UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Multi-element analysis of South African wines by ICP-AES and their classification according to geographical origin

By

Phillip P Minnaar

Supervisor:
Prof. E. Rohwer

Co-supervisor:
Dr. O. P. H. Augustyn

Submitted in partial fulfilment of the requirements for the degree of

Master of Science
Chemistry

In the Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

February 2009

CHAPTER 1

INTRODUCTION TO THE RESEARCH

1.1. INTRODUCTION

The purpose of this research is to obtain reliable information on wine-growing regions, grape varieties and wine origin in South Africa, as there appears to be a connection among these attributes and the quality of wine. More specifically, the labelling of wine has become a prominent issue in the wine industry since labelling places significant emphasis on the origin and authenticity of the product. A product from a specific area may have considerably greater value than similar products from neighbouring areas. Authenticity also implies that the information and details stated on the label should be reliable and accurate in all respects. In light of the above, the corresponding research question, which guides this research is “Can wine from the Coastal and the Breede River Valley regions in South Africa be differentiated from one another based on their trace element compositions?”

1.2. RATIONALE OF THE STUDY

South Africa’s most important wine-producing region is the Western Cape. Wine production is considered one of the most important agricultural activities in this region. The wine industry is therefore of great economic importance to both the region and the country, as it is one of the industry’s most important export commodities.

Wine, as a beverage, has gained popularity world-wide. People all over the world tend to imbue wine and the drinking of wine with an aura of tradition and romance. They prefer high quality wines to cheap, inferior wines and insist on good value and high standards for their money. For this reason, products that are viewed as exceptional in the public eye become highly sought after and tend to fetch higher prices. Many wine consumers demand guarantees in respect to naturalness and authenticity.

1.2.1. The Debate Pertaining to Certification, Authentication and Falsification of Wine

To protect the interests of all parties, wine producers and authorities have implemented systems that certify the grape variety, origin and vintage of wines. These systems have become increasingly important internationally, as authenticity problems are directly associated with the labelling of products. Most overseas countries adhere to strict compositional standards that need to be applied prior to the labelling of the product. Countries, particularly those belonging to the European Union, have done a great deal of work to provide analytical proof and support for certification systems. Unfortunately, South Africa trails its international partners in this area.

Analytical support of the certification system will assist in increasing the value of a product and the consumer's trust in the system. It will also help to trace and prove the deliberate adulteration of wine, by inclusion of illegal additives and mixing of products. In instances where significant price differences exist among products, falsification is always a possibility. For this reason, wine production is periodically subject to fraudulent activities by individuals, who falsify wines in order to sell their products or to make unjustified (or illegal) profit. These people may reconsider falsification once the cost of falsification erodes or exceeds the illegal profit that is made.

Several types of falsification in wine have been internationally discovered and disclosed. These include: the addition of exogenous sugars (which is legal in Germany) before or during the fermentation process to increase the ethanol content, the addition of synthetic aromatic substances to enhance aroma and the addition of wines of unknown origin or wines which originate from other geographical areas or countries, to high-quality wines, to so extend the volume. Fortunately, this can be traced by a variety of analytical techniques. Site-specific natural isotope fractionation-nuclear magnetic resonance (SNIF-NMR) can be used to detect the addition of sugar (Martin *et al.,* 1988). The addition of water

can be detected by means of isotope ratio mass spectrometry (IRMS) (Simpkins *et al.,* 1995) and the mixing of aromatic substances into wines to enhance the flavour can be detected by means of gas chromatography-mass spectrometry (GC-MS) (Etiévant *et al*., 1988).

1.2.2. The Need for this Research

Role-players in the wine industry world-wide have identified the urgent need for authentication and detailed labelling of wine, in an attempt to limit fraud in the wine industry and to ensure that high standards in wine production are maintained (Wittkowski, 1999). Approximately 44% of South African wines (2005) are subject to regulations laid down by the national authorities, as well as control measures enforced by the Wine and Spirit Board. These organisations certify wines complying with minimum quality requirements. One of the requirements is that the wine should reflect the typical characteristics of the grape variety from which it is produced (Sensory evaluation). It follows, therefore, that wines which have been labelled and certified, as having the same origin (*terroir*), grape variety and vintage, should have similar properties. This will provide them with specific organoleptic properties and a chemical composition that will allow them to be differentiated from other wine areas.

Isotope ratios such as $(D/H)^{Q}_{W}$ [average deuterium/hydrogen content of water in wine], $(D/H)_1$, [specific ratio of the methyl site of ethanol in the wine distillate] $(D/H)_2$, [specific ratio of the methylene site of ethanol in the wine distillate], $(\delta^{18}O\,\frac{Q}{W})$ [average oxygen-18 content of the water in wine] and $(\delta^{13}C\,\frac{Q}{A})$ [carbon–13 of the wine distillate] can be used as support in the determination of origin between large areas within a large country or between countries which are geographically significantly removed (Day *et al.,* 1995). The above-mentioned technique, however, cannot be applied in investigations involving small geographical areas, as for example, the average deuterium/hydrogen content of water in wine (which depends on aquatic environment and climate) may not differ significantly between small neighbouring production areas (Martin *et al.,* 1999).

There are, however, other techniques, which would be more usable and suitable; i.e. techniques to determine the element and phenolic composition of wine. Current indications (2005) are that element determination, particularly trace element determination and polyphenol analyses, can make meaningful contributions in this regard. Characterisation and classification of wines using trace element data as variables, and the application of pattern recognition techniques, have received considerable attention during the past decades (Noble & Brian, 1976; Kwan & Kowalski, 1978; Maarse *et al*., 1987; Martin *et al*., 1999; Sauvage *et al*., 2002; Taylor *et al.,* 2003).

Furthermore, a great deal of literature published on the element composition of wine has indicated that wines can be classified by means of multivariate data analysis (Forina *et al*., 1986; Latorre *et al*., 1994; Brescia *et al*., 2003). Most studies, however, focus on European and Californian wines, and compare wines from different countries. In the South African context, the geographical differentiation between wine producing areas such as the Coastal region and the Breede River Valley region, present a greater challenge than differentiation studies between countries. In South Africa, however, the element composition of wine has not been analysed on a routine basis and must therefore be urgently addressed.

1.3. HYPOTHESIS

My hypothesis is that it is possible to differentiate between wines from the Coastal and Breede River Valley region solely on the grounds of their trace element composition.

1.4. THE SPECIFIC AIMS OF THE STUDY

The specific aims guiding this research include:

- To determine the element composition of market-ready wines, originating from the Coastal region and the Breede River Valley region.

- To apply discriminant methods to the data in an attempt to differentiate between market-ready wines from the Coastal regions and the Breede River Valley region.

1.5. RESEARCH DESIGN AND METHODOLOGY

This section serves to inform the reader of the research design, approaches, methodology, data collection, instruments and techniques and the data analysis procedures implemented in this study.

1.5.1. The Quantitative Paradigm

A decision to implement a quantitative inquiry was justified on the premise that one needs to answer the research question quantitatively, not only by means of an in-depth literature review, but also by means of the collection of numerical data, the statistical analysis of the raw data and the interpretation thereof.

1.5.2. Approval for the Research

The Agricultural Research Council (ARC) approved the proposed project. However, the ARC did not fund the project. The researcher funded the entire project. An agreement between the ARC and the researcher was reached for the utilisation of the analytical instrumentation and laboratory facilities.

1.5.3. Gaining Access to the Research Samples and Sites

Selected wine producers in the Western Cape shall be approached in person for participation in this study. Obtaining wine samples shall be on a voluntary donation basis.

1.5.4. Obtaining the Participants' Consent

Correspondence to the wine producers shall be done by means of a written letter asking for their consent.

1.5.5. Sampling

Market-ready wines, which are bottled, labelled and government certified wines, ready for commercial markets, shall be collected directly from the producers. Wine samples will represent Cabernet Sauvignon, Merlot, Pinotage, Shiraz, Chardonnay, Chenin blanc and Sauvignon blanc from the Breede River Valley and Coastal regions. The number of wine samples representing each variety is subject to availability.

1.5.6. Chemical Analysis

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) shall be select for this study because it is increasingly being applied to element determination in analytical chemistry. At the time of the proposal submission for this study, ICP-AES was used routinely in the Analytical Laboratory of the Agricultural Research Council in Stellenbosch for the determination of trace and ultra trace elements in a variety of matrices.

1.5.7. Management of the Raw Data

All raw data shall be collected by means of the analytical instrument's built-in software programme. The data shall be stored on a network hard drive. Back-up copies shall be stored on CD-ROM. Data identification shall be done according to grape variety, locality, vintage and cellar name.

1.5.8. Data Analysis and Interpretation

Statistical pattern recognition techniques shall be applied to analyse the data. Pattern recognition techniques in particular, can be applied in situational problems where there are collections of objects and lists of measurements made on each object. Pattern recognition techniques can also be implemented to extract key elements and combinations of elements, which are characteristic of geographic origins and subtle differences in element concentrations. Methods such as stepwise discriminant analysis, canonical discriminant analysis and

linear discriminant analysis shall be applied to the data. The researcher, assisted by a statistician, shall interpret the statistical results.

1.6. QUALITY CONTROL OF CHEMICAL ANALYSIS

The laboratory where the analyses are done, shall participate in an Inter-laboratory Water, Soil, and Plant-quality Assurance Scheme, Private Bag X79, Pretoria, 0001. This participation shall confirm the competency of the laboratory and reliability of results. Water with known chemical composition and ethanol as blank samples shall be analysed to confirm the accuracy and precision of the method. The results shall be stored in Addendum A on CD-ROM. Calibration curves shall be obtained using standard multi-element solutions of 100, 200, 500, 1000 and 2000 mg/L for Potassium 10, 20, 50 and 100 mg/L for Magnesium. For the other relevant elements, 0.01, 0.05, 0.1, 1.0, 10 and 50 mg/L shall be used. Each concentration shall be analysed twice to establish a mean value.

1.7 PRESENTATION OF RESEARCH

Chapter 2 presents the theoretical framework and literature review, which guides and underpins this research. The literature review is in a three-domain format and deals with international as well as South African literature pertaining to wine characterisation and geographic differentiation. Journal articles on amino acids, polyphenols, volatile compounds, stable isotopes and element composition of wine are cited. Different techniques used to quantify these parameters are examined and explained. Different statistical methods are reviewed.

Chapter 3 presents a comparative study between atomic absorption spectroscopy and inductively coupled plasma sources. Mass spectrometry, thermal ionisation mass spectrometry, X-Ray fluorescence spectroscopy and neutron activation methods are discussed in brief.

Chapter 4 deals with multivariate statistical methods with the emphasis on pattern recognition techniques. Hypothetical examples of stepwise discriminant

analysis, canonical discriminant analysis and linear discriminant analysis are presented in addenda I, J and K on the CD-ROM.

In Chapter 5, wine samples collected for this study are noted. Sampling procedures, including analytical instrumentation are discussed. A detailed discussion of the analytical procedures, which includes reagents used, calibration methods and reference samples, is presented. Wine sample analyses are discussed under qualitative analysis and quantitative analysis. The statistical methods used in this study are mentioned.

Chapter 6 deals with the results of the calibration curves established for quantitative analyses. Quality control procedures are noted. Results of the wine sample analyses by ICP-AES are discussed. The statistical analyses results are discussed in detail under the following headings; stepwise discriminant analysis, canonical discriminant analysis and linear discriminant analysis followed by a general discussion and conclusion.

A general discussion of the results follows in Chapter 7. The strengths of the study as well as the limitations and weaknesses of the study are mentioned. Recommendations are made and conclusions drawn.

Addendum A contains the quality control data. Addenda B, C, D and E contain the raw data of the instrumental analyses of red and white wine samples. Addenda F and G contain the statistical data of the red and white wine instrumental data. Addendum H is a manuscript published in the Journal of Oenology and Viticulture (2005). Addenda I, J and K contain hypothetical examples of statistical methods.

CHAPTER 2

THEORETICAL FRAMEWORK AND LITERATURE REVIEW

A THREE-DOMAIN MODEL

2.1. APPROACHES USED IN THE LITERATURE SEARCH AND REVIEW

Literature search was conducted using Food Science and Technology Abstracts, Vitis Abstracts, the National Agricultural Library, Science Direct and South African Bibliography Information Network search engines. The search was conducted through the Agricultural Research Council's library in Stellenbosch. Wine amino acids, isotope ratios, phenolic compounds and volatile compound key words were entered in the search engines to initiate the search for the first domain model. Exactly the same procedure was followed for the second domain model, where wine mineral, element, geographic origin, wine origin, wine region and wine authenticity key words were entered. The third-domain model search was initiated with South African wine industry, South African wine of origin, wine authentication and South African wine regions as key words.

2.2. THE FIRST DOMAIN - LITERATURE DEALING WITH THE DIFFERENTIATION BETWEEN WINES ACCORDING TO GEOGRAPHICAL ORIGIN USING AMINO ACIDS, ISOTOPE RATIOS, PHENOLIC COMPOUNDS AND VOLATILE COMPOUNDS

Wine is an aqueous alcoholic solution containing a large number of chemical compounds, such as amino acids, phenols, volatile compounds, stable isotopes, and trace and ultra trace elements, which originate naturally from *terroir,* vine and grapes. Many of these chemical compounds may be used as differentiating parameters for wine origin determination. The chemical composition depends on a number of factors, which include production area, viticultural practice, and grape variety, type of soil, climate, yeasts and winemaking techniques. It appears that these factors could play an important role in the characterisation of, and differentiation between wines. Wines labelled as having the same grape variety, region and geographical origin, should have a similar or typical chemical

compositions that provide them with particular characteristics and allows the wine to be differentiated from other wines of known origin (Danzer *et al*., 1999; Sivertsen *et al*., 1999). Wine differentiation in terms of variety and geographical region of origin has been extensively investigated, as wine is an easily adulterated product. Meticulous and continuous control is required to ensure and maintain the quality of wine. There is currently a wide range of techniques used to differentiate between the origins of wines. Differentiation between wines according to grape variety can be effectively performed by determining parameters such as amino acid or protein content (Fast Protein Liquid Chromatography [FPLC] and Electrophoresis), polyphenol compounds (High-performance Liquid Chromatography, HPLC), element composition (Inductively Coupled Plasma Emission Spectroscopy, ICP-AES and ICP-MS, Inductively Coupled Mass Spectrometry), isotope-ratios (Nuclear Magnetic Resonance, NMR) and volatile compounds (Gas Chromatography-Mass Spectrometry, GC-MS) (Aleixandre *et al*., 2002).

Amino acid and protein content as well as phenolic compounds are used for both variety and geographical differentiation. Volatile compounds are used to characterise grape varieties, whereas element content is used for geographical differentiation. Furthermore, deoxyribonucleic acid (DNA) determination is also employed for differentiation between grape varieties. The use of Fourier transform infra-red (FT-IR) spectroscopy is a more recent application for determining the geographical origin and grape varieties of wine (Aleixandre *et al.,* 2002).

In this literature review, the author will focus mainly on amino acids, volatile compounds, phenolic compounds, stable isotope and element content as widely used parameters for wine origin differentiation.

2.2.1. Amino Acids

Etiévant *et al.* (1988a) have shown that amino acid concentrations of wines are dependent upon climatic conditions and the technology used in wine production. Despite climatic conditions, researchers have successfully employed amino acid composition for wine differentiation (Rizzon *et al*., 1985; Tapias *et al*., 1987; Arvanitoyannis *et al.,* 1999; Kallithraka *et al.,* 2001; Soufleros *et al*., 2003). In a study by Derde *et al.* (1983), the determination of 20 amino acids resulted in the differentiation among varietal wines originating from Bourgogne, Bordeaux and Beaujolais. These authors measured serine, ornithine, citrulline, arginine and proline by means of GC in 160 must samples from three Champagne varieties to differentiate among Pinot noir, Pinot meunier and Chardonnay. Millery *et al.* (1984*)* were able to differentiate between wine origins using arginine, which is a genetic characteristic of a grape variety and proline, which is indicative of the age of a wine. Etiévant *et al.* (1988a) analysed the amino acid composition of 34 French red wines, discovering that only proline and hydroxyproline showed clear differences among grape varieties. Studies were conducted by Soufleros *et al.* (1998) analysing 135 wines from Bourgogne, Bordeaux, Alsace and Champagne regions, measuring amino acids, biogenic amines, organic acids and volatile compounds to differentiate between wines according to geographic origin, type and vintage. After Soufleros *et al.* adapted the HPLC method for amino acid determination; it was found using fluorescence detection that the methionine, proline, asparagine, arginine and glutamic acid concentrations were successfully able to differentiate among the four regions.

De La Presa-Owens *et al.* (1995) reported the determination of amino acids for the differentiation between Spanish wines: Macabeo, Xarel-lo and Parellada from the Penedés region. Ornithine, glycine and lysine proved to be the best discriminating compounds for differentiation between grape varieties based on geographical origin. Amino acids were also effectively used in a study of 110 French wines, separating Champagnes from Sparkling wines. Champagnes are richer in amino acids than sparkling wines, except for arginine, because of the

second bottle fermentation and long lees contact (Tusseau *et al.,* 1993). Hernándes-Orte *et al.* (2002) analysed 11 amino acids in synthetic grape must solutions imitating the characteristics of amino acid profiles of 11 Spanish grape varieties. They established that threonine, serine, β-phenylalanine and methionine profiles could be used in differentiating between grape varieties.

Soufleros *et al.* (2003) found that the amino acid composition of Greek white wines was influenced by grape variety, geographic origin and vintage. They also found that malolactic fermentation had an impact on the concentration levels of certain amino acids. Within all samples, arginine and γ-amino butyric acid were the most abundant amino acids followed by lysine, alanine, glycine asparagines and leucine.

Table 2.1 summarises some of the most frequently used amino acids for wine differentiation according to region of origin.

TABLE 2.1
Most frequently used amino acids for wine origin differentiation

Wine origin	Amino acid	Reference
France	Serine, ornithine, citrulline, arginine, proline	Cordonnier & Bayonove, 1982
	Total amino acids	Derde *et al.,* 1983 Etiévant *et al.,* 1988a Soufleros *et al.,* 1998
	Proline, hydroxyproline	Valme *et al.,* 1995
Greece	Arginine, methionine, γ-amino butyric acid	Soufleros *et al.,* 2003
Italy	Glutamic acid, aspartic acid, proline, leucine, alanine, serine	Seeber *et al.,* 1991

Spain	Asparagine, proline, lysine	De la Presa-Owens *et al.,* 1995
	Threonine, serine, phenylalanine, methionine	Hernándes-Orte *et al.,* 2002

2.2.2. Volatile Compounds

Volatile compounds are extensively used for grape variety characterisation. Volatile compounds have been isolated and identified using GC techniques (De La Presa-Owens *et al.,* 1995; Garcia-Jares *et al.,* 1995; Arvanitoyannis *et al.,* 1999; Rebolo *et al.,* 2000). Soufleros *et al.* (1980) analysed the ethyl esters of fatty acids and acetates of higher alcohols in young French wines to characterise different grape varieties. Terpene alcohols are considered important contributors to varietal characterisation of French wines (Cordonnier *et al.,* 1982, as quoted by Arvanitoyannis *et al.,* 1999). High levels of terpene alcohols and terpenic oxides have been reported for Muscat, Gewürztraminer, Riesling and Muller-Thurgau wines (Marais, 1983). Simultaneous evaluation and correlation of elements, amino acids and aromatic alcohols, particularly tyrosol and tryptophol, in 34 red wines from three French regions, provided an interpretation of differences among Grenache, Carignan, Cinsault, Merlot, Cabernet Sauvignon and Cabernet Franc grape varieties related to geographic and varietal origins (Etiévant *et al.,* 1989). Differentiation between Italian Chardonnay must samples from different vintages was based on 38 volatile compounds. Among the compounds measured were alcohols, esters, amides and acids (Seeber *et al.,* 1991).

Volatile compounds in Galician wines were identified by means of gas chromatography-ion trap mass spectrometry. Forty compounds were quantified in order to characterise the wines (Garcia-Jares *et al.,* 1995). The quantification of 41 volatile compounds analysed by GC-MS in 60 Spanish wines of three grape varieties, different origins and vintages, proved that certain higher alcohols could

be used for differentiation between wines according to origin and vintage (De La Presa-Owens *et al.,* 1995). Guth (1997) analysed 44 odour compounds in Scheurebe and Gewürztraminer wines originating from Germany. Differences in odour profiles were based on *cis*-rose oxide in Gewürztraminer and 4-mercapto-4-methylpentan-2-one in Scheurebe wines. These compounds were suitable indicators for determining flavour differences and wine differentiation. Rosillo *et al.* (1999) used headspace analysis with GC-MS to determine volatile compounds in different grape varieties originating from Spain. Application of cluster analysis (CA) resulted in three groups, one for white varieties, one for Monastrell, Tempranillo and Cabernet Sauvignon and one for Dyer Grenache. Hexyl acetate, benzyl alcohol, phenylethyl alcohol and benzaldehyde were the discriminant variables for group differentiation. Hernández-Orte *et al.* (2002) found significant differences among certain volatile compounds (ethyl alcohol, ethyl acetate, acetic acid, higher alcohols and their acetates, methionol, isobutyric acid, ethyl butyrate and hexanoic acids) in experimental wines. The concentrations of certain volatile compounds were well correlated with the aromatic composition of wines made with grapes of the same variety. Classic oenological parameters, alcohols and esters were used to differentiate between Spanish varietal white wines and their corresponding sub-zones (Alvarez-Seoane *et al.*, 1988). Varietal red wines from Northwest Spain showed that terpene profiles were typical of grape character. Linalool, citronellol, α-pinene and β-pinene were important contributors to grape character. Other important compounds were norisoprenoids, phenyl-ethanol and benzyl alcohol (Calleja & Falqué, 2005).

Table 2.2 lists some of the most frequently used volatile compounds for wine differentiation according to region of origin.

TABLE 2.2

Most frequently used volatile compounds for wine origin differentiation

Wine origin	Volatiles	References
France	Terpenic alcohols	Cordonnier *et al.,* 1982
	Tyrosol, tryptophol	Etiévant *et al.,* 1989
Germany	4-Mercapto-4-methylpentan-2-one, *cis*-rosé oxide	Guth *et al.,* 1997
Portugal	2, 6, 6-trimethylcyclohex-2-ene-1, 4-dione, diacetyl (contributor to caramel descriptors)	Rogerson *et al.,* 2001
South Africa	Terpene alcohols, terpenic oxides	Marais, 1983
Spain	Thiols, sulphides, thioesters, heterocyclic compounds	Mestres *et al.,* 2000
	Methionol, isobutyric acid, ethyl butyrate, hexanoic acids	Hernández-Orte *et al.,* 2002
	Terpene profiles, linalool, citronellol, α-pinene, β-pinene	Calleja & Falqué, 2005

2.2.3. Phenolic Compounds

Phenolic compounds are another promising group of compounds widely used for the classification of wines and have been successfully employed for differentiating between wines (De La Presa-Owens *et al.,* 1995; Arozarena *et al.,* 2000; Csomós *et al.,* 2002; Rossouw & Marais, 2004). Although the concentration levels of certain grape polyphenols may vary from year to year, due to climatic and environmental variation, the polyphenol content can characterise grape varieties and provide information for geographical origin (Etiévant *et al.,* 1988b). The three principal factors affecting the phenolic content of wines are; polyphenol content of grapes, wine making techniques and reactions (polymerisation) which take place during ageing (Blanco *et al.,* 1998).

The most widely used method for polyphenol determination is HPLC analysis. Goldberg & Soleas (1999) were able to quantify catechin, epicatechin, quercetin, rutin, *cis*- and *trans*-resveratrol and *p*-coumaric acid in white wines of various countries using HPLC with diode array detection. Gu *et al.* (2000) quantified flavonoids, volatile phenols, flavonols, tannins, leucoanthocyanidins and anthocyanidins to differentiate between wine regions. Capillary zone electrophoresis was also employed to quantify epicatechin, catechin, quercetin, myricetin, rutin, gentistic acid, caffeic acid, gallic acid and trans-resveratrol.

Wines originating from the Médoc area in France were differentiated based on two-dimensional analysis of myricetin and epicatechin levels (Manterola, 1985). Pedro Ximénez and Baladi grapes originating from France were differentiated based on caftaric and coumaric acids as well as procyanidins. Caftaric and coutaric acids, as well as procyanidins were compounds, which showed considerable differentiation between the two grape varieties (Mayén *et al.,* 1997). Sivertsen *et al.* (1999) showed that improved differentiation between French wines in terms of geographical origin was achieved using polyphenolic data as opposed to sensory data. They established that wines, which are more representative of the region of origin and with better sensory descriptors, could contribute to a more useful correlation between sensory data and chemical data.

