production from three Italian regions. Canonic

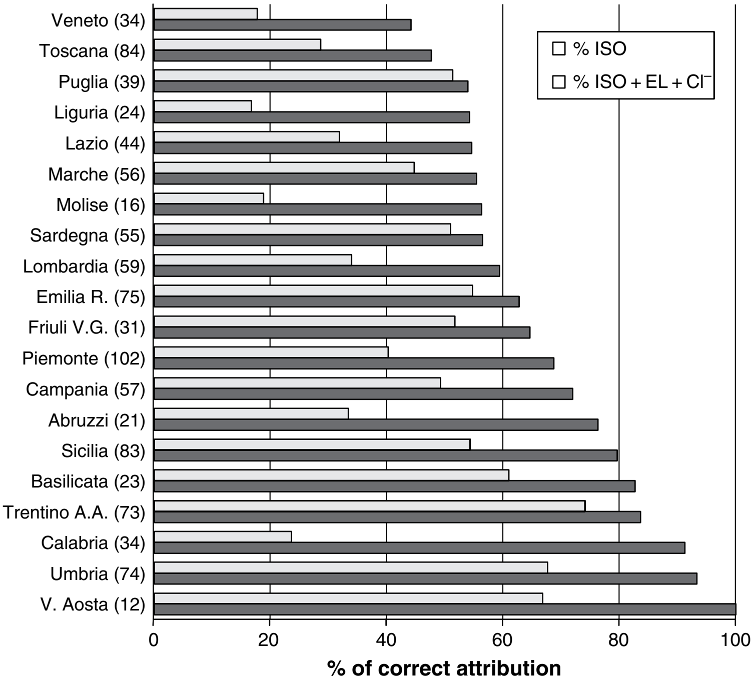

Figure 8.8 Percentages of correct re-attributions to the region of origin of Italian genuine wines using isotopic (ISO) and elemental (EL) parameters as well as chloride (Cl^-). In brackets, the number of samples analyzed is reported

discriminant analysis allowed a correct attribution of 85% of samples to their origin area and only one sample from the south was confused with the northern-B samples. 94% of southern samples were correctly attributed, while a lower performance was obtained for northern areas (Table 8.13). The plot of the canonic discriminant analysis is reported in Figure 8.9.

Table 8.13 Percentages of correct re-attribution to the geographic area of origin of musts used for balsamic vinegar.

	No. samples of certain origin	Percentage of correct re-attribution	North-A	South	North-B
North-A	28	79	22	2	4
South	17	94	0	16	1
North-B	23	87	1	2	20
Total	68	85	23	20	25

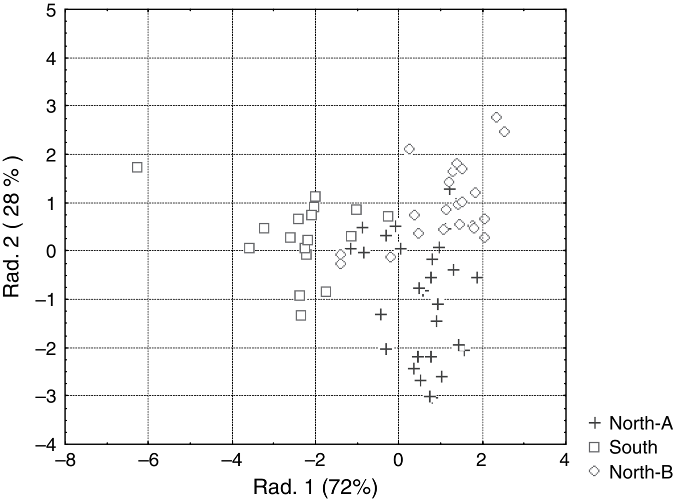

Figure 8.9 Plot of the canonic discriminant analysis carried out for mute and concentrated musts used for balsamic vinegar production from three Italian regions

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