Almela *et al.* (1996) demonstrated that the anthocyanidin content could be used as a principal parameter to differentiate between Spanish varietal young red wines. Following the method of Manterola (1985), differentiation was established between seven brands of dry sherry (Palomino variety) based on five phenolic compounds (Valme *et al.,* 1995). Gálvez *et al.* (1995) used low molecular weight phenolic compounds to differentiate between Spanish wine vinegar origins. Important differences were established as a function of age and relationships between origin and polyphenols. De La Presa-Owens *et al.* (1995) used analysis of variance (ANOVA) on polyphenol data to establish significant differences between grape varieties. Procatechuic and *trans*-coumaric acid concentrations

varied considerably depending on vintage. These parameters were used efficiently to group varietal grape musts from the Penedés region. Epicatechin, *trans*-caftaric acid, *trans*-caftaric/*trans*-coumaric acid ratios, syringic acid and *p*-coumaric acids were found to be significantly different according to grape variety, vintage and winery (locality) for white wines. In another study, Sato *et al.* (1996) differentiated between wines from different global geographical origins and vintages using the polyphenol content. Direct correlation between geographical origin and vintage was achieved, based on total polyphenols. The phenolic composition of 92 wine vinegars from different grape varieties, originating from the south of Spain was determined by HPLC. The data showed effective differentiation between geographical origin and predicted the samples according to geographical origin (Garcia-Parilla *et al.,* 1997). Flavonoids, myricetin and quercetin were determined by gradient reversed-phase HPLC and successfully used to differentiate between Spanish red wines from various geographical origins (McDonald *et al.,* 1998).

Concentration levels of phenolic acids, flavonoids, trihydroxystilbenes, *cis*- and *trans*-resveratrol and *cis*- and *trans*-polydatin were measured in four white wine varieties and five red wine varieties originating from the Niagara Peninsula, Canada. Relative patterns of individual polyphenols were determined and several significant and characteristic differences in the polyphenol content were identified (Soleas *et al.,* 1997).

Kallithraka *et al.* (2001) were able to differentiate between varietal wines from northern and southern Greece based on catechin, epicatechin, myricetin, quercetin, caffeic acid and ferullic acid composition.

Rossouw & Marais (2004) differentiated between varietal red wines originating from South Africa based on their anthocyanin composition.

Table 2.3 lists some of the most frequently used phenolic compounds for wine differentiation according to region of origin.

TABLE 2.3

Most frequently used phenolic compounds for wine origin differentiation

Wine origin	Polyphenols	Reference
Canada	Catechin, epicatechin, quercetin, caffeic acid, ferullic acid	Soleas *et al.,* 1997
France	Anthocyanidins	Etiévant *et al.,* 1989
	Coumaric acid, caftaric acid, procyanidin	Mayen *et al.,* 1997
Italy	Protocatechuic acid, *trans*-coumaric acid	De La Presa-Owens *et al.,* 1995
Greece	Catechin, epicatechin, myricetin, quercetin, caffeic acid, ferullic acid	Kallithraka *et al.,* 2001
South Africa	Anthocyanins	Rossouw & Marais, 2004
Spain	Antocyanidins	Almela *et al.,* 1996
USA	Catechin, epicatechin, quercetin	Goldberg & Soleas, 1999

2.2.4. Isotope Ratios

A comprehensive approach to wine differentiation can be provided by stable isotope determination. The most commonly employed isotope ratios for wine differentiation are carbon-13 of the wine distillate ($\delta^{13}C\frac{Q}{A}$), $(D/H)\frac{Q}{W}$, $(D/H)_1$, $(D/H)_2$ and ($\delta^{18}O\frac{Q}{A}$). The technique of nuclear magnetic resonance spectroscopy (NMR) can supply important information on the origin of a given wine (Martin *et al.,* 1988). Isotope composition is dependent on climate and grape variety. Despite

this, isotopes cannot be directly correlated with the year of production of wine (Day *et al.,* 1994). Isotope determination was conducted by Day *et al.* (1995) to determine varietal wine origin using ^{2}H-NMR spectroscopy and isotope ratio mass spectrometry. They also determined the element composition using atomic absorption spectroscopy (AAS) with flame and electro-thermal atomisation.

Deuterium/hydrogen ratios have been successfully used for detecting the presence of exogenous sugar in wines. Deuterium occurs in ethanol, which is produced by fermentation and is related to the deuterium content of wine-water and fructose molecules. Deuterium/hydrogen content of water and the methyl group of ethanol were quantified by Martin *et al.* (1999) using isotope ratio mass spectrometry (IRMS) and site-specific natural isotope fractionation (SNIF-NMR), respectively. Proton and carbon-13 NMR spectroscopy methods were applied to differentiate between German white wines from Rheinhessen, Rheingau and Mosel-Saar-Ruwer regions (Vogels *et al.,* 1993). Hermann (2003) found that besides the vintage and origin, the hydrogen isotope ratios of the methyl $(D/H)_1$ sites of ethanol were also affected by their actual locations. Typically, high $(D/H)_1$ ratios were found to cluster within borders of the Rheinhessen and Mosel-Saar-Ruwer regions, while typically low $(D/H)_1$ ratios were found predominantly in the area of Pfalz. Reliable environmental data are required on the site where grapes are grown to be able to use $(D/H)_1$ ratio values effectively. The $(D/H)_1$ value of ethanol correlates with the environmental conditions of the vintage. One of the oldest methods for determining the vintage of a wine is based on the $\delta^{18}O^{Q}_{W}$ content of wine-water and $\delta^{13}C^{Q}_{A}$ content of wine distillates. Measurements of $\delta^{13}C^{Q}_{A}$ levels have an advantage over (D/H) in that ^{13}C is considerably more abundant than deuterium (Martin *et al.,* 1999*).*

Quantitative deuterium analysis using NMR spectroscopy has been employed and natural abundance deuterium/hydrogen isotope ratios of ethanol were used to characterise the origin of Valencian (Spain) varietal grape musts. The

deuterium molecular distribution patterns of the ethanol in wine differ according to geographical and varietal origin (Gimenéz-Miralles *et al.,* 1999). Day *et al.* (1995) determined $(D/H)^{Q}_{W}$, [average deuterium/hydrogen content of water in wine], $(D/H)_1$, $(D/H)_2$ [hydrogen isotope ratios of the methyl and methylene sites of ethanol, respectively], $(\delta^{18}O^{Q}_{W})$ [Average oxygen -18 content of the water in wine] and $(\delta^{13}C^{Q}_{A})$ [carbon –13 of the wine distillate] ratios, including the element composition of wine from Burgundy, using ^{2}H-NMR and AA to determine geographical origin. Hydrogen isotope ratios of the methyl $(D/H)_1$ and methylene $(D/H)_2$, sites of ethanol determined by means of SNIF-NMR, and trace elements determined by inductively coupled plasma-mass spectrometry (ICP-MS), were measured in wines, which originated from Bordeaux to characterise the geographic origin (Martin *et al.,* 1999).

Authenticity and geographical origin of wines from Slovenia were investigated measuring $^{13}C/^{12}C$ and $(D/H)_1$ ratios by means of isotope ratio mass spectrometry (IRMS) and SNIF-NMR (Ogrinc *et al.,* 2001). Košir *et al.* (2001) applied SNIF-NMR and IRMS to 50 white wines from three wine regions in Slovenia. Košir and co-workers showed that the differentiation between wines according to geographical origin and authenticity was possible using (D/H) and $^{13}C/^{12}C$ ratios, when coastal regions were compared with inland regions.

Brescia *et al.* (2003) analysed 33 wines using HPICE, ICP-AES and NMR to differentiate between the geographical origins of red wines from three Slovenian and Apulian regions (southern Italy). They found that the best prediction of wine origin was obtained using NMR measuring $\delta^{13}C^{Q}_{A}$.

Gremaud *et al.* (2004) determined the $(D/H)_1$ ratios in ethanol, $(^{18}O/^{16}O)$ ratios in wine-water, element composition and classic oenological parameters in Swiss varietal wines. The isotope ratios $(^{18}O/^{16}O)$, elements (Sr, Rb) and ethanol percentage allowed the best differentiation between geographical origins.

Table 2.4 presents some of the most frequently used isotopes for wine differentiation according to region of origin.

TABLE 2.4

Most frequently used isotope ratios for wine origin differentiation

Wine origin	Isotopes	Reference
Germany	^{13}C	Vogels *et al.,* 1993
	$(D/H)_1$	Hermann, 2003
France	$(D/H)_1$, ^{13}C, ^{18}O	Day *et al.,* 1995
Italy	$(D/H)_1$, $(^{13}C/^{12}C)$	Gimenéz-Miralles *et al.,* 1999
	^{13}C	Brescia *et al.,* 2003
Slovenia	$(^{13}C/^{12}C)$, $(D/H)_1$	Ogrinc *et al.,* 2001
Switzerland	$(^{13}H/^{12}H)$, $(^{18}O/^{16}O)$	Gremaud *et al.,* 2004

2.3. THE SECOND DOMAIN – LITERATURE DEALING WITH THE DIFFERENTIATION BETWEEN WINES ACCORDING TO GEOGRAPHICAL ORIGIN USING TRACE AND ULTRA TRACE ELEMENTS TOGETHER WITH MULTIVARIATE ANALYSIS

A number of authors refer to the inorganic constituents in wine as elements, minerals, metals, heavy metals, major, minor, trace and ultra trace elements (Greenough *et al*., 1997; Thiel & Danzer, 1997; Frías *et al*., 2003; Taylor *et al*., 2003; Castiñeira-Gómez *et al*., 2004; Coetzee *et al*., 2005). Greenough *et al.* (1997) for example, refer to Li, Ti, Ni, As, Ba, Pb, V, Co, Y, Zr, Mo, Sn, Cs, Ga, Nb, Cd, Sb, Th and U as minerals. Castiñeira-Gómez *et al.* (2004), on the other hand, refer to Al, Cd, Ca, Cu, Fe, Pb, Zn, Cr, Na, K, Mg, Zn, Mn, Li, Rb, As, Pb, Ti and Se as metals and Taylor *et al.* (2003) refer to V, Cr, Mn, Fe, Ni, Cu, Zn, As and Pb as heavy metals. The majority of authors however refer to K, Na, Ca and Mg as major elements and Fe, Cu, Zn, Mn, Sr, Li and Rb as minor or trace elements in wine. Wine minerals normally occur in the range 10 – 1000 mg/L. Wine trace element concentrations normally occur in the range 0.001 – 10 mg/L,

whereas wine ultra-trace element concentrations are in the order of 0.001 mg/L or less.

According to Greenough *et al.* (1997) and Taylor *et al.* (2003), Ba, Rb, Cs, Sr and Li are soil-associated elements, which are the highly soluble lithophiles. Lithophile elements mainly consist of the highly reactive metals of groups one and two of the periodic table of elements. They also include a small number of reactive non-metals, and the more reactive metals of groups IVB and VB of the periodic table of elements. Copper and Bi, which are chalcophilic elements (Chalcophile elements are those metals and heavier non-metals that have a low affinity for oxygen and prefer to bond with sulphur as highly insoluble sulphides), Ni and Co, which are the siderophilic elements (Siderophilic elements are the high-density transition metals) and La, Ce, Th and U, the high-field strength lithophile elements (high-field strength lithophile elements mainly consist of the lanthanides and actinides) are also soil-associated elements. The element uptake capacity of plants does not always include all soil-associated elements.

Soil-associated elements may generally be considered good indicators of wine origin, since their concentrations are not modified during vinification processes. The element content of wines may therefore be an effective means to differentiate between wine origins. Since the research conducted by Kwan & Kowalski (1978), trace and ultra trace elements have been studied for their capacity to characterise the geographic origin of wine. Various analytical techniques have been used to measure trace and ultra trace elements of wine, which include electrochemical techniques, X-ray fluorescence, atomic absorption spectroscopy, neutron activation analysis, mass spectrometry and inductively coupled plasma spectroscopy. Inductively coupled plasma spectroscopy (ICP-AES) has been the most widely applied of all the mentioned techniques (Eschnauer *et al*., 1989; Ströh *et al*., 1994; Day *et al*., 1995; Baxter *et al*., 1997; Greenough *et al*., 1997; Thiel & Danzer, 1997; Martin *et al*., 1999; Pérez-Trujillo *et al*., 2003; Taylor *et al*., 2003; Casteñeira-Gómez *et al*., 2004).

The determination of elements by ICP-AES, in particular Na, K, Ca, Mg, Mn, Li, Fe, Cu and Pb, has been extensively used for detection of adulterated wine. Such wines display elemental profiles that differ from those found in wines of genuine variety and geographical origin (Moret *et al*., 1994; Baxter *et al*., 1997; Kallithraka *et al*., 2001; Galani-Nikolakaki *et al.,* 2002; Frías *et al*., 2003; Castiñeira-Gómez *et al*., 2004; Thiel *et al.,* 2004).

Seeber *et al.,* 1991; Moret *et al.,* 1994; Day *et al.,* 1994; Latorre *et al*., 1994; Baxter *et al.,* 1997; Greenough *et al.,* 1997; Martin *et al*., 1999; Gómez-Plaza *et al.,* 2000; Kallithraka *et al.,* 2001; Sauvage *et al.,* 2002; Galani-Nikolakaki *et al.,* 2002; Frías *et al.,* 2003; Taylor *et al.,* 2003; Castiñeira-Gómez *et al*., 2004; Thiel *et al.,* 2004; Coetzee *et al.,* 2005 have succeeded in identifying wine origin by the determination of K, Na, Fe, Zn, Rb, Ca, Mg, Mn, Cu, Cr, Co, Sb, Cs, Br, Al, Ba, Li and Ag in wine.

2.3.1. American Wines

Li & Hardy (1999) quantified trace elements by means of AAS in 41 Chardonnay and Johannisberg Riesling wines from both Ohio (Lake Erie) and California (Napa Valley and Sonoma region). Principal component analysis (PCA) and *K*-nearest neighbour analysis (*K*-NNA) revealed significant differences among regions and grape variety. The first principal component was found to discriminate wines according to type based on calcium, potassium and sodium. A classification success rate of 75% for Chardonnay and 93% for Johannisberg Riesling was established. An overall classification success rate of 93% was achieved using *K*-NNA.

2.3.2. Australian Wines

Sauvage *et al.* (2002) quantified Cu, Fe, K, Na, Mg and Ca by means of AAS in Chardonnay, Sauvignon blanc, Riesling, Gewürztraminer and Pinot gris wines originating from Melbourne. In addition, preliminary transmission near infra-red

(NIR) spectrometric analyses for the above-mentioned metals was performed to plot the correlations of metal concentration and wavelengths. Multiple linear regression analysis (MLRA) and partial least squares (PLS) were both applied to the data. Sauvage *et al.* (2002) concluded that K, Na and Ca were acceptable parameters for differentiating between geographical origins. The analytical values obtained for these variables were in agreement with those reported by other researchers.

2.3.3. Canadian Wines

Greenough *et al.* (1997) measured 33 elements in 17 white and 10 red wines originating from the Okanagan Valley using ICP-MS. Multiple dimensional scaling (MDS), cluster analysis (CA) and discriminant analysis (DA) were applied to identify relationships among chemical data. Greenough *et al.* established that wines from grapes from the same vineyard tend to be very similar, regardless of the vintage and winery that processed the grapes. Discriminant analysis has indicated that Al, V, Co, Cu, As and Cd can differentiate with up to 95% accuracy between wines according to vineyard. Magnesium, P, S, Cl, Ti, Br, Mo and Ba also correlate with grape varieties. Taylor *et al.* (2003) have provided verification of vineyard origin with 100% accuracy between Niagara and Okanagan vineyards using Sr, Rb, Mn, U, Al, V, Zn, Mo, Sb and Co.

2.3.4. French Wines

Etiévant *et al.* (1988b) quantified trace elements by means of flame emission spectroscopy and electro-thermal atomic absorption spectroscopy in 34 wines from Bordeaux, Angers and Narbonne regions. Etiévant *et al.* found that Ag, Al, As, B, Ba, Br, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Na, P, Rb, Sr, Sb, Ti and Zn were useful elements for wine region differentiation. However, they found that Rb, Mg and Li were selected by PCA and stepwise discriminant analysis (SDA) as discriminant variables. A classification success of between 94% and 100% accuracy was achieved. Potassium, Na, Ca, Mn and Cl were used to differentiate between vineyards world-wide and Rb and Li provided statistically significant

differences among wines produced in different areas within the same country. Day *et al.* (1994) analysed the trace element composition of 96 wines originating from Beaujolais, Burgundy, Bordeaux, Champagne and Alsace regions. Analysis of variance (ANOVA) revealed that the variables; Zn, Ca, Sr and Mg could be used to differentiate with up to 78% accuracy between the wine regions. Day *et al.* (1995) quantified trace elements using atomic absorption spectroscopy, flame ionisation and electro-thermal ionisation in grape must to differentiate among Alsace, Beaujolais, Burgundy and Loire Valley regions. Principal component analysis and CDA were applied to the chemical data. An 88% classification success rate was achieved using only Ba, K, Mg, Mn, Sr, Al, Fe and Zn. Martin *et al.* (1999) quantified trace elements by means of ICP-MS, graphite furnace atomic absorption spectroscopy (GFAAS) and flame atomic absorption spectroscopy (FAAS) in 97 wines and 162 grape samples from Bordeaux. Flame atomic absorption spectroscopy was used for the quantification of Na, Ca, K, Mg. Ba, Li, Sr, Al and Zn. Rubidium was determined by GFAAS. Analysis of variance and least significant difference (LSD) were applied to the data to estimate the ability of trace elements to differentiate between the origins of wine. Martin *et al.* singled out, Li, Ba, Ga and Sr as elements having the best discriminatory powers.

2.3.5. German Wines

Maarse *et al*. (1987) quantified trace elements by means of FAAS in 51 Riesling wines, originating from the Mosel and Rhein-Pfalz regions. Lithium, Rb, Fe and Na were identified as key elements for differentiation between the Mosel and Rhein-Pfalz regions. Principal component analysis, LDA and *K*-NNA were used as classification techniques. One hundred percent (100%) classification success between the two regions was established. Danzer *et al*. (1999) quantified 16 trace elements using ICP-AES in 165 wines from six regions. Cluster analysis, based on Ward's minimum variance, ANOVA, Plackett-Burman analysis (PBA) and SDA were applied to the data sets. They found that only Ba and Si could be used to achieve an overall classification success of 90%, using SDA and PBA.

Castiñeira-Gómez *et al.* (2004) quantified Li, B, Mg, Ca, V, Mn, Co, Fe, Zn, Rb, Sr, Cs and Pb in 127 white wine samples using ICP-MS. The application of quadratic discriminant analysis (QDA) proved the differentiation among four regions (Baden, Rheingau, Rheinhessen and Pfalz) with an 83% accuracy using Li, B, Mg, Fe, Zn, Sr, Cs and Pb. Magnesium and Sr played an important role in differentiating between Rheinhessen and Pfalz regions. Thiel *et al.* (2004) quantified 19 elements by means of ICP-MS in wines. They established that origin and authenticity of wines could be recognised based on typical mineral and trace element content using pattern recognition techniques. Thiel *et al.* established that As, Be, Cs, Li, Mg, Pb, Si, Sn, Sr, Ti, W and Y were relevant in differentiating between the geographical origins of wines. A prediction rate by cross validation analysis of 88% was achieved.

2.3.6. Greek Wines

Kallithraka *et al.* (2001) showed that the element content of 33 red and white varietal wines varied substantially, which indicates that element content can be employed as a reliable indicator for differentiation among wines from various regions. Principal component analysis revealed that a satisfactory classification of red wines in terms of geographical origin could be achieved. Wines were differentiated between northern Greece and southern Greece.

2.3.7. South African Wines

As early as the 1960's Zeeman & Butler quantified lead, copper and zinc in South African sweet wines using atomic absorption spectroscopy. Zeeman & Butler applied elementary statistics (mean, variance, standard deviation, coefficients of variations) to the elemental data of various wine samples. The coefficients of variations for Zn and Cu were 4.11% and 4.23% respectively. The minimum values for Cu and Zn were 0.08 mg/L and 0.23 mg/L, respectively. The maximum values were 2.5 mg/L and 3.88 mg/L, respectively. Cadmium concentrations in red and white table wines were analysed using atomic absorption spectroscopy by Zeeman & Du Plessis (1980). They found that the cadmium concentrations

were between 0.001 – 0.007 mg/L with the exception of two white wines, which exceeded 0.01 mg/L and one red wine with a concentration exceeding 0.015 mg/L. Zeeman & Du Plessis could not establish a relationship between cadmium concentrations and grape origin by means of simple statistics. Haswell & Walmsley (1998) determined the element content of South African Cabernet Sauvignon wines using X-ray fluorescence analysis. They found that K, Fe and Rb could be used to differentiate Australian, Bulgarian, Chilean, French, New Zealand, and South African wines using cluster analysis. It is uncertain why two decades passed before an in-depth investigation of elemental composition of South African wines was launched. Coetzee *et al.* (2005) quantified 40 elements in red and white young wines originating from Stellenbosch, Robertson and Swartland. Lithium, B, Mg, Al, Si, Cl, Sc, Mn, Ni, Ga, Se, Rb, Sr, Nb, Cs, Ba, La, W, Tl and U showed differences in their means across the three regions. Using stepwise discriminant analysis, Al, Mn, Rb, Ba, W and Ti were selected as the sub-set of variables that best discriminate between the three regions. Coetzee *et al*. also applied pair-wise discriminant analysis to the element data and discovered that Al, Mn, Rb, Ba, W, Se, Cs, Tl and Sr could also be used as classification parameters.

Capron *et al.* (2006) quantified macro and trace elements (ICP-MS), isotopic ratios (NMR), classical parameters (HPLC) and biogenic amines (GC) in Hungarian, Czech, Romanian and South African experimental and commercial wines in an attempt to identify the country of origin. Principal component analysis was initially applied to the data sets, where after classification and regression trees analysis, partial least squares-discriminant analysis and partial least square-uninformative variables elimination were applied. Capron *et al.* found South African samples, both experimental and commercial, formed clusters well separated from Hungary, Czech Republic and Romania when PCA is applied.

Only ethanol $(D/H)_1$, U, Y and wine $\delta^{18}O$ were selected for discrimination among the four countries.

2.3.8. Spanish Wines

Rías Baixas and non-Rías Baixas wines were successfully differentiated, using pattern recognition techniques, according to geographical origin and wine type, using Li and Rb as key elements (Latorre *et al.,* 1992). Latorre *et al.* (1994) quantified trace elements using ICP-AES and AAS in 42 wines from Rías Baixas, Galicia, Ribeiro and Valdeorras regions. Cluster analysis, PCA, LDA, *K*-NNA and soft independent modelling of class analogy (SIMCA), were applied to the element data. A classification success rate of between 90% and 100% was achieved using PCA. Lithium was dominant in the first principal component (PC1) and Rb dominated the second principal component (PC2). Linear discriminant analysis gave a 100% correct classification, whereas *K*-NNA and SIMCA gave 94% and 95%, respectively.

Baxter *et al.* (1997) quantified 48 trace elements in 112 red and white English and Spanish (originating from Somontano, Rioja and Cariñena regions) wines, using flow injection (FI), ICP-MS and thermal ionisation mass spectrometry (TIMS). A statistical package for social sciences (SPSS) was applied to the trace element data to establish certain trace element ratios. Canonical discriminant analysis was used to differentiate between regions using Cd, Cr, Cs, Er, Ga, Mn and ^{86}Sr. A classification success of 95% for Cariñene, 100% for Rioja and 84% for Somontano regions was achieved.

Peña *et al.* (1999) quantified trace elements in 39 red wines from Galicia by means of AAS. The element data were processed using PCA, DA and *K*-NNA to develop a typification for wine samples of Ribeira Sacra origin. Peña *et al.* were able to differentiate between Ribeiro and Valdeorras wines. Two distinct groups of sample were observed - Ribeira Sacra and non-Ribeira Sacra wines. Using Li

and Fe content as discriminant variables, satisfactory differentiation between the two regions was achieved. A classification success rate of between 76% and 95% was achieved between non-Ribeira and Ribeira wines using PCA. Iron, Co, Zn and Li were dominant variables in the first principal component (PC1) and Mn, Rb and Na were dominant variables in the second principal component (PC2). *K*-nearest neighbour analysis classified 87% of the samples correctly and LDA classified 91% of the samples correctly.

Rebolo *et al.* (2000) quantified the element content of 39 Galician red wines by means of AA. Cluster analysis, PCA, LDA, *K*-NNA and SIMCA were applied to the data sets. Lithium and Fe were dominant in the first PC. Zinc and Rb were dominant in the second PC. Iron, Li and Rb were selected as key features using stepwise Bayesian analysis (BASTEP). *K*-nearest neighbour analysis classified 82% correct (K = 3). Soft independent modelling of class analogy obtained a classification success rate of 87%.

Pérez-Magariño *et al*. (2002) analysed the element content in 71 rosé wines from Ribeira del Duero, Rioja, Valdepeñas and La Mancha regions, using AAS. Stepwise discriminant analysis and LDA were applied to the chemical data sets. Calcium, Fe, Zn and Na were selected as discriminatory variables. The best discriminatory variable for differentiation between the three regions was Ca, with an 85% classification success.

Condo *et al.* (2002) quantified K, Na, Ca, Mg, Fe, Cu, Zn, Mn and Sr by means of FAAS and Li and Rb by means of flame atomic emission spectroscopy in 125 white and rosé wines originating from the Valle de Gűimar, Valle de La Orotava, Tacoronte-Acentejo and Ycoden-Daute-Isora regions. Principal component analysis and SDA were used to differentiate between wines according to variety and origin. A classification rate of 63% was achieved using PCA and a 90% classification rate was achieved using LDA and SDA.

Frias *et al.* (2003) measured 11 elements in 45 dry and sweet wines from the Canary Islands, using FAAS, flame atomic emission spectroscopy and ICP-AES. Frias *et al.* found significant differences between dry and sweet wines from La Palma Island in all the analysed elements with the exception of Na, Fe and Rb. No significant differences in element composition were found between sweet and dry wines from Lanzarote Island, with the exception of Sr. Significant differences in mean content were found between the two islands, with the exception of Fe and Cu. Cluster analysis (CA) and PCA showed differences between wines according to the island of origin. It was established that Rb, Na, Sr and Li had the best discriminatory powers. A classification success of 100% was achieved using CA, PCA (PC1), with Rb, Li, Na, Sr and Mn as variables and K and Mg as variables (PC2). *K*-nearest neighbour analysis and SIMCA, using Sr, Mn, Mg, Zn, Li, Rb as variables and LDA, using Rb, Na, Mn, Sr as variables. Linear discriminant analysis, using Rb, Na, Mn and Sr as the discriminatory variables, showed 100% recognition ability and 95% prediction ability.

Table 2.5 lists some of the most frequently used elements for wine differentiation according to region of origin with multivariate statistical methods.

TABLE 2.5
Most frequently used element variables with multivariate methods for wine origin differentiation

Wine origin	**Elements**	**Multivariate method**	**Reference**
America	Ca, K, Na, Mn (Regional differentiation)	PCA, *K*-NNA	Li & Hardy, 1999
Australia	K, Na, Ca	MLRA, PLS	Sauvage *et al.*, 2002
Canada	Mg, P, S, Cl, Ti, Br, Mo, Ba (variety differentiation)	MDS, DA, CA	Greenough *et al.*, 1997

	Al, V, Co, Cu, As, Cd (Vineyard differentiation)		
	Sr, Rb, Mn, U, Al, V, Zn, Mo, Sb, Co (Vineyard differentiation)	PCA, DA	Taylor *et al.,* 2003
France	Rb, Mg, Li	PCA, SDA	Etiévant *et al.,* 1988
	Zn, Ca, Sr, Mg	ANOVA	Day *et al.,* 1994
	Ba, K, Mg, Mn, Sr, Al, Fe, Zn (Grape must)	PCA, CDA	Day *et al.,* 1995
	Li, Ba, Ga, Sr (Wine & grape)	ANOVA, LSD	Martin *et al.,* 1999
Germany	Li, Rb, Fe, Na	PCA, LDA, *K*-NNA	Maarse *et al.,* 1987
	Ba, Si	CA, ANOVA, PBA, SDA	Danzer *et al.,* 1999
	Li, B, Mg, Fe, Zn, Sr, Cs, Pb	QDA	Castiñeira-Gómez *et al.,* 2004
	As, Be, Cs, Li, Mg, Pb, Si, Sn, Sr, Ti, W, Y	PCA, CA	Thiel *et al.,* 2004
Greece	K, Na, Ca, Mg, Zn, Mn, Fe, Cu (Regional differentiation)	PCA	Kallithraka *et al.,* 2001
South Africa	K, Fe, Rb	PCA, CA	Haswell & Walmsley, 1998

	Al, Mn, Rb, Ba, W, T, , Al, Mn, Rb, Ba, W, Se, Cs, Tl, Sr	SDA, PDA	Coetzee *et al*., 2005
	U, Y	PCA, PLS-DA	Capron *et al*., 2006
Spain	Li, Rb	PCA, CA, LDA, SIMCA, *K*-NNA	Latorre *et al.,* 1994
	Cd, Cr, Cs, Er, Ga, Mn, Sr	SPSS, CDA	Baxter *et al.,* 1997
	Co, Zn, Li, Fe, Mn, Rb, Na	PCA, DA, *K*-NNA	Peña *et al.,* 1999.
	Rb, Na, Sr, Li, Mn, Mg, Zn	CA, PCA, SIMCA, *K*-NNA	Frias *et al.,* 2003

2.4. THE THIRD DOMAIN – LITERATURE DEALING WITH THE SOUTH AFRICAN WINE INDUSTRY

In this section, the history of South African wine will be briefly highlighted. The types of wine produced will also be mentioned and the statistics on wine production in South Africa will be discussed. The scheme of origin and wine certification with the emphasis on "Wine of Origin Scheme" will be discussed. The ten most popular wine grape varieties are considered followed by a discussion on the wine regions, districts and wards. Lastly, some important annual events in the wine industry and important wine organisations will be mentioned.

2.4.1. Overview of the South African Wine Industry

According to Kench *et al.* (1983), the establishment of the first vineyards in South Africa coincided with the arrival of the European settlers. Commander Jan van Riebeeck of the Dutch East India Company planted the first vines in 1655, three years after his arrival in Table Bay. He pressed the first wine at the Cape on 2nd February 1659 from cuttings imported from France. In 1659, he wrote his famous

report, which read, “Today, praise the Lord, wine was pressed from Cape grapes for the first time.” Governor Simon van der Stel succeeded Van Riebeeck and firmly established the wine industry in the Cape. In 1685, Simon van der Stel established a wine estate on the lower slopes of Table Mountain. It was here that he built a model farm, which he named Constantia.

In 1688, two hundred French Huguenots arrived in South Africa, bringing their wine culture with them to the Cape. Their arrival further enhanced the already thriving wine industry as they expanded viticulture beyond the boundaries of the Cape Peninsula by introducing wine farms to the Stellenbosch and Franschhoek areas. Today, many viticulture scholars continue to consider these areas as influential in the making of quality wine.

During the 18th Century, Constantia’s famous dessert wines contributed to the establishment of the Cape as an important wine-producing region. In the meantime, the Stellenbosch area developed into a centre of viticulture interest. Unfortunately, however, by the end of the 19th century the establishment of vineyards and the production of wine declined significantly because of a microscopic plant aphid infestation called *Phylloxera.* As in Europe, *Phylloxera* had taken its toll on the South African wine industry. In South Africa, steps were taken to control production and the market with the introduction of a large farmers’ co-operative. In light of this, the Ko-operatiewe Wijnbouwers Vereniging van Zuid-Afrika, Beperk, better know as KWV, was established in 1918 followed by the founding of the Stellenbosch Farmers’ Winery in 1925. South Africa's most valuable contribution to the history of the *Vitis vinifera* vine was the successful crossing of Pinot noir and “Hermitage” grape variety by Professor Al Perold in 1925. The new variety was subsequently named Pinotage, much later it became evident that what the locals had previously considered to be Hermitage, was in fact Cinsaut noir. However, the widely accepted name Pinotage remained unaffected (South African Wine Industry Directory, 2006/2007).

CHAPTER 3

A REVIEW ON INSTRUMENTAL TECHNIQUES

3.1. INTRODUCTION

Chapter 3 serves as a comparative study among atomic absorption spectroscopy, inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Thermal ionisation mass spectrometry, X-ray fluorescence and neutron activation methods are also discussed.

According to Skoog & Leary (1992), spectroscopy is the use of absorption, emission or fluorescence spectra of electromagnetic radiation by atoms to study atoms or molecules or physical processes. An absorption spectrum is the absorption of light as a function of wavelength. The spectrum of an atom or molecule depends on its energy level structure. The two regions of the spectrum, which yield atomic information, are the ultra-violet-visible region and the X-Ray region.

Ultra-violet or visible atomic spectra are obtained by converting the chemical components of a sample into gaseous atoms by suitable heat treatment. The emission, absorption or fluorescence of the resulting gaseous mixture serves as quantitative and qualitative determinations of one or more of the elements present in the sample. The process by which the sample converts firstly into a fine mist or aerosol and then into a gas of free atoms, is called atomisation. There are generally six methods of sample atomisation, namely flame atomisation, inductively- coupled argon plasma atomisation, direct-current argon plasma atomisation, electro-thermal atomisation, electric-arc atomisation and electric-spark atomisation (Alkemade & Hermann, 1979). Only flame, inductively-coupled argon plasma and electro-thermal atomisation methods are discussed because they are applicable to this study.

3.2. SAMPLE TREATMENT, NEBULISATION AND ATOMISATION

This section will focus on sample treatment, nebulisation and atomisation, since they are the initial steps that need to be taken prior to the analysis of samples by atomic absorption spectroscopy.

3.2.1. Sample Treatment

Before samples are nebulised and atomised, samples are first decomposed and converted into an aqueous solution. More popular methods used by Kment *et al*. (2005) and Capron *et al.* (2006) for sample decomposition are treatment with hot mineral acids and oxidation with liquid reagents such as sulphuric, nitric or perchloric acid. This is generally referred to as wet ashing. Combustion in an oxygen chamber, ashing at high temperature and high-temperature fusion reagents such as boric oxide, sodium carbonate, sodium peroxide or potassium pyrosulphate are used by Aceto *et al.* (2002) and Frias *et al.* (2003) for decomposition purposes. Refer to Section 3.7 for details on wine treatment and decomposition.

3.2.2. Sample Nebulisation

Once the sample is in solution, it is sprayed into a flame using a nebuliser. The nebuliser converts the sample solution into an aerosol, mist or vapour, which consists of tiny droplets. The droplets feed into a flame through a burner. Nebulisation usually leads to a sample mixture of atoms, ions and molecules formed by the fuel, oxidant and sample.

The more common types of nebulisers are the concentric tube nebuliser or pneumatic type nebuliser as shown in Figure 3.1. In these nebulisers, the liquid sample is sucked through a capillary tube by a high-pressure stream of gas, flowing around the tip of the tube. Cross-flow nebulisers are also used in spectroscopy for sample nebulisation. In cross-flow nebulisers, the high-pressure gas flows across a capillary tip at right angles. This process of liquid transport is

called aspiration. The high velocity gas breaks the liquid up into fine droplets of various sizes and carries it into the flame or atomiser (Bock, 1979).

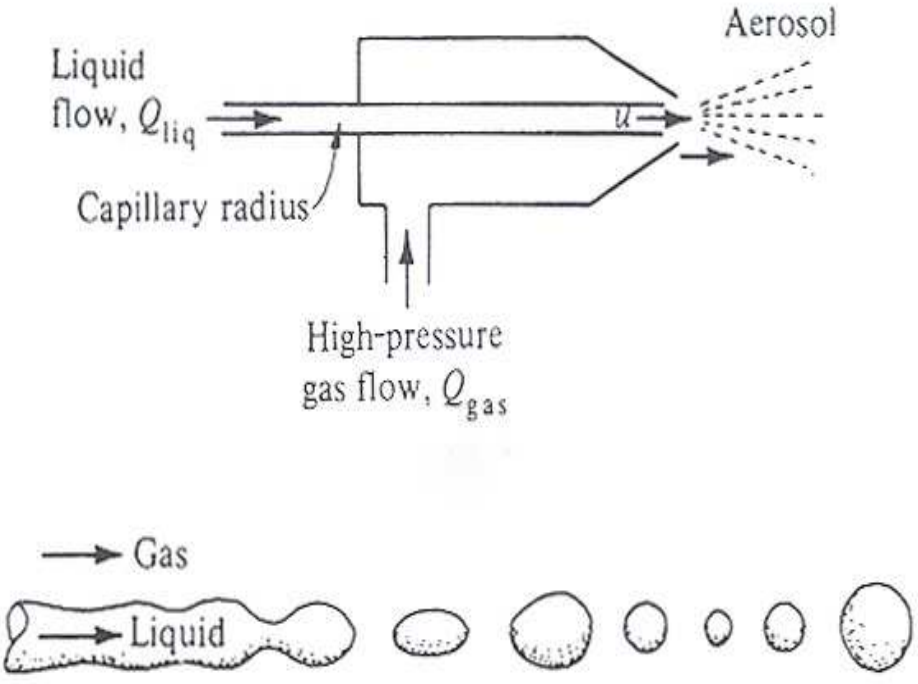


FIGURE 3.1

A Concentric Tube or Pneumatic Nebuliser (Willard *et al.,* 1988)

3.2.3. Sample Atomisation

There are two types of sample atomisers, namely continuous and discrete atomisers. In continuous atomisers, which include flame atomisation and inductively-coupled argon plasma atomisation, the sample continuously feeds into the atomiser at a constant rate. The spectral signal is therefore constant with time. In discrete atomisers, which include electro-thermal atomisers, a measured quantity of a liquid or solid sample is introduced into the atomiser. The spectral

signal rises to a maximum then decreases to zero as the atomic vapour is carried out of the heated region (Alkemade & Hermann, 1979).

3.2.3.1. Flame Atomisers

In flame atomisation, a sample solution is sprayed into a flame by means of a nebuliser. Flame atomisers are used for emission, absorption or fluorescence measurement spectroscopy.

3.2.3.2. Electro-Thermal Atomisers

According to Fuller (1978), electro-thermal atomisers generally provide better sensitivity than flame atomisers, because the entire sample is atomised in a short period and the average residence time of the atoms in the optical path is usually more than one second. Electro-thermal atomisers are used for atomic absorption and atomic fluorescence measurements, but have not been generally applied for emission spectroscopy (Skoog & Leary, 1992). Electro-thermal atomisers have also been used for atomising samples for inductively-coupled plasma emission spectroscopy in the determination of element composition (Skoog & Leary, 1992). In electro-thermal atomisers, the sample is pipetted directly into the furnace for ashing and atomisation. Solid samples are weighed directly into a cup-type atomiser and are introduced into the furnace. Further detail on electro-thermal atomic absorption spectroscopy will follow in Section 3.6.

3.3. ATOMIC EMISSION AND ABSORPTION SPECTRA

According to Willard *et al.* (1988), when a sample is atomised, a substantial fraction of the sample constituents are reduced to gaseous atoms and only a certain fraction is ionised, resulting in a gaseous mixture of atoms and ions. The emission, absorption or fluorescence spectra of these gaseous atomic particles consist of well-defined narrow lines arising from electronic transitions of the outermost electrons. At room temperature, most of the atoms of a sample are in the ground state. For example, the single outer electron of metallic sodium occupies the 3*s* orbital. The heat of a flame or an electric arc or electric spark

brings about the excitation of this electron to a higher orbital. When the electron returns to the ground state, it emits a photon of radiation. In a hot gaseous medium, sodium atoms are also capable of absorbing radiation of wavelengths characteristic of electronic transitions from, for example, the 3*s* orbital, ground state, to the higher 3*p* and *4p* orbital, excited states (Harvey, 1950).

3.4. ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Flame AAS is predominantly a single-element technique that uses a flame to generate ground-state atoms. When light passes through an atom mist, specific wavelengths of light are absorbed which are characteristic of the presence of the metal of interest. The amount of light absorbed indicates the amount of analyte present (Tyler, 1991). Atomic absorption principles and procedures will be discussed following Section 3.4.

Atomic absorption spectroscopy may be divided into two processes, namely the production of free atoms and the absorption of radiation from an external source by these atoms. Sample treatment and the conversion of sample analyte into free atoms are discussed in Section 3.2.

3.4.1. Radiation Sources for Atomic Absorption Methods

The two types of radiation sources used in atomic absorption spectroscopy are the hollow-cathode lamp and the electrode-less discharge lamp.

3.4.1.1. The Hollow-Cathode Lamp

According to Caroli (1985), the most common radiation source for atomic absorption measurements is the hollow-cathode lamp. Hollow-cathode lamps get their name from the cup-shaped cathode, which is made from the element of interest. This type of lamp consists of a tungsten anode and a cylindrical cathode sealed in a glass tube. The tube is filled with inert neon or argon gas at a pressure of between 1 – 5 torr. Hollow-cathode lamps are types of discharge lamps that produce narrow emission lines from atomic species. The electric

discharge ionises rare gas atoms, which accelerates into the cathode and sputter the metal atoms into the gas phase. Collisions with gas atoms or electrons excite the metal atoms to higher energy levels, which then decay to lower levels by emitting light. Figure 3.2 depicts a schematic diagram of a typical hollow-cathode lamp (Caroli, 1985).

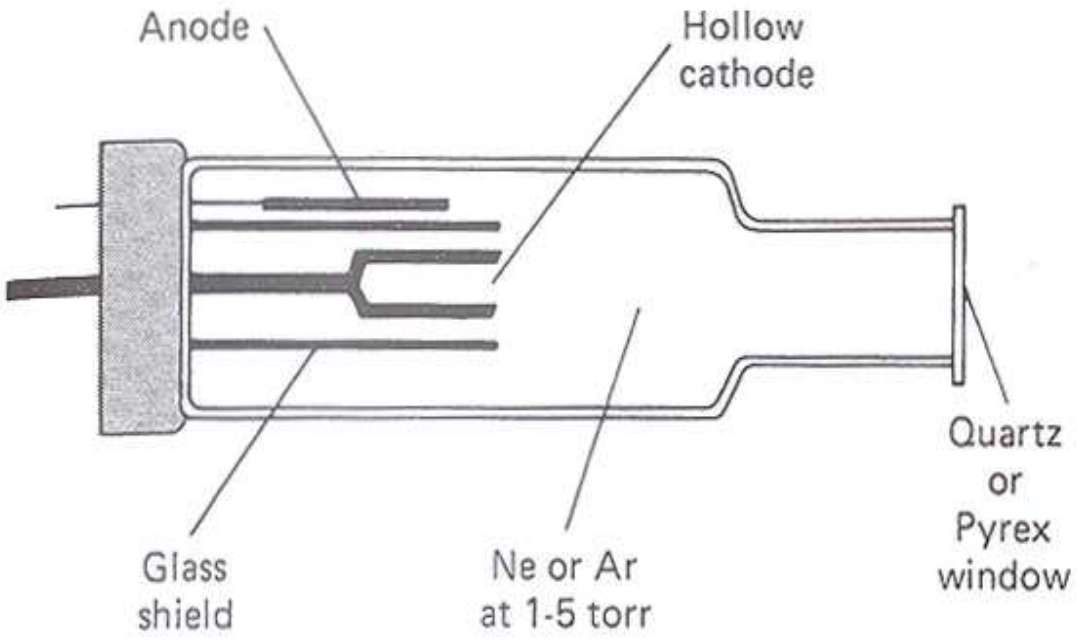


FIGURE 3.2

Schematic Diagram of a Hollow-Cathode Lamp (Skoog & Leary, 1992)

3.4.1.2. The Electrode-Less Discharge Lamp

Electrode-less discharge lamps are useful sources of atomic line spectra and provide radiant intensities that are normally greater than the hollow-cathode lamps' intensities. A typical lamp is constructed of a sealed quartz tube containing an inert gas such as argon under pressure. The cathode tube also contains a small quantity of the metal or salt to produce the required atomic spectrum (Caroli, 1985).

3.4.2. Spectrophotometers Used for Atomic Absorption Spectroscopy

According to Willard *et al.* (1988), there are generally two types of spectrophotometers used for atomic absorption, namely single-beam and double-beam spectrophotometers. A single-beam spectrophotometer (Figure 3.3a) consists of several hollow-cathode sources, a pulsed power supply, an atomiser and a single grating spectrometer with a photomultiplier transducer. A double-beam spectrophotometer (Figure 3.3b) also consists of several cathode lamps but a mirrored splitter splits the beam from the hollow-cathode source, so that one-half of the beam passes through the flame and the other half of the beam passes around the flame. The two beams merge by means of a half-silvered mirror and then pass into a grating monochromator.

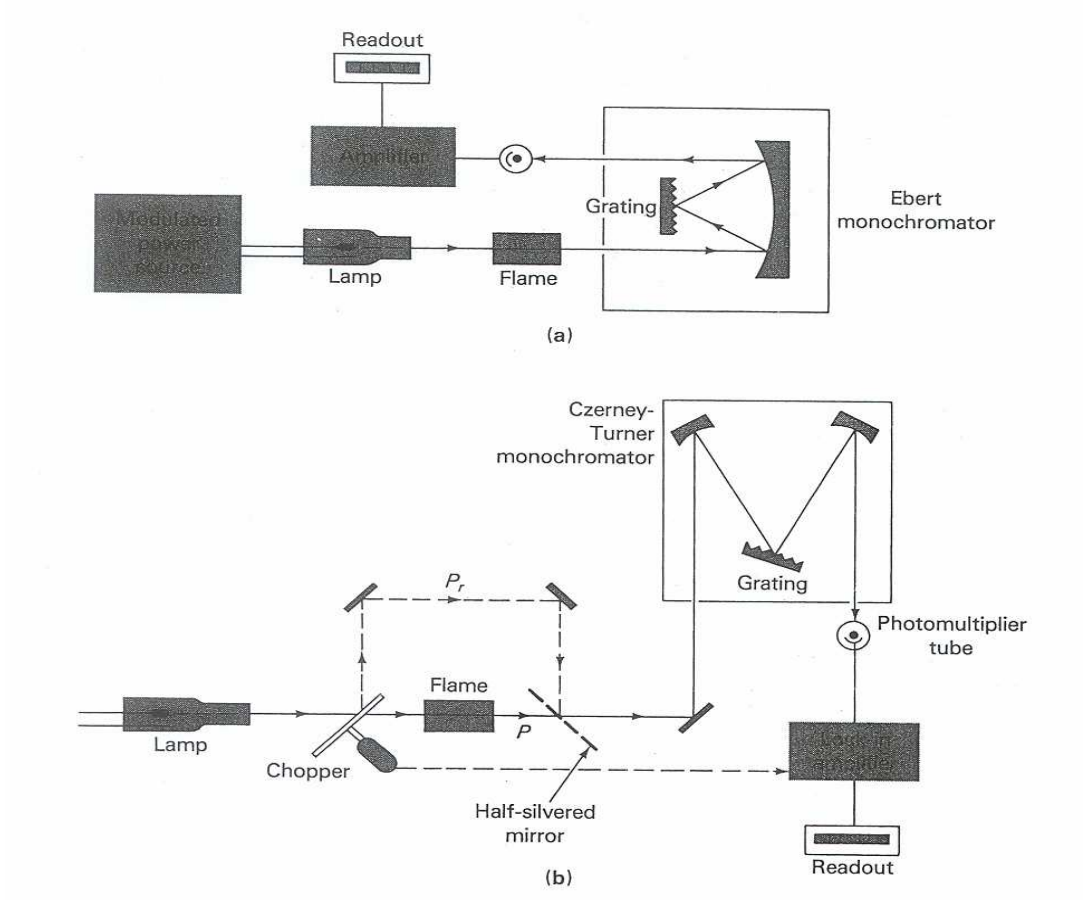


FIGURE 3.3
Schematic Diagram of a Single-beam (a) and Double-beam (b) Spectrometer (Skoog & Leary, 1992)

3.5. ATOMIC EMISSION SPECTROSCOPY (AES)

In (flame) atomic emission spectroscopy, the sample is introduced into a low-temperature flame where it is desolvated, vaporised and atomised. Subsequently, the atoms rise to the excited state via thermal collisions with the constituents of the partially burnt flamed gases. When the atoms return to a lower electronic state, the excited atoms emit radiation characteristic of the sample components. A low-temperature flame eliminates excitation of most other metals of non-interest, resulting in a simple spectrum. The emitted radiation passes through a monochromator that isolates the specific wavelength for the desired analysis and a photo detector measures the radiant power of the selected radiation (Dean & Rains, 1969).

3.5.1. Sample Treatment and Sample Introduction

Sample treatment for flame emission spectroscopy is similar to that of atomic absorption spectroscopy. Refer to Section 3.2 for discussion on sample treatment.

3.5.2. Instrumentation

Instruments used for flame emission spectroscopy are similar in design to those used in flame absorption spectroscopy, except that the flame now serves as the radiation source instead of the hollow-cathode lamps or electrode-less discharge lamps. There are generally three types of instruments used for emission spectroscopy namely Spectrophotometers, Photometers and Instruments for Simultaneous Multi-element Analysis. Current instruments (2007) are adaptable to either emission or absorption measurements

3.6. GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROSCOPY (GFAAS)

Graphite furnace AAS is a single-element technique, although some multi-element instruments are available. Graphite furnace AAS is a modification of

flame atomic absorption spectroscopy and works on precisely the same principle as flame AAS, except that a small heated graphite tube to generate ground-state atoms replaces the flame. Since the ground state-atoms are concentrated in a smaller area than the flame, greater light absorption takes place resulting in detection limits approximately 100 times lower than those of flame AAS.

Graphite furnace atomic absorption spectroscopy, also referred to as electro-thermal atomic absorption spectroscopy is normally of the discrete type, in which a measured volume of a solution is deposited in a small graphite tube, which can be heated to vaporise and atomise the analyte. The temperature is increased significantly, so that atomisation steps occur over a brief period (Fuller *et al*, 1989). See also section 3.2.3.2.

3.6.1. Sample Treatment and Sample Introduction

Sample treatment and introduction for GFAAS is similar to that of flame AA. Refer to Section 3.2 for a discussion on sample treatment and sample introduction.

3.6.2. Instrumentation

A graphite atomiser consists of a hollow graphite cylinder positioned in such a manner that the radiation from the external source, the hollow cathode-tube, passes through the centre of the cylinder. The interior of the cylinder is coated with pyrolytic graphite. Electrodes at the end of the cylinder are connected to a low-voltage, high-current power supply.

The inert gas, usually argon, enters the graphite cylinder at both ends ensuring the removal of vaporised matrix components, which takes place during the ashing step. Figure 3.4 demonstrates a cross-section of a graphite atomiser (Fuller, 1989).

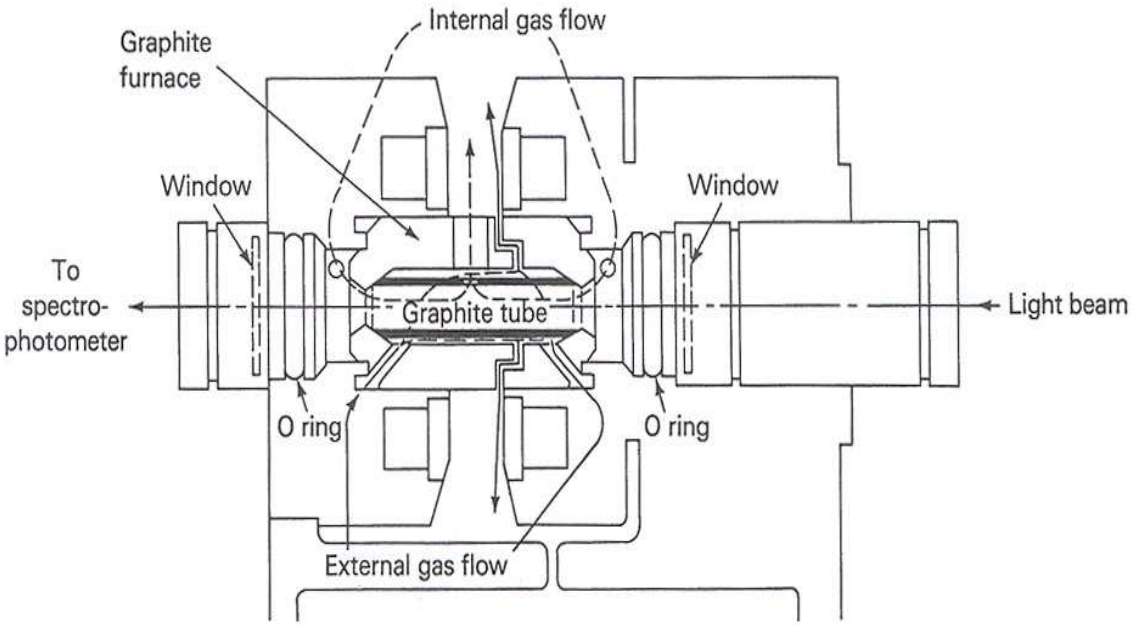


FIGURE 3.4

Cross Section of a Graphite Atomiser (Perkin-Elmer Corporation, 1995)

3.6.3. Detection Limits for Flame AA and GFAAS

Refer to Table 3.1 for discussion, which focuses on detection limits for flame emission and absorption spectroscopy as well as furnace atomic absorption spectroscopy.

3.7. WINE TREATMENT

Wine samples for major element determination are usually analysed without any decomposition steps. However, the wine is stabilised prior to analysis by adding 1 ml of concentrated hydrochloric acid, perchloric acid or nitric acid solution to 50 ml of undiluted wine (Peña *et al*., 1999; Aceto *et al*., 2002; Conde *et al*., 2002; Frías *et al*., 2003).

Acidified wine can also be evaporated to dryness over heat prior to analysis. The wine residue is re-dissolved in 5 – 10 ml distilled water prior to analysis (Galani-Nikolakaki *et al*., 2002; Kment *et al*., 2005; Lara *et al*., 2005).

During the determination of trace and minor elements, however, wine samples are first decomposed. In decomposition, 1 – 2 ml concentrated nitric acid (63%) and 1 ml hydrogen peroxide (40%) or concentrated perchloric acid (60%) are added to 5 ml of wine. The suspension is heated for three hours at 150 °C in a Teflon vessel or the suspension can be decomposed in a microwave oven.

3.7.1. Elements Quantified in Wine Samples

Buldini *et al.* (1998), Aceto *et al.* (2002), Frías *et al.* (2003) and Kment *et al.* (2005) have successfully quantified Na, K, Ca, Mg, Mn, Cu, Zn, Sr and Fe in wine using AAS. The metals Li and Rb are usually quantified using AES (Peña *et al.,* 1999; Aceto *et al*., 2002; Conde *et al.,* 2002; Frías *et al.,* 2003).

Buldini *et al.* (1999) quantified Zn, Ni, Co, Fe and Cu in wine using graphite furnace AAS. Graphite furnace AAS was also used to quantify As, Cd, Cr, Hg and Pb (Aceto *et al.,* 2002).

ATOMIC EMISSION SPECTROSCOPY BASED ON PLASMA SOURCES

3.8. INTRODUCTION

Plasma is an electrical conducting gaseous mixture containing a large concentration of cations and electrons. The concentrations of the cations and electrons are such that the net charge tends to zero. In the argon plasma, which is employed for emission analyses, argon ions and electrons are the principal conducting species. Cations from the sample will also be present, but to a lesser degree. Argon ions, once formed in the plasma, are capable of absorbing sufficient power from an external source to maintain the temperature at a level at which further ionisation sustains the plasma indefinitely. Three power sources have been employed in argon plasma spectroscopy namely, direct-current

electrical source, inductively-coupled plasma or radio-frequency fields and microwave-frequency fields (Slavin, 1989). Only inductively-coupled plasma sources are dealt with in this study.

3.9. THE INDUCTIVELY-COUPLED PLASMA SOURCE

Inductively-coupled plasma emission is a multi-element technique that uses an extremely hot plasma source to excite atoms to the point that they emit wavelength-specific photons of light characteristic of a particular element. The number of photons produced is directly related to the concentration of that element in the sample (Tyler, 1991). Figure 3.5 shows a schematic representation of an inductively-coupled plasma source referred to as a torch. The torch consists of three concentric quartz tubes. A stream of argon flows through the tubes, normally at a flow rate of between 11 - 17 litres per minute. A water-cooled induction coil is assembled around the top of the tubes, which is powered by a radio-frequency generator. A spark from a Tesla coil, also called a resonant transformer, initiates ionisation of the flowing argon gas. The resulting ions and electrons, interact with the fluctuating magnetic field (labelled H in Figure 3.5) produced by the induction coil. This interaction causes the ions and electrons within the coil to flow in a closed circular path. Due to resistance to movement of the ions and electrons, heat is generated. The plasma temperature is high enough to melt quartz and requires thermal isolation from the outer quartz cylinder. This isolation is achieved by the flowing of argon around the walls of the tube as indicated by the arrows in Figure 3.5. The argon flow centralises and stabilises the plasma. This characteristic of high-frequency inductively-coupled plasma facilitates the introduction of the sample in aerosol form through the inner tube of the torch. The inductively-coupled plasma takes on a toroidal shape. This toroidal plasma shaping lengthens the resident time of the sample in the interior high-temperature zone of the plasma and improves the detection limits for many elements. A long, well defined plasma extension emerges from the high-temperature plasma on the tip of the torch. This plasma extension contains all the analyte atoms and ions that have been excited by the heat of the plasma.

The area used for analysis falls just above the tip of the primary plasma cone and just below the base of the flame-like after-glow (Thompson & Walsh, 1989; Olesik, 1991).

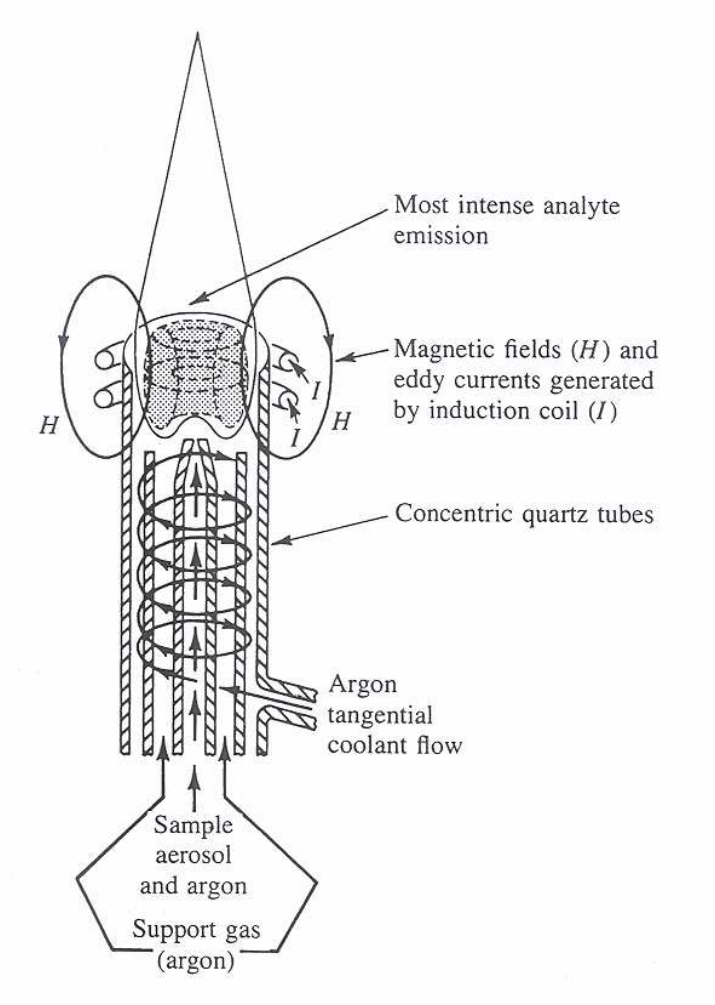


FIGURE 3.5

A typical Inductively-Coupled Plasma (American Association for the Advancement of Science)

3.9.1. Sample Preparation, Introduction and Atomisation

3.9.1.1. Sample Preparation

The sample preparation is similar to that described for atomic absorption and emission spectroscopy. Decomposition methods such as microwave, high-pressure fusion and acid decomposition are employed for sample preparation of solid and aqueous samples.

3.9.1.2. Sample Introduction

The sample is carried into the hot plasma at the head of the tubes by the argon that flows through the central quartz tube. The sample may be an aerosol, a thermally or spark-generated vapour or a fine powder. The apparatus used for sample injection is similar in construction to the nebuliser employed for flame methods. Figure 3.6 shows a typical example of a nebuliser used for inductively-coupled plasma spectroscopy. The sample is nebulised by the flow of argon and the fine droplets are carried into the plasma by the argon flow. Another method of introducing liquid and solid samples into the plasma is by electro-thermal vaporisation. Here the sample is vaporised in a furnace. In plasma applications, however, the furnace is used for sample introduction rather than sample atomisation (Browner & Boorn, 1984).

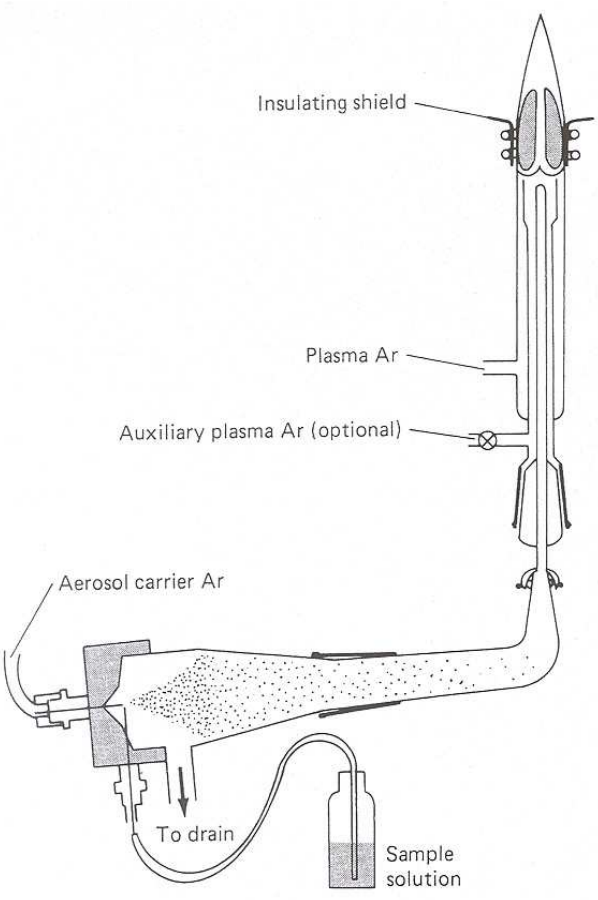


FIGURE 3.6

A Typical Nebuliser for Sample Injection into a Plasma Source (American Association for the Advancement of Science)

3.9.1.3. Sample Atomisation

Figure 3.7 shows temperatures at various parts of the plasma. When the sample atoms reach the observation point, which is just above the plasma, the atoms reside for approximately two milliseconds at temperatures ranging from 4000 – 8000 degrees Kelvin. These residing times and temperatures are about two to three times greater than the temperatures found in the hottest combustion flames employed in flame methods. Atomisation is therefore more complete and fewer chemical interference problems are encountered (Fassel, 1978).

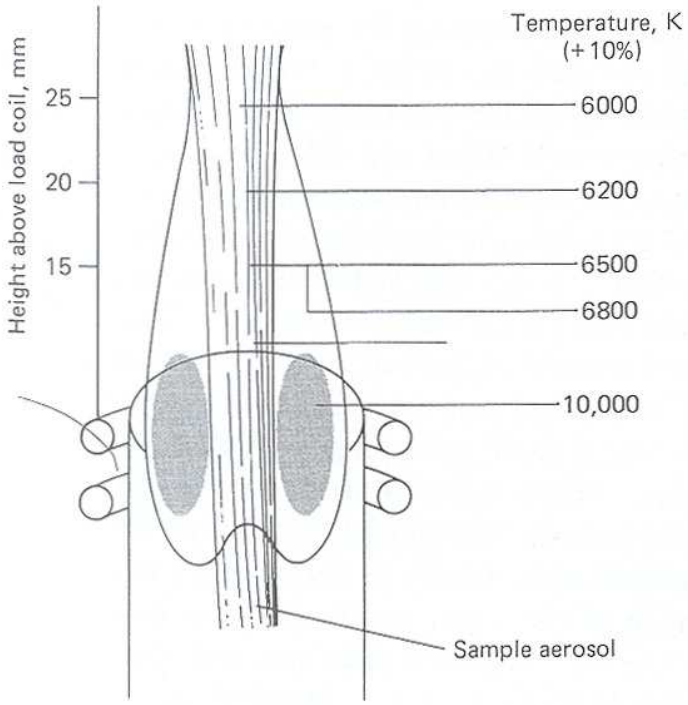


FIGURE 3.7

Temperatures in a Typical Inductively Plasma Source (V. A. Fassel, Science, 1978).

3.9.2. Instrumentation

There are two types of plasma emission instrument according to Browner & Broon (1984), which are used with plasma emission sources. They are sequential and simultaneous multiple-channel instruments. Sequential

instruments are less complex and less expensive than simultaneous multi-channel instruments. Simultaneous instruments measure line intensities on a one-by-one basis. Sequential instruments are programmed to move from the line for one element to that of a second element, pausing long enough (a few seconds) at each element to obtain a satisfactory signal-to-noise ratio. Multiple-channel instruments are designed to measure the intensities of emission lines for a large number of elements simultaneously.

3.9.2.1. Sequential Instruments

Figure 3.8 represents an optical diagram of a sequential instrument, which is used either for emission analyses with an inductively-coupled plasma source or for atomic absorption analyses with a flame or graphite furnace atomiser.

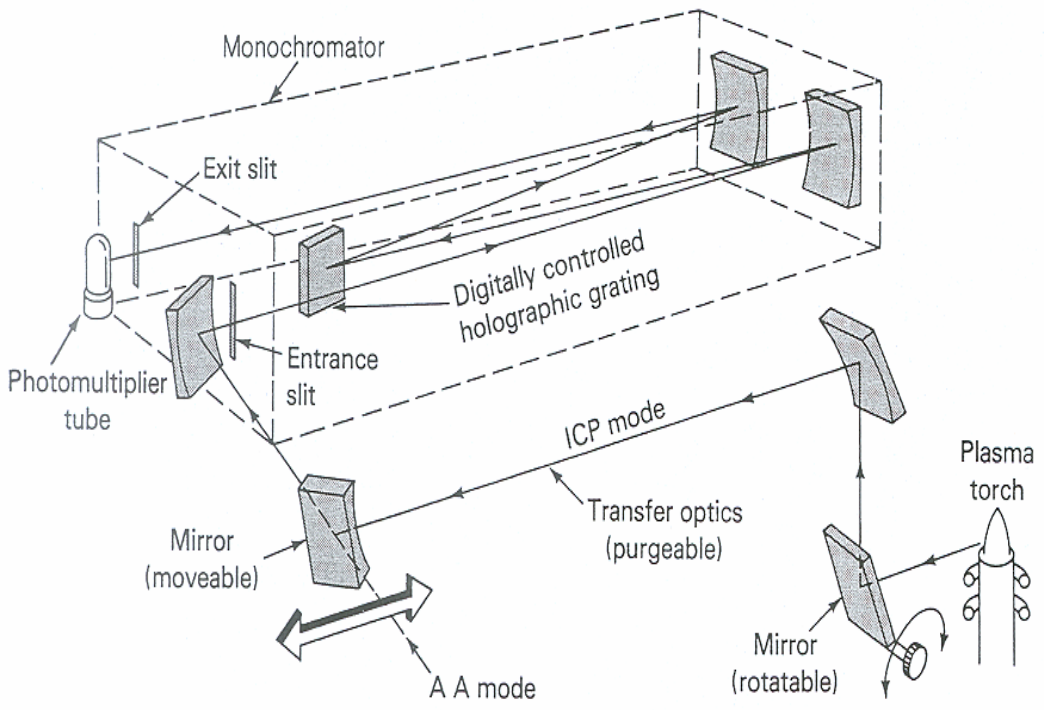


FIGURE 3.8

A Sequential Spectrometer for ICP Emission and Atomic Absorption Spectroscopy (Perkin-Elmer Corporation, 1995)

3.9.2.2. Multiple-Channel Instruments

Figure 3.9 depicts a typical multiple-channel spectrometer. The entrance slit, exit slit and grating surface are located along the circumference of a Rowland Circle. The Rowland Circle is a curvature that corresponds to the focal curve of the concave grating. Mirrors reflect the radiation from the several fixed slits to photomultiplier tubes. The slits are fixed by the manufacturer to transmit lines for elements chosen by the operator. In this instrument, the pattern of lines can be changed to accommodate new elements or delete others. Spectrometers as the one shown in Figure 3.9 may be used with plasma, arc or spark sources (Skoog & Leary, 1992).

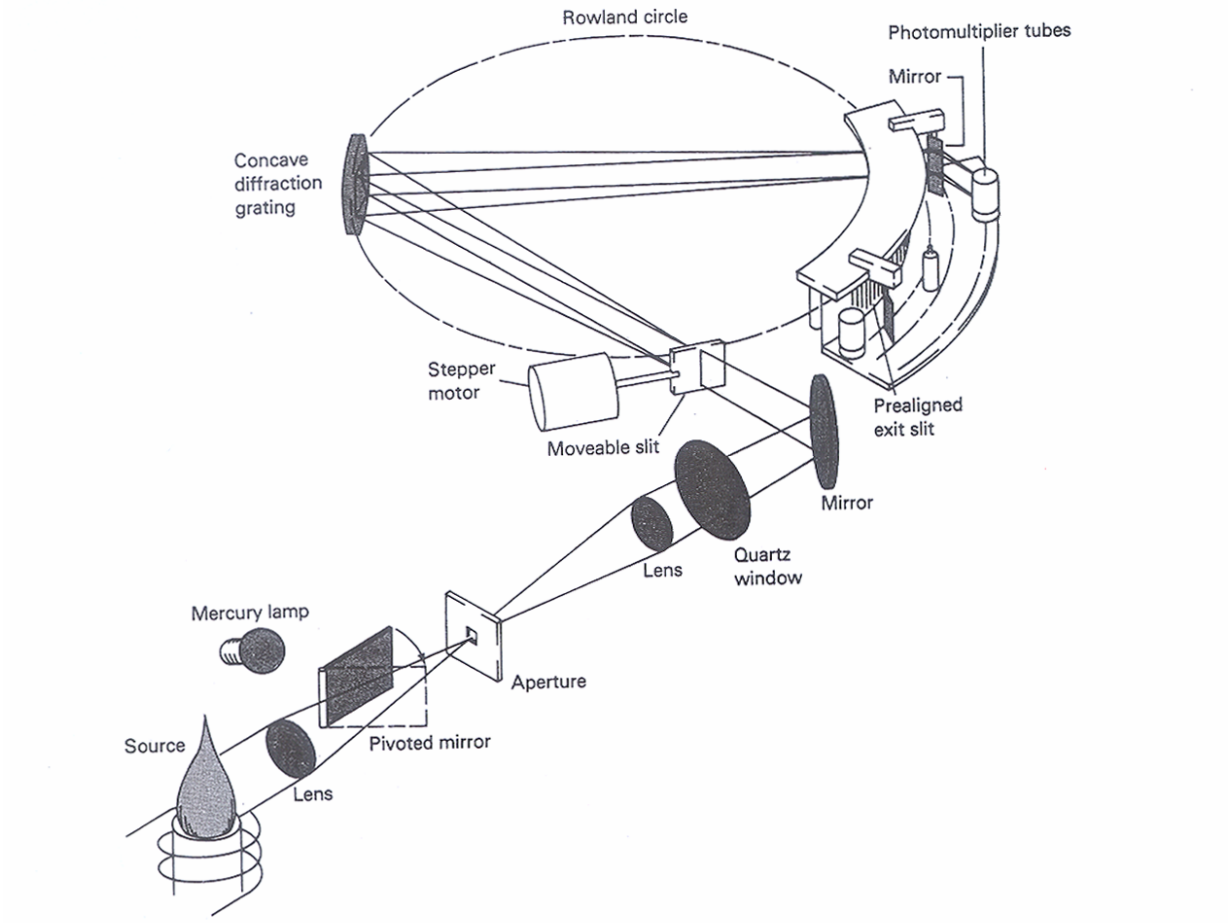


FIGURE 3.9

Multiple Channel Spectrometers Based on Rowland Circle Optics (Skoog & Leary, 1992)

3.9.3. Detection Limits

Modern ICP-AES instruments (2007) are available in radial and axial configurations, which can cover the range of mg/L to µg/L detection limits. The radial configuration uses a traditional vertical plasma source in which the emitting light is viewed from the side, whereas the axial configuration uses horizontal plasma, in which the light is viewed directly down the centre. The benefit of the axial design is that the detector "sees" more photons and as a result, it offers 5 – 10 times lower detection limits than the radial configuration. According to Tyler (2001) in comparison to flame AA, furnace AA and ICP-AES, the best detection limits are obtained with furnace AA. However, conventional or radial configuration ICP-OES and flame AAS offer very good detection limits for most elements, but generally not as low as GFAAS. Table 3.1 lists the comparative detection limits among ICP-AES, flame AA and furnace AA in µg/L and picograms for furnace AAS.

TABLE 3.1

Comparison of detection limits for ICP-AES, flame AAS and furnace AAS of concentrations in micrograms per litre. Furnace AAS detection limits are reported in picograms (Geoff Tyler, Varian Australia, 2001).

	ICP-AES	**Flame AA**	**Furnace AA**	
***Element**	**Detection limit in µg/L**	**Detection limit in µg/L**	****Concentration in µg/L**	**Mass in Picogram/L**
Silver	3	2	0.035	0.7
Aluminium	1.5	30	0.25	5
Arsenic	12	300	0.5	10
Boron	1.5	500	43	855
Barium	0.07	20	0.85	17
Beryllium	0.2	1	0.025	0.5
Bismuth	12	50	0.45	9
Bromine	6000	N D	N D	N D
Carbon	65	N D	N D	N D
Calcium	0.03	1	0.03	0.6
Cadmium	1.5	2	0.01	0.2

Chlorine	200 000	N D	N D	N D
Cobalt	5	5	0.21	4.2
Cesium	3200	4	0.55	11
Chromium	4	6	0.075	1.5
Copper	2	3	0.3	6
Iron	1.5	6	0.06	1.2
Gallium	6.5	100	0.23	4.5
Iodine	60	N D	N D	N D
Indium	18	40	0.35	7.0
Potassium	10	3	0.02	0.4
Lanthanum	0.02	2000	N D	N D
Lithium	0.6	2	0.2	4
Magnesium	0.1	0.3	0.01	0.2
Manganese	0.3	2	0.03	0.6
Molybdenum	4	20	0.35	7
Nitrogen	50 000	N D	N D	N D
Sodium	1	0.2	0.005	0.1
Niobium	4	2000	N D	N D
Nickel	5.5	10	0.24	4.8
Lead	14	10	0.28	5.5
Palladium	7	10	0.43	8.6
Rubidium	35	10	0.05	1
Platinum	20	100	3.5	70
Sulphur	20	N D	N D	N D
Antimony	18	40	0.5	10
Selenium	37	500	0.7	14
Silicon	5	300	0.75	15
Tin	15	100	0.5	10
Strontium	0.02	2	0.1	2
Tantalum	9	2000	N D	N D
Titanium	0.6	100	2.5	50
Thallium	16	20	0.75	15
Uranium	18	40 000	N D	N D
Vanadium	2	100	1.1	22
Zinc	0.9	1.0	0.0075	0.15
Zirconium	1.5	1000	N D	N D

*Not all elements are listed. **The furnace AA data are also reported in concentrations by assuming a 20µL sample size.

ICP = Inductively Coupled Plasma, AA = Atomic Absorption, N D = Not Detected

The comparison between detection limits in Table 3.1 highlights the following differences:

- Furnace AA detection limits are generally better for most elements.
- Detection limits for group 1 elements of the periodic table, for example, Na, K, Rb and Cs are generally better by flame AAS than ICP-AES.
- Detection limits for refractory elements for example Al, B, Mo, V, Nb, Ta, and W are better by ICP-AES than by flame AAS.
- Non-metals such as S, N, C and the halogens such as I, Cl and Br can only be determined by ICP-AES.

3.10. WINE TREATMENT

Wine samples are normally decomposed by adding 2 ml hydrogen peroxide solution (40%) and 5 ml nitric acid solution (70%) to 50 ml undiluted wine. This suspension is decomposed on a hotplate or in a microwave oven and evaporated to dryness. The decomposed wine sample is re-dissolved in 50 ml distilled water and analysed (Brescia *et al.,* 2003; Šperkowá & Suchánek*,* 2005).

3.10.1. Elements Quantified in Wine

Inductively-coupled plasma is sufficiently sensitive to determine the major elements K, Na, B, Ba, Ca, Cr, Cu, Fe, Mn, Mg, P, Pb, Si, Sn, V and Zn in wine samples (Brescia *et al.,* 2003; Šperkowá & Suchánek*,* 2005).

3.11. ADVANTAGES AND DISADVANTAGES OF ICP-AES, FLAME AAS AND GFAAS

Table 3.2 provides a convenient reference guide to the advantages and disadvantages of ICP-AES, flame AAS and furnace AAS. An in-depth discussion on flame AA, plasmas and furnaces follows the table.

TABLE 3.2

Advantages and Disadvantages of Atomic Spectroscopy Methods

	METHODS		
Advantage	**ICP-AES**	**Flame AAS**	**Furnace AAS**
Large linear dynamic range	Yes		
Minimal chemical interference	Yes (> 3000 °C)		
Minimal ionisation interference	Yes		Yes
Minimal spectral interference		Yes	Yes
Good detection limits for most elements			Yes
No combustion gases	Yes		Yes
Fast sample throughput	Yes		
Low operating costs		Yes	
Unattended Operation	Yes		Yes
Least experienced operator		Yes	

Disadvantage	**ICP-AES**	**Flame AAS**	**Furnace AAS**
Limited dynamic range		Yes	Yes
Chemical interference		Yes	Yes
Ionisation interference		Yes	
Spectral interferences	Yes		
Specific-element detection limits	Yes	Yes	
Combustion gases		Yes	
Slow sample throughput		Yes	Yes
High operating costs	Yes		Yes
Attended Operation		Yes	
Experienced operator	Yes		Yes

3.11.1. Flame AA

An advantage of flame AAS is that it has fewer interferences in comparison to furnaces and plasmas. Flame AAS techniques also require the least experienced operator of the three techniques.

A disadvantage is that elements such as V, B, Mo, Ta and W are only partially dissociated in the flame because of their high melting points and are therefore not readily accessible by flame AAS. This partial dissociation can lead to interferences. Another disadvantage is elements such as P and Cl, which have resonance lines in the furthest UV range, and the halogens, are not easily determined by flame AAS. Although flame AAS techniques are fast, the instruments are seldom automated to allow simultaneous analysis (Tyler, 2001).

3.11.2. Plasmas

According to Tyler (2001), several kinds of plasmas are considered suitable for analytical spectroscopy, but the most widely used is the ICP-AES. An advantage of the ICP-AES is that it provides good detection limits for most elements, whereas flame AA provides good detection limits for some elements. Refer to Table 3.1 for a comparison of detection limits. Refractory elements and those elements that are partially dissociated in flames are normally completely dissociated by the high temperatures of the ICP-AES, is an advantage. However, other types of interferences are present in the ICP-AES, which are considered a disadvantage. For example, high concentration of inorganic matrices can shift the location of the hot portion of the plasma and therefore alter the signal for specific analytes (Tyler, 2001).

Spectral interferences are an important consideration for the ICP-AES and different lines are chosen for a specific element in different matrices. This often requires that spectroscopic skills must be applied to solve difficult analytical problems. However, the ICP-AES is essentially a multi-element technique and modern instrumentation takes advantage of this by using many detectors arranged along the focal plane of the monochromator or by very rapid sequential scanning of the monochromator.

Another advantage of the plasmas is that special sources are not necessary. For example, one can determine certain elements once a year without having to

retain a lamp inventory. The analytical range of the ICP-AES usually extends three to four orders of magnitude, whereas flame AA extends only two to three orders of magnitude (Tyler, 2001).

3.11.3. Furnaces

Based on concentration levels, furnace detection limits are generally 10 – 100 times better than flame AA or ICP-AES. Furnace detection limits on a mass basis, are often 100 times more sensitive because only a small sample is required for the furnace. However, the graphite furnace is not totally interference free, but the level of interferences for the graphite furnace is no greater than flame AA or ICP-AES. Nevertheless, furnace determinations are slow, typically several minutes per element per sample. Graphite furnace AAS is generally single element determination and the analytical range is not very wide, less than two orders of magnitude. Furnaces are normally used when flame or ICP-AES provides an inadequate detection limit (Tyler, 2001).

No single method is best for all applications. A variety of sources should be available for the analyst to choose the method best suited for the analysis. Various factors, such as the expected concentration of elements, the elements that need to be quantified, excitation potentials of the atomic lines and the physical condition of the sample are factors to be considered when selecting a source.

MASS SPECTROMETRY

3.12. INTRODUCTION

A mass spectrum is obtained by converting components of a sample into gaseous ions and separating the components based on a mass-to-charge ratio. Mass spectrometry provides information about the qualitative and quantitative composition of both inorganic and organic analytes in a sample, isotope ratios, the structures of a wide variety of complex molecular species and the structure and composition of solid surfaces.

In mass spectrometry, in contrast to most types of analyses, one is often interested in the precise mass of a particular isotope of an element or the precise mass of compounds containing a particular set of isotopes. Figure 3.10 depicts a flow diagram of the major components of a mass spectrometer. The inlet accepts a small sample and converts it to a gaseous sample. The ion source converts the components of the sample into ions by bombardment with electrons, ions, molecules or photons. Ionisation can be brought about by thermal or electrical energy. The result is a stream of positive or negative ions that are accelerated into the mass analyser. The function of the mass analyser is similar to that of the grating in an optical spectrometer. Dispersion is based on a mass-to-charge basis of the analyte ions. The detector converts the beam of ions into an electrical signal, which can be processed. A characteristic feature of mass spectrometers is the requirements of a vacuum system to maintain low pressures in all of the instrument's components, except the signal processor and read-out (Skoog & Leary, 1992).

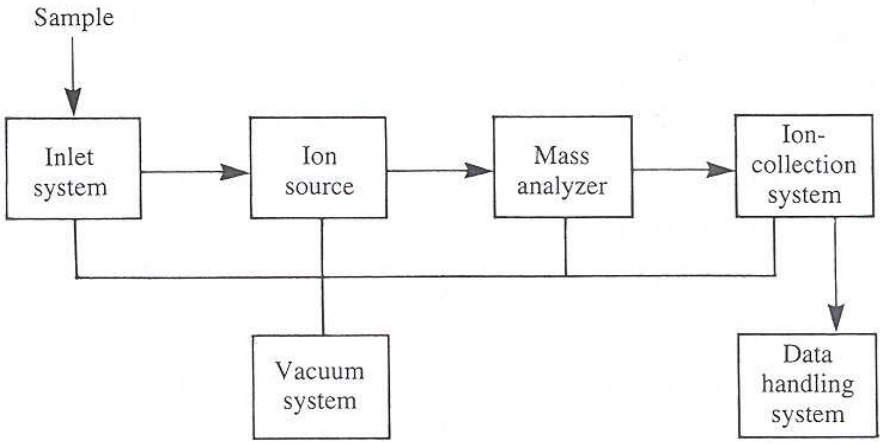


FIGURE 3.10

Flow Diagram for Mass Spectrometry (Willard *et al*., 1988)

3.12.1. Sample Inlet-Systems

The purpose of the inlet system is to allow the introduction of a representative sample into the ion source with minimal loss of vacuum. The most modern types of mass spectrometers are equipped with three types of inlet systems to accommodate various kinds of samples, namely batch inlet systems, direct probe inlet systems and chromatographic inlet systems.

3.12.2. Ionisation Sources

The mass spectrum of a molecular species is dependent on the method used for ionisation. Normally, ions for mass determination are produced by bombarding the components of gaseous samples with electrons. Ion sources are categorised into:

- Gas-phase sources, which include (a) electron, (b) chemical and (c) field ionisation sources
- Desorption sources, which include (a) field, (b) laser, (c) plasma and (d) thermal desorption sources.

In gas-phase sources, the sample is first volatilised and then ionised. With desorption sources, ions are formed from samples in the condensed phase within the ion source (Lattimer & Schulter, 1989).

3.12.3. Mass Analysers

Several methods are available for separating ions with different mass-to-charge ratios, namely magnetic sector, double-focusing, quadrupole, ion trap and time-of-flight mass spectrometers. The function of the mass analyser is to separate ions produced in the ion source according to their different mass-to-charge rations. When the ions exit the ion source, they are accelerated by a system of electrostatic slits and then enter into the particular spectrometer (Willard *et al*., 1988).

3.12.4. Detectors

There are generally three types of detectors commercially available for mass spectrometers. These include electron multipliers, the faraday cup and scintillation-type detectors. According to Skoog & Leary (1992), the electron multiplier is the detector of choice for most routine analysis.

3.12.4.1. Electron Multipliers

Figure 3.11 schematically depicts a discrete-dynode electron multiplier. Electron multipliers are similar to the photo-multiplier detectors used for ultra-violet and visible radiation. The cathode and dynodes normally have copper and beryllium surfaces. According to Watson (1985), electron multipliers are capable of providing high-current outputs and nano-second response times. These detectors are placed, for example, directly behind the exit slit of a magnetic sector mass spectrometer because the ions reaching the detector normally have sufficient kinetic energy to emit electrons from the first stage of the device. Electron multipliers are also used with mass analysers that utilise low-kinetic-energy ion beams such as quadrupole mass spectrometers.

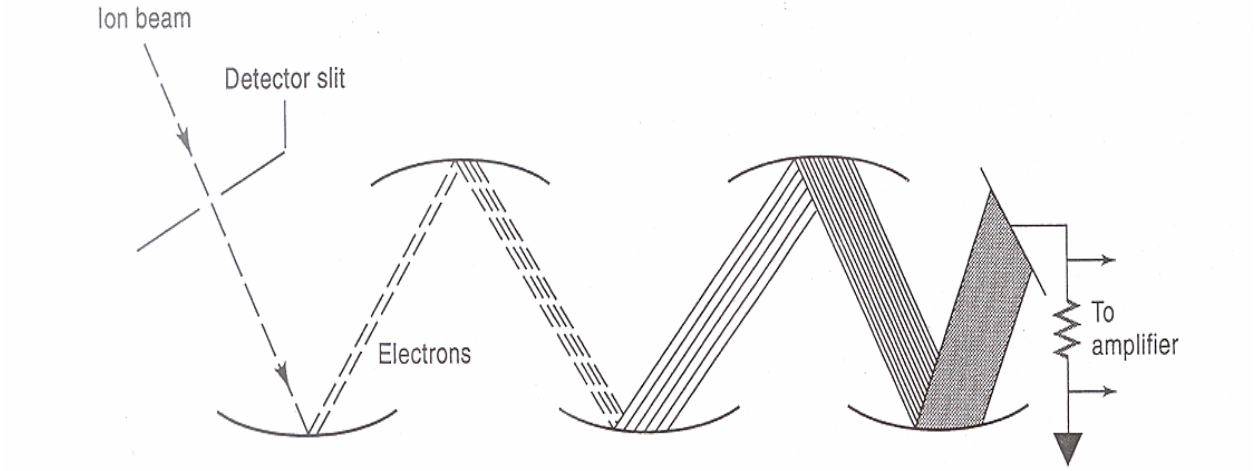


FIGURE 3.11

A Representation of a Discrete Dynode Electron Multiplier (Skoog & Leary, 1992)

3.13. APPLICATION OF MASS SPECTROMETRY

Mass spectrometry can be used for the quantitative determination of elements or molecules. Element analyses are mainly based on radio-frequency spark and inductively-coupled plasma sources, although laser, thermal, secondary ion and glow discharge sources can also be used (Skoog & Leary, 1992). Determination of element concentrations based on inductively-coupled plasma sources will be discussed in Section 3.13.1, since this particular technique was used for the quantification of wine element data.

3.13.1. Determination of Element Concentrations

Mass spectrometry is applied in determining the concentrations of elements in a variety of matrices such as soil, water, wine and fossil fuel samples. In such applications, the samples are exposed to a high-energy source that atomises the sample and converts the resulting atomic vapour into ions that are then determined in a mass spectrometer. One of these sources for the production of elementary ions is inductively-coupled plasma and is discussed in Section 3.13.2.

3.13.2. Analyses Based on Inductively-coupled Plasma Sources

Inductively-coupled mass spectrometry (ICP-MS), like ICP-AES, uses a plasma source for sample atomisation. However, the fundamental difference between ICP-AES and ICP-MS is that the plasma is not used to generate photons of light but rather to generate trace metal ions. In inductively-coupled mass spectrometry, positive metal ions produced in a conventional inductively-coupled plasma torch, are sampled through a differentially pumped interface linked to a mass analyser, such as a quadrupole spectrometer. The spectra produced in this way, which are remarkably simple compared to conventional ICP-AES spectra, consist of a simple series of isotope peaks.

3.13.3. Detection Limits

According to Tyler (2001), the best detection limits are obtained with ICP-MS when compared to GFAAS, ICP-OES and flame AAS. Certain ICP-MS systems exhibit detection limits in the order of 25 - 50 parts per trillion, other systems offer detection limits of 10 - 100 parts per quadrillion, depending on the manufacturer specifications (Tyler, 2001). However, for most routine laboratories, detection limits of nanograms per litre and picograms per litre are not necessary.

3.14. WINE TREATMENT

In direct wine analysis, Perez-Trujillo *et al.* (2003), Marengo & Aceto (2003), Taylor *et al.* (2003) and Thiel *et al.* (2004) diluted wine samples to a 1:1 and a 1:10 fold concentration with 0.2 mol/L nitric acid for the determination of trace elements. The dilution procedure reduced the ethanol concentration, which reduced the plasma instability associated with introducing ethanol into the plasma.

In sample decomposition, however, nitric acid and hydrogen peroxide were added to wine with similar specifications as for AAS, AES and GFAAS analyses (Castiñeira *et al*., 2004; Thiel *et al.,* 2004; Coetzee *et al.,* 2005).

3.14.1. Elements Quantified in Wine

Wine trace elements namely As, Be, Cd, Co, Cs, Ga, Li, Mo, Nb, Ni, Rb, Sb, Te, Ti, Tl, U, W, Y and Zr and the rare-earth elements La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm and Lu were determined on a routine basis using ICP-MS. Most of the above-mentioned elements are utilised for wine origin determinations (Castiñeira *et al*., 2004; Thiel *et al.,* 2004; Coetzee *et al.,* 2005; Angus *et al.,* 2006).

THERMAL IONISATION MASS SPECTROMETRY

3.15. INTRODUCTION

According to Cotter (1988), thermal ionisation sources, also referred to as surface ionisation source, are used for inorganic solid material analysis. Samples are coated on a tungsten ribbon filament and then heated until ionisation or evaporation takes place. When an atom or molecule is evaporated from the surface at about 2000 °C, it maintains a certain probability of being evaporated as a positive ion. This probability is predicted and is a function of the ionisation potential of the sample and the surface work function of the filament material. The relationship between the ratio of ions (Y_1) and the neutral species (Y_o) is given by the Saha-Langmuir equation as:

$\frac{Y_1}{Y_o} = \frac{g_1}{g_o} \exp\left[\frac{(WF - IP}{kT}\right]$, where $\frac{Y_1}{Y_0}$ is the ion to neutral species ratio, $\frac{g_1}{g_0}$ is the statistical weights of ion and neutral states, *k* is Boltmann's constant, *T* is surface temperature, WF is the work function and IP is the ionisation potential. One application of thermal ionisation is thermal ionisation mass spectrometry. The ions, which come off the filament, are directed into a mass spectrometer for the determination of elements or isotopes present in the sample (Cotter, 1988).

3.15.1. Sensitivity

Thermal ionisation is used for the determination of inorganic compounds that generally have low ionisation potentials. Thermal ionisation analysis is also particularly useful in determining isotope ratios in inorganic compounds. However, thermal ionisation analysis is inefficient for organic compounds with ionisation potentials between 7 – 16 electron volts (Cotter, 1988).

NEUTRON ACTIVATION METHODS

3.16. INTRODUCTION

Activation methods are based on the measurements of radioactivity, which is induced into a sample through irradiation with neutrons or charged particles such as hydrogen, deuterium or helium ions. The two types of activation methods

include destructive and non-destructive methods. In both methods, the sample is irradiated with neutrons. In the non-destructive method, the sample is counted after cooling. In destructive methods, the components present in the analyte are first separated be=fore counting by means of precipitation, extraction, ion exchange or chromatographic methods. The type of sample is usually a solid, liquid or gas. Exposure times are normally three to five times the half-life of the analyte product (Skoog & Leary, 1992).

3.16.1. Neutrons and Neutron Sources

Three sources of neutrons are generally used in neutron activation methods namely, nuclear reactors, radioactive isotopes and neutron-beam accelerators. All three sources produce highly energetic neutrons, which pass through a moderating material that reduces their energies. Energy loss to the moderator occurs by “elastic scattering”, in which neutrons bounce off nuclei in the moderator material, transferring part of their kinetic energy to each nucleus they strike. Eventually, the nuclei will come to a thermal equilibrium with the surroundings. Neutrons having this energy are called “thermal neutrons”. Most activation methods are based on thermal neutrons, which react efficiently with most elements (Ehmann & Vance, 1989).

3.16.2. Sample Procedure

A sample is weighed directly into a plastic or quartz container and sealed. The sample is placed near the centre of the source and irradiated for a period. The placing of the sample near the centre results in "saturating" the sample with neutrons. If a neutron approaches the nucleus of an atom in the sample, it may be absorbed. When absorption occurs, the sample element will become a different isotope of the same element. Sometimes this "new" isotope is unstable or is radioactive and decays by emitting gamma rays.

The sample decays after irradiation and is then counted using a gamma-ray spectrometer. The resulting gamma-ray spectrum is similar to a mass-spectrum

indicating counts and channel instead of relative intensity and mass on the y and x axes respectively. The position of each “peak” determines the energy of the gamma ray, identifying the responsible element of interest (Ehmann & Vance, 1989).

3.16.3. Detection Limits

The most important characteristic of neutron activation methods is the high sensitivity for many elements (Refer to Table 3.3). The disadvantages of neutron activation methods, however, are the requirement for large and expensive equipment and special facilities for handling and disposing of radioactive materials. These aspects are not conducive to the operation of an oenological laboratory. Another disadvantage of neutron activation is when heavy metals are determined at low levels. Considerable precautions must be taken to ensure the reagents used are specially purified and prevent dust from settling on the samples (Ehmann & Vance, 1989).

TABLE 3.3

Estimated Detection Limits for Neutron Activation Analysis using Decay Gamma Rays (Glascock, 2007)

Sensitivity in picograms	Elements
1	Dy, Eu
1 – 10	In, Lu, Mn
10 -100	Au, Ho, Ir, Re, Sm, W
100 – 10^3	Ag, Ar, As, Br, Cl, Co, Cs, Cu, Er, Ga, Hf, I, La, Sb, Sc, Se, Ta, Tb, Th, Tm, U, V, Yb
10^3 – 10^4	Al, Ba, Cd, Ce, Cr, Hg, Kr, Gd, Ge, Mo, Na, Nd, Ni, Os, Pd, Rb, Rh, Ru, Sr, Te, Zn, Zr
10^4 – 10^5	Bi, Ca, K, Mg, P, Pt, Si, Ti, Tl, Xe, y
10^5 – 10^6	F, Fe, Nb, Ne
10^7	Pb, S

X-RAY SPECTROSCOPY

3.17. INTRODUCTION

X-rays are short-wavelength electromagnetic radiation, produced by the deceleration of high-energy electrons or by electronic transitions involving electrons in the inner orbital of atoms. The wavelength range of X-rays is from approximately 10^{-6} nm to 10 nm. Spectroscopy (X-ray) is based on the 0.01 nm to 2.5 nm region (Anzelmo *et al.,* 1987).

3.18. EMISSION AND ABSORPTION OF X-RAYS AND X-RAY FLUORESCENCE

X-rays are obtained in three ways, namely the bombardment of a metal target with a beam of high-energy electrons, the exposure of a substance to a primary beam of X-rays to generate a secondary beam of X-ray fluorescence and the employment of a radioactive source where the decay process results in X-ray emission.

When a beam of X-rays passes through a thin layer of matter, its intensity or power diminishes due to absorption and scattering. The effect of scattering, except for the lightest elements, is minimal and is neglected in the wavelength regions where appreciable absorption occurs.

The absorption of X-rays produces electronically excited ions that return to their ground state by transitions, involving electrons from higher energy levels. Thus, an excited ion with a vacant K shell is produced when, for example, lead absorbs radiation of wavelengths shorter than 0.014 nm. After a brief period, the ion returns to its ground state via a series of electronic transitions characterised by the fluorescence of wavelengths identical to those that result from excitation produced by electron bombardment. The wavelengths of the fluorescent lines are slightly greater than the wavelength of the corresponding absorption lines. Absorption requires a complete removal of the electron, whereas fluorescence involves transitions of an electron from a higher energy level within the atom.

Thus, X-ray fluorescence is initiated by the excitation of a sample by radiation with an X-ray from an X-ray tube or radioactive source. Under these conditions the elements in the sample are excited by absorption of the primary beam and emit their own characteristic fluorescence X-rays (Jenkins, 1988).

3.19. INSTRUMENTATION

There are three types of X-ray fluorescence instruments namely, wavelength dispersive, energy dispersive and non-dispersive instruments. Energy dispersive and non-dispersive instruments are sub-divided into X-ray tube sources or radioactive substance sources depending upon which sources serve as a radiation source.

3.19.1. X-Ray Sources

X-ray tubes, radio-isotopes and secondary fluorescence sources are the three sources used in X-ray instruments.

3.19.1.1. The X-Ray Tube

According to Jenkins (1988), the most common source of X-rays for analytical methods is the X-ray tube. The X-ray source is a tube under vacuum in which is mounted a tungsten filament cathode and an anode. The anode consists of a block of copper with a metal target plated on the surface. Figure 3.12 shows schematically one of many different shaped X-ray tubes.

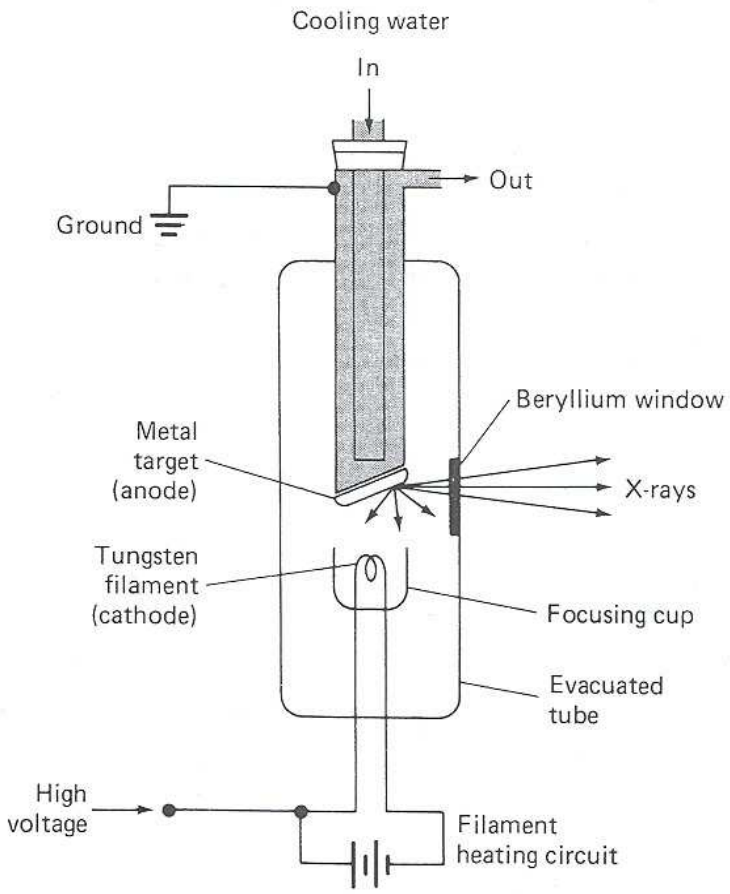


FIGURE 3.12

Schematic Representation of an X-Ray Tube (Skoog & Leary, 1992)

3.19.1.2. Radio-isotopes

A number of radioactive substances are employed as sources in X-ray fluorescence methods. Many radioactive sources provide simple line spectra and others like $^{147}_{61}$Pm-Al produce a continuous spectrum.

3.19.1.3. Secondary Fluorescent Sources

Fluorescence spectra of elements that are excited by radiation from an X-ray tube can serve as a source of absorption of fluorescence. This is known as a secondary fluorescence source.

3.19.2. X-Ray Detectors

X-ray detectors convert radiant energy into an electrical signal. Three types of detectors are used for X-ray methods. They include gas-filled detectors, scintillation counters and semi-conductor detectors.

3.20. X-RAY METHODS

According to Skoog & Leary (1992), three X-ray methods are generally used, namely X-ray Fluorescence, X-ray absorption and X-ray diffraction methods. Only X-ray fluorescence will be discussed.

3.20.1. X-Ray Florescence

Excitation occurs when a sample is irradiated by a beam of X-rays from an X-ray tube or radioactive source. Under these conditions, the elements in the sample are excited by absorption of the primary beam and subsequently emit their own characteristic X-rays. This is referred to as X-ray fluorescence.

X-ray fluorescence, according to Anzelmo & Lindsay (1987), is one of the most widely used of all analytical methods for the qualitative identification of elements having atomic numbers greater than oxygen (>8). It is often employed for semi-quantitative or quantitative elemental determination.

3.20.2. Detection Limits

According to Gruber *et al.* (2006), X-ray fluorescence methods are generally not as sensitive as optical methods. In most cases, concentrations of a few parts per million can be measured. X-ray fluorescence spectra are relatively simple which implies that spectral line interferences are unlikely.

Generally, X-ray fluorescence methods are used on a variety of objects without destruction of the sample. Analyses are also performed on samples ranging from a barely visible speck to a massive object. X-ray fluorescence is used to measure

the major metal content of many different samples. It is also applied to measure trace metals in samples.

A disadvantage of X-ray fluorescence is that flame AA and ICP-AES are simpler to use than X-ray methods and flame AA and ICP-AES are less expensive than X-ray methods.

3.21. WINE TREATMENT

Wine samples (5 ml) are normally digested with 1 ml hydrogen peroxide (40%) and evaporated to dryness on a hot plate at 80 °C. The evaporated sample is re-dissolved in concentrated nitric acid. The acidified sample is diluted to 100 ml with distilled water. A droplet of this solution is placed on a carbon foil or glass quartz reflector and allowed to dry before analysis.

Wine samples can also be analysed in their original form by placing a droplet of undigested, undiluted acidified wine onto the reflector and allowed to dry by evaporation with an infra-red light (Haswell & Walmsley, 1998; Galani-Nikolakaki *et al*., 2002; Orescanin *et al*., 2003; Gruber *et al.,* 2006).

3.21.1. Elements Quantified in Wine

The most frequently determined elements by X-ray fluorescence methods in wine samples are Al, As, Br, Ca, Cd, Cl, Co, Cu, Cr, Fe, K, Mn, Ni, P, Pb, Rb, S, Se, Sr, Ti, V and Zn (Haswell & Walmsley, 1998; Galani-Nikolakaki *et al.,* 2002; Anjos *et al.,* 2003; Gruber *et al.,* 2006).

3.22. CONCLUSION OF CHAPTER 3

The literature reviews on instrumental techniques reveal both positive as well as negative aspects. When one considers the individual requirements for laboratories, one requirement deserves special mention. In a large laboratory, a combination of ICP-AES and electro-thermal AAS would be more suitable,

whereas flame and electro-thermal AAS would be adequate for a small laboratory, particularly concerning cost and sample number.

The literature review demonstrates that it is important that the analyst define both the present and future analytical requirements for the laboratory before deciding on an appropriate technique. The requirements one needs to consider prior to the purchase of an analytical instrument are the following:

- The number of samples to be analysed per week or day.
- The type of matrices that need to be analysed.
- The number of elements that need to be determined.
- The type of elements that need to be determined.
- What are the typical sample volumes?
- The concentration ranges that are present in the matrices.
- What levels of expertise do the operators require?
- The funds that have been budgeted for the purchase of an instrument?

Appropriate answers to the above questions may assist the analyst to decide on the most suitable technique. Laboratories often require more than one technique to meet their analytical needs.

During the Apartheid years, the wine industry in South Africa became isolated as international trade with South Africa diminished and sanctions took hold. Since South Africa's first democratic elections in 1994 and the demise of Apartheid, the wine industry, which was largely controlled by white owners and producers, was forced to introduce reform measures to be more representative of a democratic South Africa. The KWV was restructured into a commercially driven venture in 1997 and together with other role-players, formed the South African Wine Industry Trust in 1999 to promote transformation in the wine industry. In 2001, a hands-on project, the Vineyard Academy, was launched by the South African Wine Industry Trust (SAWIT) to provide vineyard workers with skills and training in various fields to advance transformation of the wine industry and to promote South African wine exports. Most wine farm owners are at present primarily white people, however, black partnerships are becoming evident in the wine industry. During 2001, the Stellenbosch Farmers' Winery merged with the Distillers Corporation, another large wine producer, to establish a new company named Distell. Distell currently ranks among the top ten liquor companies in the world with an annual turnover of approximately R4 billion. There are now positive indications that some of the bigger brand producers are looking at the potential of the black urban market (South African Wine Industry Directory, 2006/2007).

2.4.2. Historical Background

According to Simon (1973), many European and American wine drinkers consider wines from Africa as unusual, Europe being the traditional wine-producing continent. One may encounter vineyards all over the continent of Africa. Algeria and Morocco have been producing wines for decades. In Zimbabwe and Kenya, modern winemaking techniques are established to keep abreast with other winemaking countries. In the Western Cape, however, where climatic and topographic conditions are similar to those of the old wine countries, such as France, Italy and Spain, Africa's finest wines are produced. Today South African wines compete with the best in the world.

Wine culture in the Western Cape may be traced 350 years in history, reflects the country's colonial and apartheid past, but at the same time is filled with the potential and expectation of the modern wine world. From this history, a unique wine tradition of tastes and styles developed with its roots in France, Italy and Spain. With it developed an awareness of the contemporary consumer as defined by winemaking styles in countries like America and Australia.

The South African wine industry is in the unique position to apply both old and new world winemaking techniques due to its colonial influences. It therefore offers marketing possibilities that are able to exploit the challenges the new global economy demands. It can also offer the wine-drinking world new experiences in flavour.

2.4.3. Types of Wine Producers

The South African wine industry offers products such as wine, rebate wine, distilling wine, brandy and other spirits distilled from distilling wine, grape juice and grape juice concentrate for use in wine and non-alcoholic products. According to the South African Wine Industry Information and Systems (SAWIS), there are currently 4 360 grape farmers, 572 wine cellars, which produce their own wine and 118 bulk wine buyers in South Africa. The 572 wine cellars are divided into three types, namely:

- Co-operative cellars (66), which are large units that operate on a communal basis and process members' grapes,

- Private wine estates (93) which are wine cellars that produce wine that is grown, made and bottled on the estate. The cellar must be registered as a unit for the production of estate wines.

- Private wineries and wholesalers (413) buy grapes, wine, and produce wine for bottling under their brand names in addition to producing wine from grapes grown on their own land.

In 2005, the grape crop totaled approximately 1.17 million tons, from which 628 million litres of wine were made by 355 active cellars, of which 185 were non-estate private producers, 93 registered estates, 66 co-operatives and nine grape producing wholesalers (SAWIS, 2006).

2.4.4. Wine Production

Since the first democratic election in 1994, the South African wine industry has resumed its trade on the world market with significant impact. Exports have increased from approximately 50.7 million litres in 1994 to approximately 312.6 million litres in 2007.

By international standards, the South African wine industry is considered comparatively small and is ranked 15th with about 1.7% of global grape vine plantings. However, with a wine production ranking at ninth position it accounts for 3.3% of the world's wine.

According to the South African Wine Industry Directory (2006/2007) there are 126 075 hectares under wine grapevines in South Africa. According to the latest official statistics (SAWIS) of the 126 075 hectares under wine grapevines, compared with 98 203 hectares in 1997, 18.8% is Chenin blanc, which is the country's most widely planted variety, with Colombard at 11.3% followed by Chardonnay grape variety at 7.8%.

Cabernet Sauvignon grape variety is the most planted of red variety at 13.5% of total vineyard plantings followed by Shiraz and Merlot grapes.

White grape varieties continue to represent more than two thirds of the total plantings in South Africa. In 2005, 74% of all new plantings were white grape varieties, with Chenin blanc, Chardonnay and Sauvignon blanc grapes representing the majority of plantings. At the same time, 63% of all vines up-rooted were white, comprising mostly Chenin blanc and Colombard varieties.

The major geographic distribution of South African wine grape vineyards per region during 2006 was the Breede River Valley with the highest percentage at 39.24%. The Paarl-Stellenbosch region was slightly less at 35.71%. The Swartland region represented 12.66% and the Olifants River at 8.99%. The Orange River represented 3.38% and the Little Karoo 3.02% of wine grape distribution.

Red grape varieties planted during 2005 represented 945 hectares with the majority planted in the Stellenbosch and Worcester areas. White grape varieties represented 2706 hectares with the majority planted in the Worcester area. The highest percentage of grapes planted in South Africa occurred in the Western Cape Province (South African Wine Industry Directory, 2006/2007).

2.4.5. Area of Origin and Wine Certification

With the introduction of the Wine of Origin System in 1973, South Africa's wine lands were divided into regions, districts and wards (Table 2.6). The region of origin can be described as production units and vary in size from a ward (e.g. viticultural area) to a geographical unit (e.g. Western Cape). Each unit is demarcated and described by legislation before it may officially be recognised and used on a label. There are currently three geographical units namely Northern-Cape, which includes the production areas Hartswater, Douglas, Lower Orange and Rietrivier (Free State), Kwazulu-Natal, which includes no other production areas and Western Cape, which includes all other production areas.

Wines of origin are produced under strict control, from the harvesting of the grapes to the final product, in order to comply with the requirements of the Wine of Origin Scheme with respect to origin, variety and vintage. The Wine and Spirit Board, which is a state entity, issues an official seal, which is displayed on the bottle neck after certifying a wine for origin, vintage and variety. The certification seal on the bottle confirms that at the time of its assessment by the Wine and Spirit Board, the wine conformed to the prescribed standards enforced by the Wine and Spirit Board. In other words, if a wine producer claims origin, the statutory regulations ensure that the wine is definitely from that origin. When the term “Wine of Origin” or the abbreviation “W. O.” together with the name of the production area, such as Robertson or Paarl appears on the label, it confirms that 100% of the grapes from which the wine is produce come from that specific region, district or ward. Labels may also reveal the composition of grape varieties in a blend. Varieties are named in descending order of their percentage. Regulations allow the blending of up to 15% of another variety or varieties in a wine. Such a blend is still labelled as a single variety wine (Liquor Products Act 60 of 1989). An identification number appears on the seal to record the history of each wine to the day it was harvested. Vineyards are subject to inspections and wines are subject to monitoring in the cellar. Certification is approved only after final, official chemical analysis and tasting. The analysis ensures that a wine complies with certain legal chemical requirements, for example: sulphur dioxide levels (bound and free), fructose and glucose content, pH and volatile acidity. During tasting, a panel evaluates the wine for minimum quality standard and varietal character. During the 2005 harvest, over 300 million litres of wine were certified (SAWIS, 2006/2007).

2.4.6. The Ten Most Popular Wine Grape Varieties in South Africa

According to the South African Wine Industry Statistics (2004), Chenin blanc is the most planted wine grape variety in South Africa. Chenin blanc is versatile and is used for white wines, sherry and jerepigos. Chenin blanc dry, noble rot and

sparkling wines from the Loire valley in France are also well known. The leading districts for Chenin blanc in South Africa are Worcester, Malmesbury and Paarl.

Cabernet Sauvignon grape variety is the most planted red variety in South Africa. It is often regarded as a very sought after red wine variety of the world and high quality red wine is produced in most of the wine producing countries. The Bordeaux wines are the best known in Europe. Good quality rosé wines are also produced from Cabernet Sauvignon grapes, especially those originating from the Loire valley in France. In South Africa, Stellenbosch, Paarl and Robertson are the most important regions for Cabernet Sauvignon grapes.

Colambard grapes rank third in South Africa as the most popular wine grape variety planted. It is rarely sold as Colambard variety wine as it is normally produced as dry or sweet white wine blends or used for brandy production. Internationally, it is widely used as a variety for the production of cognac. In South Africa, the districts of Orange River, Worcester, Robertson and Olifants River are where the Colambard grape variety is most planted.

Shiraz ranks fourth as the most widely planted wine grape variety in South Africa. It was traditionally only grown in the Rhône valley of France before the Australians popularised it. Stellenbosch, Paarl and Malmesbury are the main regions in South Africa for Shiraz grape plantings.

Chardonnay grape varieties rank fifth in South Africa as the most planted wine grape variety. Traditionally in Europe, it is known for champagne and white Burgundy wines. Robertson, Worcester, Stellenbosch and Paarl are the main districts for Chardonnay grape plantings.

Sauvignon blanc ranks sixth in South Africa as the most wine grape variety planted. Internationally renowned Sauvignon blanc wines come from Marlborough New Zealand, Sancerre and Pouilly Fumé valley in France. Blends

with Sémillon and Sauvignon blanc from the Graves and Sauternes regions of Bordeaux are also well known. In South Africa, the main districts for Sauvignon blanc grapes are Stellenbosch, Worcester and Robertson.

Merlot ranks seventh as the most planted wine grape variety in South Africa. Château Pétrus, the most expensive wine in the world, is normally produced from Merlot. Merlot, in its own right, is mostly used in blends with Cabernet Sauvignon. Stellenbosch, Paarl and Worcester are the most important districts for Merlot grapes.

Pinotage, a grape variety developed in South Africa, ranks eighth as the most planted wine grape variety in South Africa. Malmesbury, Stellenbosch and Paarl are the main regions for Pinotage grapes.

Cinsaut noir ranks as the ninth most planted variety in South Africa. Cinsaut noir wines are presently very limited due to replacement by other red grape variety wines. When Cinsaut noir grape vines have high yields, it is only suitable for brandy or wine alcohol production. Paarl, Worcester and Malmesbury are the main districts for Cinsaut noir grapes.

Ruby Cabernet ranks tenth in South Africa as the most planted wine grape variety. Ruby Cabernet produces acceptable wines, but will never produce the ultimate quality of Cabernet Sauvignon wines according to SAWIS. Worcester and Robertson are the main districts for Ruby Cabernet grape plantings (South African Wine Industry Statistics, 2006).

2.4.7. Wine Regions, Districts and Wards

There are currently three geographical units, four regions, eighteen districts and fifty-three wards, which have been delineated in South Africa for the production of grapes.

2.4.7.1. Summary of Important Districts and Wards in South Africa

a. **Constantia** is the historic heart of South African wine according to Kench *et al*. (1983). It includes some of the most famous estate wine cellars such as Groot- and Klein Constantia and Buitenverwachting in the Western Cape.

b. **Durbanville**, which is situated northeast of Cape Town, has a winemaking history dating back 280 years. Sauvignon blanc wines as well as Shiraz and Merlot are produced in this ward.

c. **Stellenbosch,** one of the finest wine areas in South Africa, which is home to the co-operative cellar, Distell and commercial Berg Cellar, produce good red wines. Designated wards within the district are Jonkershoek Valley, Simonsberg-Stellenbosch, Bottelary, Devon Valley and Papegaaiberg.

d. **Franschhoek**, a town founded by the French Huguenots in 1688. Presently it is a wine region with small producers.

e. **Paarl** another of the Cape's historic towns where wine has been made for centuries. It is home to the original KWV head office, as well as the Nederburg brand. Winemakers have been concentrating on producing Shiraz, but some fine Chenin blanc, Pinotage, Cabernet Sauvignon, blends, and even unusual varieties such as Viognier and Mourvèdre have been produced.

f. **Robertson** situated in a valley where white wines such as Chardonnay and sparkling wines are produced. Currently, more cellars in the Robertson area are producing red wines, especially Shiraz.

g. **Worcester** and the surrounding areas comprise 20% of all South Africa's vineyards. Brandy is mainly produced in this region and wine for wholesalers. Small volumes of wine are bottled under estate cellar names.

h. **Swartland**, traditionally a wheat-producing area north-west of Cape Town, is currently producing white wines and red wines on a small scale.

i. **Walker Bay**, which is situated near the coastal town of Hermanus, has become an important white wine producing area. It was recently elevated to district status with four wards.

j. **Klein Karoo** is a semi-desert inland area. Fortified wines such as Muscadels and Portuguese "port" styles are produced in places such as Calitzdorp.

k. **Olifants River** is a fast-developing wine-grape region stretching a few hundred kilometres up the west coast from Cape Town.

l. **Orange River** area represents large white-wine producing vineyards.

A detailed list of the wine regions, districts and wards of South Africa is presented in Table 2.6.

TABLE 2.6

Production areas defined in terms of the wine of origin scheme with an indication of the regions, districts and wards (SAWIS, 2006).

Region	District	Wards
BREEDE RIVER VALLEY	ROBERTSON	Agterkliphoogte
		Bonnievale
		Boesmansrivier
		Eilandia
		Hoopsrivier
		Klaasvoogds
		Le Chasseur
		McGregor
		Vinkrivier
	WORCESTER	Aan-de-Doorns
		Goudini
		Nuy

		Scherpenheuvel
		Slanghoek
	SWELLENDAM	Buffeljags
		Stormsvlei
KLEIN KAROO	-	Montagu
	-	Tradouw
	CALITZDORP	-
	-	Upper Langkloof
	-	Outeniqua
COASTAL REGION	CAPE POINT	-
	-	Constantia
	TYGERBERG	Durbanville
		Philadelphia
	PAARL	Franschhoek Valley
		Wellington
		Simonsberg-Paarl
		Voor Paardeberg
	STELLENBOSCH	Jonkershoek Valley
		Papegaaiberg
		Simonsberg-Stellenbosch
		Bottelary
		Devon Valley
		Banghoek
	DARLING	Groenekloof
	SWARTLAND	Riebeekberg
		Malmesbury
	TULBAGH	-
OLIFANTS RIVER	LUTZVILLE VALLEY	Koekenaap
	-	Spruitdrift
	-	Vredendal
	CITRUSDAL MOUNTAIN	Piekenierskloof
	-	Bamboes Bay
	CITRUSDAL VALLEY	-
No Region	OVERBERG	Elgin
		Klein River
No Region	CAPE AGULHAS	Elim
No Region	WALKER BAY	Hemel en Aarde Valley
		Upper Hemel en Aarde valley
		Bot River
		Sondags Kloof
No Region	DOUGLAS	-
No Region	No District	Hartswater
		Lower Orange
		Cederberg
		Ceres
		Herbertsdale

	Rietrivier FS
	Ruiterbosch
	Swartberg
	Prince Albert Valley
*BOBERG	

*BOBERG (For use in respect of fortified wines from Paarl and Tulbagh); NORTHERN CAPE: A geographical unit that includes the production areas Hartswater, Douglas, Lower Orange and Rietrivier (Free State), while the WESTERN CAPE geographical unit includes all other production areas.

2.4.8. Important Annual Wine Events

- The annual Veritas Wine Show held in Paarl, is the most representative of the wine industry, awarding double gold, gold, silver and bronze medals for the best-judged wines.

- The Absa Top 10 Pinotage competition is to encourage the improvement of local Pinotage and is widely supported by the wine industry.

- The Diners Club Wine-maker of the Year award focuses on a specific wine grape each year. A new Young Winemaker-of-the-Year award was recently incorporated into the Diners Club Wine-maker of the year award.

- The South African Airways Selection is highly regarded as a measure of quality and prestige due to the participation of international judges.

- As with Pinotage, *Wine* Magazine's yearly Chenin Blanc Challenge encourages improved wines from Chenin blanc grapes.

- Air France-Preteux Bourgeois Classic is an annual competition that judges Cape wines according to European standards.

2.4.9. Organisations

- **Wines of South Africa (WOSA).** This is an organisation, which represents all South African wine producer-exporters. Comprehensive information including an overview of wine-growing areas, varieties and soil types is available.
- **South African Wine Industry Information & Systems (SAWIS).** A non-profit company that administers the industry's Wine of Origin System.
- **Pinotage Association.** The association provides a comprehensive guide to this variety, with a listing of Pinotage wineries.

2.5. DISCUSSION

The literature review (section 2.3) clearly shows that the most frequently analysed elements were Na, K, Ca, Mg, Mn, Li, Fe, Cu, Zn, Rb, Co, Cs, Al, Sr and Ba, and to a lesser extent, Pb, B, Mo, Br, Cr, Sb, Si, Ti, Ga, Ag, As and V. The most frequently quantified elements, used as key variables to differentiate between wine regions were Na, Fe, Li, Mg, Rb, Sr and Zn. Multivariate techniques such as PCA, CA and *K*-NNA were most frequently employed for data analysis. The majority of studies were conducted in Mediterranean and European countries, such as Spain, France, Italy, Slovenia, Germany and Greece, owing to their interest in wine authenticity. In South Africa, however, the regional differentiation between varietal wines of known origin, by means of routine chemical analysis, has not been addressed, except for Zeeman & Butler (1962), who determined the lead, copper and zinc content in South African fortified wines by means of AAS but for non-origin purposes. Haswell & Walmsley (1998) included South African Cabernet Sauvignon wines in their investigation with X-Ray fluorescence analysis in an attempt to discriminate the country of origin. Coetzee *et al.* (2005) published data on the differentiation between red and white wines originating from the Stellenbosch, Robertson and Swartland areas. Their study was based on wine trace element composition using ICP-MS. Capron *et al*. (2006) included South African wines in their determination of organic and inorganic parameters in an attempt to differentiate the country of origin.

There are certain measures, however, which are taken by SAWIS (South African Wine Information and Systems) to ensure the grape origin, variety and quantity of wine in South Africa, prior to certification. In addition, the national authorities also provide strict guidelines, which must be adhered to concerning the quality of wine, since the production of quality wines is of economic importance to South Africa. These guidelines include official tasting (organoleptic properties), chemical analyses such as ethyl alcohol concentrations, fructose and glucose content, sulphur dioxide levels (bound and free), pH and volatile acid determinations. Although these guidelines are strictly enforced, and do, to a certain extent guarantee the quality of wine, they cannot be considered a substitute for chemical analyses to determine the origin of wine. The aim of this study was to investigate the use of element determination by ICP-AES to differentiate between the geographic origins of wines produced in the Western Cape (Breede River Valley and Coastal regions).

Based on the information in the third-domain model of the literature review, which is the differentiation between wines originating from the Coastal and the Breede River Valley regions in South Africa, differentiation between market-ready wines (commercial wines) originating from these two regions has not been addressed using wine trace-element composition.

The literature review has shown that South African market-ready wines (commercial wines, as opposed to tank wines analysed by Coetzee *et al.* (2005)) originating from the Breede River Valley and Coastal regions have not been differentiated based on their trace element composition. Consideration of the facts in the literature review, indicate that it should be possible to differentiate between wines from different geographical wine regions based solely on the grounds of their trace element composition. The literature review also showed that quantitative analysis of wine element data with the appropriate statistical analyses will support my research design.

2.6. CONCLUSION OF CHAPTER 2

From the literature review is it clear that volatile compounds and amino acids are not as popular compounds for wine origin differentiation as are polyphenols, isotopes and element composition. Most authors applied principal component analysis, linear discriminant analysis and canonical discriminant analysis. It is clear from the review that isotope analysis in combination with element composition give the most reliable and accurate origin determination of wine.

CHAPTER 4

A REVIEW ON MULTIVARIATE STATISTICAL METHODS

4.1. INTRODUCTION

The information age has resulted in masses of data in every discipline. Today's researchers are able to obtain multi-element data of samples rapidly and efficiently by means of sophisticated, easy-to-use, automated, analytical instrumentation coupled with data acquisition systems.

The challenge is thus how to generate data that are more objective. Collecting more data does not necessarily mean your chances of solving problems are improved. Despite the quantity of data available, the ability to obtain a clear picture of what is going on and make intelligent decisions is a challenge. What is needed is a better understanding of what the data really means - that is, a tool to find significant meaning in the large amount of data we already have. One tool an increasing number of researchers are turning to in order to help them get the most useful information from their data is multivariate analysis. It has proven to be one way to work better, faster and more efficiently.

Univariate measurements (analysis), which are often straightforward direct properties of a sample, can be a useful source of information. Univariate analysis explores each variable in a data set, separately. It looks at the range of values, as well as the central tendency of the values. It describes the pattern of response to the variable. It describes each variable on its own. In many cases, however, simple univariate measurements do not provide enough information to resolve the problem at hand for these reason multivariate methods are applied. Multivariate measurements (analysis) are superior to univariate analysis because it simultaneously processes information from multiple variables in a meaningful way. Multivariate analysis describes a collection of procedures, which include any statistical technique used to analyse data that arises from more than one variable at a time.

Multivariate statistical methods involve a statistical approach that develops statistical models to characterise some property in multivariate data that is difficult to measure directly. The use of these methods in the statistical analysis of instrumental data, often results in a faster and more precise interpretation of data or variables.

Multivariate analysis is generally concerned with two areas, descriptive and inferential statistics. The descriptive techniques are exploratory and they essentially generate hypotheses rather than test them. Descriptive statistics simply describe what is going on in the data. Inferential techniques make deductions and reach conclusions that extend beyond the immediate data. The goal is to make use of inferential techniques for formal hypothesis testing. In the inferential area, many multivariate techniques are extensions of univariate procedures (Rencher, 2002).

4.2. PATTERN RECOGNITION TECHNIQUES

Initially, exploratory data analysis is performed on the data set. This step involves the computation and graphical display of patterns of association in multivariate data sets. Often the exploratory phase is accomplished with two techniques namely hierarchical cluster analysis and principal component analysis. Hierarchical cluster analysis finds clusters of observations within a data set and principal components analysis is a technique used to reduce multidimensional data sets to lower dimensions for analysis. Both can be used to identify the dominant patterns in the data, such as groups, outliers and trends.

Classification modelling can be conducted, which involves the computation and graphical display of class assignments based on the multivariate similarity of one sample to others. Two different classification models that are commonly used are *K*-nearest neighbour and soft independent modelling of class analogy (SIMCA). In *K*-nearest-neighbour classification, the training dataset is used to classify each

member of a "target" dataset or dataset to be classified. Soft independent modelling by class analogy is a statistical method for supervised classification of data. In addition, regression techniques such as partial least squares can be used to measure the degree of predictability. With regression models, the analyst is interested in predicting some value, rather than assigning a class designation for an unknown sample.

The first successful pattern recognition application on wine chemical data was conducted by Kwan & Kowalski (1978). Kwan & Kowalski applied a classification method known as least squares multi-linear classifier to atomic absorption and gas chromatographic data. Separation by vintage and wine regions was achieved. Their findings showed that pattern recognition techniques could effectively be applied to problems encountered in the wine industry. For example element concentrations, which comprise a collection of objects (wine samples) with a number of measurements that need to be made on each wine sample? Subsequent to the publication of the research findings of Kwan & Kowalski, numerous authors (Peña *et al.*, 1999; Frías *et al.,* 2003; Taylor *et al.*, 2003; Thiel *et al.*, 2004; Coetzee *et al.*, 2005; Šperková & Suchánek, 2005; Capron *et al.,* 2006) have successfully applied pattern recognition techniques in the analysis of wine data. Consequently, this application has become a valuable analytical technique in oenological studies.

4.3. DISCRIMINANT ANALYSIS

This section includes a discussion of inferential multivariate methods with specific focus on stepwise discriminant analysis, linear discriminant analysis and canonical discriminant analysis.

According to Flury (1997) and Rencher (2002), discriminant analysis is used to classify observations into two or more groups based on one or more quantitative variables. Classification is possible by either a parametric method or a non-parametric method. A parametric method is appropriate only for approximately

normal within-class distributions. The method generates a linear discriminant function, where the within-class covariance matrices are assumed as equal, or a quadratic discriminant function where the within-class covariance matrices are assumed as unequal. Non-parametric methods may be used to derive classification criteria where the distribution within each group is not assumed to have any specific distribution or is assumed to have a distribution different from the multivariate normal distribution. Non-parametric methods are also known as *distribution free* methods as they do not rely on assumptions that the data are drawn from a given probability distribution.

In discriminant analysis, the data collected are primarily from distributions different from the normal distribution. Various forms of non-normality can arise, such as qualitative variables or variables with underlying continuous but non-normal distributions. If the multivariate normality assumption is disobeyed, the use of parametric discriminant analysis may not be appropriate. When a parametric classification criterion such as a linear or quadratic discriminant function derives from a non-normal population, the resulting error rate estimates may be biased (Rencher, 2002).

Krzanowski (2000) states, that discriminant analysis may be used to predict group-memberships from a set of measurements. Discriminant analysis requires finding a transform, which gives the maximum ratio of differences between a pair of group multivariate means to the multivariate variance within the groups. An attempt is made to describe based upon maximising between group variance while minimising within group variance. The measurements' characteristics are related to form groups based on similarities of distribution in a normal-dimensional space, which are then compared to groups, which are entries by the user as known values. This enables the user to test the validity of groups based upon actual data, which have been created or to place objects into groups.

According to Rencher (2002), prior probabilities may be assigned to determine unequal sample sizes. The size of the smallest group should still be larger than the number of the predictor variables. The assumption of multivariate normality holds that the scores on predictors are randomly distributed and that the sampling distribution of any linear combination of predictors is linearly distributed. Discriminant analysis is relatively robust to non-normality due to skewness, yet not that which is due to outliers. Discriminant analysis is significantly sensitive to outliers. Variables with significant outliers necessitate transformation, which is a remedy for outliers prior to analysis. Linearity, which indicates a straight-line relationship between variables, is also assumed for discriminant analysis.

The inclusion of redundant variables in the computation of discriminant analysis normally results in automatic exclusion of multi-collinear or highly correlated variables and singular or perfect correlated variables. A tolerance test is undertaken to assess the viability of all independent variables prior to analysis with most statistical application programmes.

The purpose of discriminant analysis is therefore to classify objects (wine samples) into groups based on a set of features or measurements, that describes the objects. In general, we assign an object to one of a number of predetermined groups or origins based on observations made on the object.

The following discussion serves as an example to illustrate the use of discriminant analysis We need to determine whether wines (object) can be differentiated from one another based on several element concentration measurements. The class category, group or wine region is what needs to be identified (also called the dependent variable). Each measurement on the wine (object) is referred to as a feature that describes the object (also called independent variable). It follows that in discriminant analysis, the dependent variable (Y) is the group and the independent variables (X) are the object features that may describe the group. The dependent variable is always a

category, in our case the wine regions, while the independent variables can be any measurement. If we assume that the groups are linearly separable, we can apply linear discriminant analysis. However, stepwise discriminant analysis must be applied to the data before discriminant analysis can be calculated, to establish the best sub-set of discriminant variables for use in linear and canonical discrimination analyses (Rencher, 2002).

4.3.1. Stepwise Discriminant Analysis

According to Flury (1997) and Rencher (2002), stepwise discriminant analysis selects a sub-set of variables from the quantitative variables (data) to discriminate among classes or groups. The set of variables that makes up each class is assumed multivariate normal with a common covariance matrix. Stepwise selection is a combination of the forward and backward approaches. If there are no variables that require priority interest in the testing for significance, a data–directed search for variables that best separate the groups may be conducted.

The first approach to stepwise discriminant analysis is called **forward selection.** At the first selection step, lambda $\Lambda(y_i)$ is calculated for each individual variable and the variable with the minimum or maximum $\Lambda(y_i)$ associated partial *F*-value. During the second step, $\Lambda(y_i \mid y_1)$ is calculated for each of the *p*-1 variables, which were not entered at the first step, where y_1 indicates the first variable entered. For the second variable, the minimum or maximum $\Lambda(y_i \mid y_1)$ associated partial *F*-value is chosen. This variable adds the maximum separation to the variable entered at the first step. Let the variable entered at the second step be y_1. At the third step calculate $\Lambda(y_i \mid y_1, y_2)$ for each of the *p*-2 remaining variables and choose the one that minimises or maximises $\Lambda(y_i \mid y_1, y_2)$ the associated partial *F*-value. Continue this process until the *F*-value falls below a predetermined threshold value.

Backward selection, also referred to as elimination, is a procedure in which one begins with all the variables in the model. At each step, the variable that contributes the least to the discriminatory power of the model as measured by Wilks' Lambda is removed, as indicated by the partial *F*-value. When all remaining variables meet the criterion to stay in the model, the backward elimination process stops. See addendum I for a hypothetical example of stepwise discriminant analysis**.**

4.3.2. Linear Discriminant Analysis

According to Teknomo (2006), linear discriminant analysis is used to find linear combination of features (measurements), which best separate two or more objects (wine samples). The resulting combinations may be used as a linear classifier or more commonly a dimensionality reduction procedure. This classification technique maximises the variance (difference) between groups and minimises the variance (difference) within groups. Linear discriminant analysis is closely related to analysis of variance and regression analysis, which attempt to express a dependent variable as a linear combination of other features or measurements. Linear discriminant analysis is also closely related to principal component analysis and factor analysis in that both detect linear combinations of variables which best explain the data. Linear discriminant analysis explicitly attempts to develop the difference between the classes of data.

The term "linearly separable" suggests that the groups may be separated by a linear combination of features that describe the objects. When only two features are present, the separators between object groups will become lines. When three features are present, the separator becomes a plane. When the number of features is more than three, the separators become a hyper-plane. For example, we need to determine the probability *P* (*i* I x) that an object belongs to group *i,* given a set of measurements x. In practice, however, the quantity of *P* (*i* I x) is difficult to obtain. What we may get is *P* (x **|** *i*). This is the probability of obtaining a particular set of measurements x given that the object comes from group *i*. For

example, once we are certain that the wine originates from a specific region we can measure the object (wine). What we need to determine in our case is whether groups of the wine originate from a specific region or not, based on element measurements. Fortunately, a relationship exists between the two conditional probabilities, which are known as Bayes' Rule, which states that:

$$P(i \mid x) = \frac{P(x \mid i).P(i)}{\sum_{\forall} P(x \mid j).P(j)}$$

Bayes' classification rule is used to assign an object to the group with the highest conditional probability. This rule also minimises the total error of classification. If there are *g* groups, the Bayes' rule is to assign the object to group *i*. where *P* (*I* Ix) > *P* (j I x), for $\forall j \neq i$. Where *P* = Prior probability vector; x = features (measurements); *i, j* = groups. The prior probability *P* (*i*) is the probability about the group *i* known without making any measurements. In practice, we may assume the prior probability is equal for all groups based on the number of samples in each group (Teknomo, 2006).

In practice, however, the direct use of Bayes' rule is impractical because to obtain *P* (x | *i*), requires a large amount of data to obtain the relative frequencies of each group for each measurement. It is more practical to assume the distribution and derive the probability theoretically. If we assume that each group has multivariate normal distribution or distribution of the same general form and all groups have the same covariance matrix we obtain what is called a linear discriminant analysis formula which is $f_i = \mu_2 C^{-1} x^T_{\hat{k}} - ½ \mu_i C^{-1} \mu^T_i + 1n (p_i)$. The second part of the above equation ($\mu_i C^{-1} \mu^T_i$) is actually the Mahalanobis distance, which is the distance to measure dissimilarity among several groups. Mahalanobis distance is also known as *quadratic distance*. It measures the separation of two groups of objects. See addendum J for a hypothetical example of linear discriminant analysis.

4.3.3. Canonical Discriminant Analysis

According to Rencher (2002), the term canonical implies that something has been reduced to its simplest form. Canonical discriminant analysis is a dimension-reduction technique related to principal component analysis and canonical correlation. In canonical discriminant analysis, we find linear combinations of the quantitative variables that provide maximal separation between classes or groups. Two output data sets are produced. The first data set contains the canonical coefficients and the second data set contains the scored canonical variables. The canonical coefficients output data set can be rotated by the FACTOR procedure (also a data reduction technique), which is a statistical approach used to analyse inter-relationships among large numbers of variables and describe these variables in terms of their common underlying dimensions or factors.

Canonical discriminant analysis derives a linear combination of the variables that have the highest possible multiple correlation of two or more groups of observations with measurements on several quantitative variables. The maximal multiple correlations are called the first canonical correlation. The coefficients of the linear combination are the canonical coefficients or canonical weights. The variable defined by the linear combination is the first canonical variable or canonical component. The second canonical correlation is obtained by finding the linear combination uncorrelated with the first canonical variable that has the highest possible multiple correlation with the groups. The process of removing canonical variables can be repeated until the number of canonical variables equals the number of original variables or the number of classes minus one, whichever is the least (Rencher, 2002).

The first canonical correlation is at least as large as the multiple correlations among the groups and any of the original variables. If the original variables display high within-group correlations, the first canonical correlation may be large even if all the multiple correlations are small. In other words, the first canonical

variable may show substantial differences between the classes, even if none of the original variables does. A property of the canonical variables is that they are uncorrelated whether the correlation is calculated from the total sample or from the pooled within-class correlations. Canonical discriminant analysis is also used as a means of distinguishing among a group of samples from potentially different populations.

The purpose of group discrimination is firstly to find the axis of the greatest discrimination between groups, identified as *a priori*, secondly to test whether the means of those groups along that axis are notably different and thirdly to assign individual samples to groups. When two groups need to be separated, the technique is known as discriminant function analysis. Where there are more than two groups the same question is addressed through canonical variate analysis. See addendum K for an example of canonical discriminant analysis.

4.4. CONCLUSION OF CHAPTER 4

The general sequence for discriminant analysis procedure of data may be presented as follows:

The first step is to acquire data by means of instrumental analyses. In the second step, statistical methods are applied to the data, which include:

4.4.1. A correlation procedure, which include:

a. Simple statistics and,

b. The calculation of Pearson correlation coefficients.

4.4.2. Discriminant analysis with the application of:

a. Stepwise discriminant analysis.

b. Canonical discriminant analysis and,

c. linear discriminant analysis.

4.4.3. Principal Component Analysis (optional), with the application of:

a. Simple statistics and,

b. Correlation matrix.

4.4.4. *K*-nearest Neighbour Analysis (optional) with the application of:

a. Frequencies; Weights; Proportions; Prior Probabilities.

b. Squared distance function..

c. Posterior probability of membership in each locality and,

d. Proportion of observations in group *k.*

In this study, I have applied only stepwise discriminant analysis, linear discriminant analysis and canonical discriminant analysis, because the data were assumed normally distributed.

Acknowledgement

My sincere appreciation is conveyed to Marieta van der Ryst and Mardé Booyse for their explanation of the statistical concept.

4.5 PREVIEW OF CHAPTER 5

Chapter 5 serves to inform the reader of the wine samples collected for this study. Sampling procedures, including analytical instrumentation are discussed. A detailed discussion of the analytical procedures, which includes reagents used, calibration methods and reference samples, is presented. Wine sample analysis is discussed under qualitative analysis and quantitative analysis. The statistical methods used in this study are mentioned.

CHAPTER 5

RESEARCH DESIGN AND METHODOLOGY

5.1. INTRODUCTION

In chapter 5, I shall explain how the samples were collected; where they were collected and the type of sample collected. I shall also inform the reader on the type of analytical instrument used for the data collection. I shall explain in detail the procedure of wine analysis. The data processing methods shall also be discussed.

5.2. WINE SAMPLES

All wine samples used in the study were generously donated by the wine industry. The wine producers were personally approached and donations were on a voluntary basis. The participating wine cellars as well as the wine samples collected for use in this study are listed in Table 5.1. Wines collected for this study include the following:

- Eight Pinotage wine samples,
- Nineteen Merlot wine samples,
- Nine Shiraz wine samples,
- Twelve Cabernet Sauvignon wine samples,
- Fifteen Sauvignon blanc wine samples,
- Twenty-five Chardonnay wine samples and
- Eight Chenin blanc wine samples.

TABLE 5.1

Wine samples used in this study, with wine origin, grape variety and vintage.

Region	District	Ward	Cellar	Grape variety	Vintage
Breede River Valley	Worcester	Goudini	Deetlefs	Pinotage	99/00
	Robertson	Le Chasseur	Le Grand	Chardonnay	00/01
			Chasseur	Shiraz	00/01
		Bonnievale	Van Zylshof	Chardonnay	00/01
		No ward	Zandvliet	Shiraz	98/99/00
Coastal	No district	Constantia	Groot Constantia	Merlot	99/00

Paarl	No ward	De Zoete Inval	Sauvignon blanc	99/00/01
			Cabernet Sauvignon	97/98
	Wellington	Hildenbrand	Chardonnay	99/00
			Cabernet Sauvignon	99/00
	Franschhoek Valley	La Motte	Chardonnay	97/98
			Shiraz	97/98
	Franschhoek Valley	L'Ormarins	Sauvignon blanc	99/00/01
			Cabernet Sauvignon	95/96/97
	No ward	Rhebokskloof	Chardonnay	98/99/00
			Merlot	98/99
	No ward	Ruitersvlei	Chenin blanc	00/01
Stellenbosch	Devon Valley	Clos Malverne	Sauvignon blanc	99/00/02
	Jonkershoek	Klein Gustrouw	Merlot	00/01
			Cabernet Sauvignon	00/01
	Bottelary	Hazendal	Chardonnay	98/99/00
			Merlot	99/00/01
	No ward	Klawervlei	Chenin blanc	96/98/01
			Merlot	00/01
	No ward	Meerlust	Chardonnay	96/97/98
			Merlot	95/96/97
	Bottelary	Mooiplaas	Sauvignon blanc	98/99/00/01
			Pinotage	98/99/00
	Simonsberg-Stellenbosch	Morgenhof	Chenin blanc	98/99/00
			Merlot	98/99
	No Ward	Neethlingshof	Chardonnay	00/01
			Merlot	00/01
	Simonsberg-Stellenbosch	Nietvoorbij	Chardonnay	97/98/00
			Pinotage	97/98/99
	No ward	Vredenheim	Cabernet Sauvignon	92/93/97
	Stellenbosch	Warwick	Chardonnay	98/99/00
			Merlot	98
	Bottelary	Zevenwacht	Shiraz	98/99
			Sauvignon blanc	00/01

5.2.1. Wine Sampling Procedure

The wine samples were personally collected between 2000 and 2001, directly from estate cellars in sealed, labelled bottles. Confirmation of the wine's grape variety, origin and vintage was obtained from the cellar master or wine-maker. More than one vintage of a wine and location were included wherever possible to

facilitate data comparison. A laboratory number was allocated to each wine sample, after which it was packed into crates, taken to the laboratory and stored at 4 °C until required for analysis.

5.3. ANALYTICAL INSTRUMENTATION

Inductively-coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was the method of choice. A Varian Liberty series II ICP-AES, equipped with a Varian grating with 1800 lines/mm for the sequential mode in the Paschen-Runge configuration, a Varian auto-sampler, Model SPS-5 and a concentric silica torch with a V-groove nebuliser, were used to determine the element composition of the collected wine samples. The system was managed by Varian “Plasma 96” software version.

5.3.1. Operating Conditions

Operating conditions were as follows: Pump rate, 15 revolutions per minute; forward radio frequency power, 1000 watt; argon flow in the plasma and nebuliser, 2 litres per minute; spray chamber temperature, ambient; observation height, 10 millimetres above load coil; sample size, 1.5 ml; sample uptake rate, 3.0 millilitres per minute; purge time, 0.5 minutes; rinse time, 15 seconds; signal integration time, 0.167 minutes; wash cycle, 0.5 minutes with de-ionised water as the rinsing solution.

5.4. ANALYTICAL PROCEDURE

5.4.1. Reagents Used

The multi-element solution, Merck catalogue no. 10365 and ethyl alcohol, Merck, catalogue no. 100983, Merck/NT Laboratories, South Africa, supplied both analytical grades.

De-ionised water with conductivity between 0.06 and 0.08 m S/m was prepared by passing single-distilled water through a Millipore milli-R020 system, which was equipped with two ion exchange filters. Water purity was verified on a monthly

basis, which formed part of the analytical laboratory's good laboratory practice scheme. The water purification system was supplied and serviced by Microsep, South Africa.

All glassware used in the procedure was rinsed with de-ionised water, washed with 3% nitric acid solution, rinsed three times with de-ionised water. All glassware was allowed drying in a drying oven at 80 ºC.

5.4.2. Calibration

Calibration curves for the measurements of emission from standard solutions were created. Calibration standards were prepared from a stock solution with a concentration of 100 mg/mL multi-element solution, using sequential dilutions with a solution containing 13% ethanol in de-ionised water and stored at room temperature. Most South African red and white wines contain between 12-15 percent ethanol. The purpose of this matrix matching procedure was to minimise the viscosity differences between the wine samples and the calibration standard solutions in terms of ethanol. Samples with high concentrations of organic matter such as wine can have an effect on the plasma stability. However, due to the high temperature of the plasma, less matrix interferences transpire. All calibration standards were prepared immediately before analysis. Results of the semi-quantitative measurements (minimum and maximum values) were used to determine the calibration range values for the quantitative analysis. The highest values were identified, after which the values were rounded off and used as the highest calibration point for a particular element. Four to five concentration points for calibration curves were established.

All blank and standard solutions were prepared with a solution of de-ionised water and ethanol. An aqueous ethanol concentration of 13% (volume per volume) was used for matrix matching of the alcohol content of the undiluted wine samples.

5.4.3. Reference Samples

Water samples (no ethanol added) of known chemical composition supplied by Agrilasa, Private Bag X79, Pretoria, 0001 were used as reference samples in the absence of appropriate reference material for wine samples. The water reference samples were analysed at intervals of ten wine samples. Refer to Addenda B and C on CD-ROM.

5.5. WINE ANALYSIS

5.5.1. Qualitative Analyses (Multi-element Scan)

Initially, all wine samples were analysed qualitatively for element composition by means of a multi-element scan using ICP-OES. The atomic emission lines used to determine each element were chosen according to Eschnauer *et al.* (1989). The selected atomic lines are also those most frequently used for routine analysis of each element in a variety of matrices (Maartens, R., Dept. Physics, University of Stellenbosch, personal communication, 2004).

5.5.2. Quantitative Analysis

After qualitative analyses were performed, the results were visually examined. All measurements, which were below the limit of detection, indicated by negative values and zero values, due to rounding off (Varian software) were disregarded. The wine samples were re-analysed quantifying the remaining elements.

5.6. STATISTICAL METHODS

Stepwise discriminant analysis, canonical discriminant analysis and linear discriminant analysis were applied to the element data acquired from the ICP-AES.

Each grape variety was considered as a data set on its own. In each data set, a univariate procedure, normal probability plots and Shapiro-Wilk tests were used to test the normality assumption in each variable or element. Two-dimensional

scatter plots were used to verify bivariate elliptical shapes and multivariate normalities. These analyses indicated that normality assumption was valid.

Stepwise discriminant analysis (SDA) was used to select a subset of variables from the initial variables obtained. The subset of variables contained those elements which best differentiate or discriminate between geographic origins. Stepwise discriminant analysis is considered a preliminary analysis and the resulting subset of elements from SDA was used in canonical discriminant analysis (CDA) and linear discriminant analysis (LDA).

Canonical discriminant analysis is a parametric dimension-reduction technique related to principal component analysis. Canonical discriminant analysis discriminates between a given set, group or geographic origin, based on a few linear combinations of variables or elements. These linear combinations are known as canonical variables, which can be plotted on an axis to obtain a two-dimensional graph, which depicts the discrimination between groups. The first two canonical variables are plotted against each other, since they account for the most significant discrimination between groups. Canonical discriminant analysis was applied to data for each grape variety using the subset of discriminating variables identified by SDA.

Linear discriminant analysis is also a parametric technique used to differentiate between groups. Linear discriminant analysis provides a discrimination function, which makes it a useful tool for classification purposes (Srivastava & Carter, 1983). Linear discriminant analysis was performed on the same data set as the CDA, using the same variables. Where discrimination between groups is possible, LDA will classify the data into the correct groups, and consequently obtain its classification accuracy.

All the statistical analyses in this study were done using Statistical Analysis System (SAS) Base and Stats, version 8.2 (SAS Institutes, 1999).

5.7. PREVIEW OF CHAPTER 6

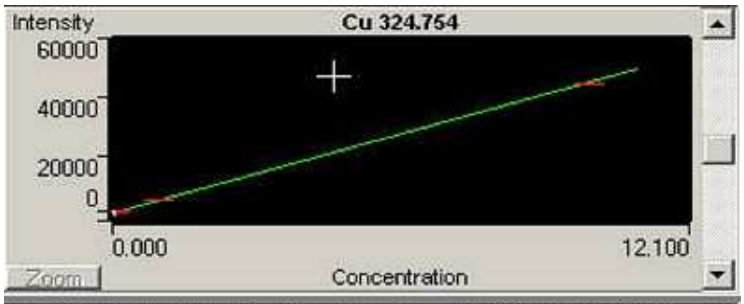
Chapter 6 shows calibration results of two elements established for quantitative analyses. Quality control procedures are noted. Results of the wine sample analyses by ICP-AES are discussed. The statistical results are discussed in detail under the following headings: stepwise discriminant analysis, canonical discriminant analysis and linear discriminant analysis.

CHAPTER 6

RESULTS AND DISCUSSION

6.1. CALIBRATION

Prior to analysis, calibration curves were established for quantitative analysis (not all calibration curves are shown). The calibration curves were linear over four to five orders of magnitude with a correlation coefficient of between 0.9974 and 0.9999. Calibration plots were obtained using standard solutions of 100, 200, 500, 1000 and 2000 mg/L for Potassium; 10, 20, 50, 100 and 150 mg/L for Magnesium and Calcium and for the remaining elements, 0.01, 0.05, 0.1, 1.0, 10 and 50 mg/L. Each standard solution was analysed twice to establish a mean value. Figures 6.1 and 6.2 represent examples of calibration curves for copper and calcium standards, respectively.



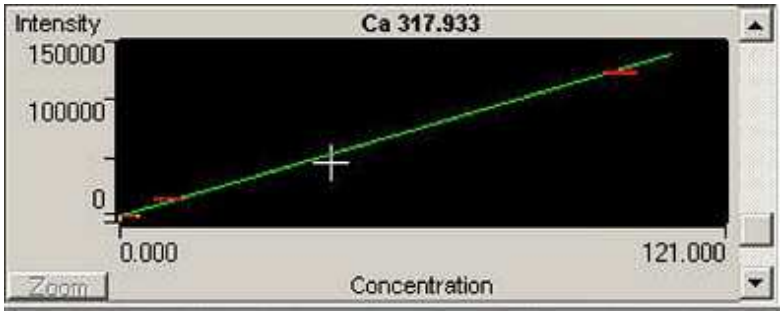
Cu 324.754 Calibration (mg/L) on May 12 2004, 12:23:06 pm

Standard	Flags	Int (c/s)	Std Conc
0.01 ppm	e	0.0	0.01000
0.05 ppm	---	281.7	0.05000
0.1 ppm	---	483.2	0.10000
1 ppm	---	4480.3	1.00000
10 ppm	---	44115.5	10.0000
Blank	---	6.6	0.00000

Correlation Coefficient	0.999999
Status	Calibrated.
Curve Type	Linear
Curve Coefficient 1:	6.593
Curve Coefficient 2:	4494.703
Blank Offset	6.6

FIGURE 6.1

Calibration Curve for Copper (Mean values)



Ca 317.933 Calibration (mg/L) on May 6 2004, 11:07:56 am

Standard	Flags	Int (c/s)	Std Conc
0.1 ppm	e	0.0	0.10000
1 ppm	e	0.0	1.00000
10 ppm	---	13166.0	10.0000
100 ppm	---	122452.2	100.000
Blank	---	3.8	0.00000

Correlation Coefficient	0.999974
Status	Calibrated.
Curve Type	Linear
Curve Coefficient 1:	3.805
Curve Coefficient 2:	1259.975
Blank Offset	3.8

FIGURE 6.2

Calibration Curve for Calcium (Mean values)

6.2. QUALITY CONTROL

Regular participation in an Inter-laboratory water, soil, and Plant Quality Assurance Scheme (Private Bag X79, Pretoria, 0001) confirmed the competency of the laboratory and the reliability of results. Refer to Addendum A, on CD-ROM for quality control results.

6.3. WINE ANALYSES

There are four important details, which have to be considered and attended to before sample analysis can be performed. The first is that the samples must be in solution. Secondly, the sample-containing solution must be stable. Thirdly, care must be taken to ensure that the analyte concentration falls within the working range of the instrument. Fourthly, ensure that the sample-containing solution can be nebulised in a reproducible manner. All the above criteria were verified before analyses commenced.

6.3.1. Qualitative Analyses (Multi-element Scan)

Qualitative information is related to the wavelength at which the radiation is emitted. All samples were initially subjected to qualitative analysis.

Samples were obtained by shaking the sealed bottles, which were inverted three to four times, before the capsule and cork were removed with a corkscrew. The bottleneck was wiped clean with paper towel and the wine was poured (ca. 50 ml) directly into an ICP-AES glass sample tube after the first few millilitres of wine ("Rinsing" the bottle neck) were discarded. All glass tubes as well as the sample racks were coded. The initial number of elements analysed and the atomic emission lines used to determine each element are listed in Table 6.1. The selected elements and atomic lines were chosen according to Eschnauer *et al.*, 1989. The selected atomic lines are those most frequently used for routine determination of each element in a variety of matrices (Maartens, R., Dept. Physics, University of Stellenbosch, personal communication, 2004). Refer to Addenda B and C on CD-ROM for qualitative data.

TABLE 6.1

Selected elements with atomic lines for qualitative analysis

Element	Atomic line (nm)	Element	Atomic line (nm)	Element	Atomic line (nm)
Silver	328.07	Iron	259.94	Platinum	265.94
Aluminium	396.15	Potassium	769.90	Antimony	252.85
Arsenic	188.97	Lanthanum	408.67	Selenium	196.09
Gold	267.59	Lithium	670.78	Silicon	251.61
Boron	208.95	Magnesium	285.21	Tin	189.92
Barium	455.51	Manganese	257.61	Strontium	407.77
Beryllium	234.86	Molybdenum	202.03	Titanium	334.94
Calcium	317.93	Sodium	589.59	Vanadium	292.46
Cadmium	214.43	Nickel	231.60	Yttrium	371.03
Cobalt	228.61	Phosphorous	214.91	Zinc	213.85
Chromium	267.71	Lead	220.35	Zirconium	343.82
Copper	324.76	Palladium	340.45		

6.3.2. Quantitative Analysis

Quantitative information is related to the amount of electromagnetic radiation that is emitted. After qualitative analyses were performed, the results were visually examined. Measurements below the limit of detection [indicated by negative values and zero values, due to rounding off (Varian software)] were disregarded. After visual examination of the results, 12 elements were disregarded due to values below the detection limits indicated as negative values or non-detection for all the wine samples. Table 6.2 lists the elements selected for quantitative analysis.

TABLE 6.2

Selected elements with atomic lines for quantitative analysis

Element	Atomic line (nm)	Element	Atomic line (nm)
Aluminium	396.15	Phosphorous	214.91
Arsenic	188.97	Lead	220.35
Boron	208.95	Palladium	340.45
Barium	455.51	Sodium	589.59
Calcium	317.93	Antimony	252.85
Copper	324.76	Selenium	196.09
Iron	259.94	Silicon	251.61
Potassium	769.90	Tin	189.92
Lanthanum	408.67	Strontium	407.77
Magnesium	285.21	Titanium	334.94
Manganese	257.61	Zinc	213.85
Molybdenum	202.03		

The wine samples were re-analysed, quantifying the remaining 23 elements. The same sample procedure was followed as in qualitative analysis (Section 6.2.1). Fresh wine samples were poured from each bottle into clean sample tubes and placed onto the auto-sampler rack. A typical analysis batch comprised, one blank sample, which is a 13% aqueous ethanol solution, six water reference samples (one water reference sample placed after every tenth wine samples) and fifty wine samples. Each wine sample was analysed in duplicate to establish a mean

concentration. Final quantification was performed using the quantitative programme of Varian built-in software of the ICP-AES instrument. Refer to Addenda D and E on the CD-ROM for ICP-AES quantitative data. All quantitative data were submitted for statistical analysis. Table 6.3 lists the average elemental concentrations with standard deviations of the analysed wines.

Table 6.3

Average Elemental Concentrations in mg/L and Standard Deviations for 23 Elements in White Wines and 22 Elements in Red Wines from Stellenbosch, Paarl Valley and the Breede River Valley Wine Regions.

	Sauvignon blanc		**Chardonnay**		
Element	Stellenbosch	Paarl Valley	Stellenbosch	Paarl Valley	Breede River Valley
Mg	65.77 (±21.79)	42.19 (±0.94)	77.43 (±13.91)	60.51(±19.52)	70.02 (±17.14)
K	977.15 (±375.76)	821.95 (342.87)	1132.39 (±204.38)	958.67 (±234.85)	1125.65 (±63.28)
Ca	10.93 (±0.21)	9.71 (±0.17)	6.21 (±0.38)	7.29 (±0.03)	10.23 (±0.25)
Ti	0.09 (±0.07)	0.02 (±0.02)	0.05 (±0.03)	0.09 (±0.05)	0.04 (±0.02)
Mn	0.83 (±0.01)	0.45 (±0.01)	0.15 (±0.08)	0.13 (±0.04)	0.86 (±0.01)
Cu	0.33 (±0.02)	0.45 (±0.03)	0.71 (±0.05)	0.57 (±0.04)	0.12 (±0.09)
Zn	0.41 (±0.02)	0.18 (±0.01)	0.11 (±0.12)	0.42 (±0.01)	0.33 (±0.01)
B	0.34 (±0.11)	0.22 (±0.01)	0.27 (±0.07)	0.25 (±0.07)	0.41(±0.01)
Al	0.37 (±0.17)	0.34 (±0.03)	0.44 (±0.17)	0.31 (±0.06)	0.31 (±0.02)
Si	0.81 (±0.18)	0.81 (±0.63)	1.46 (±1.22)	1.47 (±0.68)	1.61 (±0.21)
P	N D	N D	N D	N D	N D
As	N D	N D	N D	N D	N D
Se	0.16 (±0.12)	0.32 (±0.036)	0.52 (±0.92)	0.24 (±0.08)	0.21 (±0.17)
Sb	0.033 (±0.75)	0.032 (±2.57)	0.033 (±3.42)	0.061 (±2.81)	0.066 (±0.91)
Sn	0.05 (±0.02)	0.06 (±0.05)	0.04 (±0.03)	0.04 (±0.01)	N D
Pd	N D	N D	N D	N D	N D
Mo	N D	N D	N D	N D	N D
Sr	0.28 (0.02)	0.21 (±0.02)	0.25 (±0.13)	0.21 (±0.09)	0.34 (±0.04)
Ba	0.04 (±0.02)	0.04 (±0.02)	0.05 (±0.03)	0.05 (±0.02)	0.04 (±0.01)
La	N D	N D	N D	N D	N D
Pb	N D	N D	N D	N D	N D
Fe	0.15 (±0.04)	0.24 (±0.19)	0.13 (±0.13)	0.11 (±0.03)	0.09 (±0.063)
Na	N D	N D	N D	N D	N D

Table 6.3 Continued

Average Elemental Concentrations in mg/L and Standard Deviations for 23 Elements in White Wines and 22 Elements in Red Wines from Stellenbosch, Paarl Valley and the Breede River Valley Wine Regions.

	Chenin blanc			Pinotage	
Element	Stellenbosch	Paarl Valley	Element	Stellenbosch	Breede River Valley
Mg	63.96 (±5.07)	93.26 (±6.66)	Na	108.19 (±34.43)	54.61 (±29.88)
K	1200.25 (±174.3)	750.65 (±94.19)	Mg	62.464 (±12.48)	57.50 (±7.05)
Ca	8.93 (±0.08)	8.45 (±0.13)	K	1479.78) (±270.4)	1485.23 (± 575.68)
Ti	0.14 (±0.07)	0.14 (±0.01)	Ca	8.26 (±2.64)	6.94 (±0.09)
Mn	0.89 (±0.07)	0.87 (±0.07)	Sr	0.38 (±1.82)	0.26 (±11.87)
Cu	0.29 (±0.01)	0.46 (±0.01)	Ba	0.07 (±0.038)	0.061 (±0.31)
Zn	0.21 (±0.02)	0.17 (±0.01)	Ti	N D	N D
B	0.24 (±0.3)	0.31 (±0.07)	Mo	N D	N D
Al	0.40 (±0.08)	0.51 (±0.01)	Mn	0.11 (±0.02)	0.87 (±0.48)
Si	0.74 (±0.05)	1.05 (±0.39)	Fe	0.21 (±0.02)	3.33 (±2.41)
P	N D	N D	Pd	0.01 (±0.002)	0.10 (±0.001)
As	N D	N D	Cu	0.15 (±0.07)	0.71 (±0.42)
Se	0.23 (±0.09)	0.09 (±0.020)	Zn	0.03 (±0.01)	0.21 (±0.09)
Sb	0.031 (±0.22)	0.042 (±1.58)	Pb	N D	N D
Sn	0.01 (±0.02)	0.03 (±0.01)	Sn	N D	N D
Pd	N D	N D	Sb	0.041 (±1.97)	0.04 (±0.29)
Mo	N D	N D	As	N D	N D
Sr	0.19 (±0.03)	0.41 (±0.11)	Se	0.18 (±0.05)	0.23 (±0.32)
Ba	0.04 (±0.01)	0.09 (±0.03)	P	2.21 (±0.91)	2.63 (±0.88)
La	N D	N D	Si	1.01 (±0.48)	0.99 (±0.72)
Pb	N D	N D	Al	0.33 (±0.1)	3.28 (±0.19)
Fe	0.11 (±0.01)	0.17 (±0.01)	B	0.32 (±0.06)	2.48 (±0.38)
Na	N D	N D			

N D = Not Detected

Table 6.3 Continued

Average Elemental Concentrations in mg/L and Standard Deviations for 23 Elements in White Wines and 22 Elements in Red Wines from Stellenbosch, Paarl Valley and the Breede River Valley Wine Regions.

	Merlot		**Cabernet Sauvignon**	
Element	Stellenbosch	Paarl Valley	Stellenbosch	Paarl Valley
Na	78.35 (±41.78)	81.39 (±27.66)	113.96 (±22.46)	105.05 (±66.24)
Mg	70.22 (±5.78)	77.96 (±16.63)	70.05 (±8.61)	81.59 (±22.47)
K	1765.61 (± 212.07)	1324.74 (±417.84)	1665.44 (± 519.18)	1789.46 (±718.96)
Ca	6.85 (±0.74)	6.57 (±0.93)	6.51 (±1.23)	9.17 (±4.42)
Sr	0.37 (±1.53)	0.51 (±0.14)	0.38 (±0.49)	0.36 (±1.750)
Ba	0.08 (±0.02)	0.021 (±0.67)	0.11 (±0.08)	0.13 (±0.1)
Ti	N D	N D	N D	N D
Mo	N D	N D	N D	N D
Mn	0.82 (±0.04)	0.17 (±0.11)	0.11 (±0.040)	0.10 (±0.03)
Fe	0.19 (±0.04)	2.58 (±0.12)	0.30 (±0.15)	0.44 (±0.39)
Pd	0.01 (±0.002)	0.05 (±0.01)	N D	N D
Cu	0.88 (±0.03)	0.19 (±0.140)	0.11 (±0.03)	0.74 (±0.060)
Zn	0.03 (±0.01)	0.18 (±0.80)	0.04 (±0.03)	0.02 (±0.01)
Pb	N D	N D	N D	N D
Sn	N D	N D	N D	N D
Sb	0.04 (±1.59)	0.08 (±0.71)	0.02 (±0.94)	0.04 (±2.36)
As	N D	N D	N D	N D
Se	0.16 (±0.08)	0.16 (±0.19)	0.19 (±0.02)	0.16 (±0.12)
P	2.76 (±0.49)	2.11 (±0.41)	2.80 (±0.31)	2.00 (±0.91)
Si	0.98 (±0.38)	2.14 (±0.17)	0.60 (±0.23)	1.08 (±0.57)
Al	0.28 (±0.04)	0.34 (±0.53)	0.38 (±0.03)	0.51 (±0.29)
B	0.34 (±0.13)	0.35 (±0.22)	0.34 (±0.02)	0.29 (±0.09)

N D = Not Detected

Table 6.3 Continued

Average Elemental Concentrations in mg/L and Standard Deviations for 23 Elements in White Wines and 22 Elements in Red Wines from Stellenbosch, Paarl Valley and the Breede River Valley Wine Regions.

	Shiraz		
Element	Stellenbosch	Paarl Valley	Breede River Valley
Na	114.99 (±6.34)	102.65 (±73.76)	23.19 (±19.51)
Mg	74.63 (±7.89)	54.91 (±3.11)	54.82 (±14.93)
K	1059.23 (± 72.78)	1571.75 (± 24.32)	1227.14 (±675.44)
Ca	7.96 (±0.39)	6.60 (±1.71)	10.82 (±4.91)
Sr	0.51 (±0.07)	0.36 (±1.11)	0.53 (±1.17)
Ba	0.05 (±0.01)	0.05 (±0.02)	0.09 (±0.02)
Ti	N D	N D	N D
Mo	N D	N D	N D
Mn	0.74 (±0.01)	0.18 (±0.05)	0.78 (±0.02)
Fe	0.26 (±0.04)	0.18 (±0.01)	0.11 (±0.05)
Pd	N D	N D	N D
Cu	0.96 (±0.01)	0.16 (±0.17)	0.32 (±0.03)
Zn	0.05 (±0.01)	0.03 (±0.01)	0.02 (±0.01)
Pb	N D	N D	N D
Sn	N D	N D	N D
Sb	0.03 (±0.24)	0.04 (±5.39)	0.05 (±0.19)
As	N D	N D	N D
Se	0.17 (±0.02)	0.24 (±0.01)	0.24 (±0.03)
P	1.81 (±0.06)	2.04 (±1.36)	2.15 (±0.11)
Si	0.83 (±0.01)	1.22 (±1.33)	1.00 (±0.41)
Al	0.44 (±0.02)	0.28 (±0.03)	0.15 (±0.19)
B	0.28 (±0.01)	0.36 (±0.05)	0.32 (±0.05)

6.4. STATISTICAL ANALYSIS

6.4.1. Stepwise Discriminant Analysis (Region and Cultivar Data)

Stepwise discriminant analysis identified thirteen discriminant elements or variables, which had the most effective discriminatory powers and that provided the best combinations for subsequent analysis. Refer to Table 6.4 for concentrations levels and minimum and maximum values of the stepwise-

selected elements. Table 6.4 lists the concentration levels of selected elements in the analysed red and white wine samples

TABLE 6.4

Mean concentrations of selected elements in collected red and white wines, Including minimum, maximum and standard deviations.

White wine (n = 48)					Red wine (n = 48)				
Element	Mean (mg/L)	S. D.	Min. (mg/L)	Max. (mg/L)	Element	Mean (mg/L)	S. D.	Min. (mg/L)	Max. (mg/L)
Magnesium	67.9557	18.143	38.481	101.150	Sodium	8.226	4.191	0.655	18.855
Boron	0.297	0.087	0.166	0.492	Magnesium	67.382	13.132	43.890	95.32
Aluminium	0.392	0.147	0.215	0.886	Potassium	1605.127	286.35	730.300	2449.23
Selenium	0.225	0.172	0.020	0.990	Strontium	0.391	0.145	0.159	0.720
Tin	0.067	0.092	0.011	0.620	Iron	0.260	0.189	0.065	0.578
Barium	0.053	0.022	0.025	0.119	Copper	0.096	0.076	0.001	0.289
					Zinc	0.310	0.192	0.030	0.571
					Phosphorous	2.282	0.706	0.656	4.082
					Aluminium	0.329	0.149	0.018	0.648
					Barium	0.095	0.058	0.033	0.251

S. D. = Standard deviation; Min. = Minimum; Max. = Maximum; n = number of samples; mg/L = milligrams per litre

Stepwise discriminant analysis also provided squared roots, *F*-values and *P*-values of the selected variables (Refer to Table 6.5). According to Krzanowski (1987), the *F*-values could be used as indicators for inclusion in the subset, even though the probabilities of the *F*-values are not significant. Krzanowski suggests using the largest *F*-values for inclusion. It is known that key elements among chemical data sets may offer an increased reliability. Usually, a sample to variable ratio higher than three would be ideal (Kwan & Kowalski, 1978). Owing to the low ratio in this study, selecting those variables, which exhibited high *F*-values, determined by the initial one-way analysis of variance, reduced the number of variables.

TABLE 6.5

Summary of the variables, Including R^2, *F* and *P*-values that best discriminate among wine regions, districts and wards for each grape variety obtained from SDA.

Grape variety	Discriminating variables	R^2	*F*-values	*P*-values
Chardonnay	Selenium	0.8848	23.04	<0.0001
	Boron	0.7858	10.40	<0.0001
	Tin	0.4835	2.03	0.1343
Chenin blanc	Tin	0.9602	120.77	0.0001
	Magnesium	0.8933	50.21	0.0004
	Barium	0.8889	32.01	0.0048
Sauvignon blanc	Boron	0.9473	65.91	<0.0001
	Aluminium	0.8909	27.22	<0.0001
	Magnesium	0.6045	4.59	0.0327
Cabernet Sauvignon	Magnesium	0.8696	11.67	0.0032
	Iron	0.7178	3.81	0.0709
	Potassium	0.7974	4.92	0.0553
Merlot	Sodium	0.7036	8.31	0.0012
	Barium	0.6961	7.45	0.0024
	Copper	0.6364	4.81	0.0172
	Potassium	0.6074	4.464	0.0170
	Phosphorous	0.5849	3.52	0.0483
	Zinc	0.5719	3.01	0.0785
	Iron	0.5503	2.45	0.1309
	Strontium	0.5191	2.16	0.1645
Pinotage	Magnesium	1.000	Infinity	<0.0001
	Potassium	1.000	Infinity	<0.0001
	Zinc	1.000	31006	0.0004
	Iron	1.000	89129	0.0024
Shiraz	Sodium	0.9965	188.53	0.0053
	Aluminium	0.9779	73.74	0.0001
	Potassium	0.9750	52.09	0.0012
	Magnesium	0.9533	20.39	0.0169

R^2 = R squared; *P* = Probability of the F-value; *F* = Statistics of decision-making criteria.

The selected variables of all seven grape varieties were subjected to CDA and LDA, to establish whether discrimination between wines regions could be achieved using these methods. Results of CDA are discussed in section 6.4.2 below for each grape variety.

6.4.2. Canonical Discriminant Analysis (Region and Cultivar Data)

Pinotage

Total dispersion of a 100% was defined with the first two canonical functions. Eigenvalues for the two functions were 29.5744 and 2.5158, respectively, and the canonical correlations were 0.98 and 0.85, respectively. The total canonical structure coefficients of the two functions were potassium; Can. 1 (= Canonical variable) = -0.410373 and Can. 2 = -0.220561. Magnesium; Can. 1 = 0.475670 and Can. 2 = 0.000039. Zinc; Can. 1 = 0.453714 and Can. 2 = -0.088616. Iron; Can. 1 = -0.162982 and Can. 2 = 0.634389.

The discriminant analysis was carried out considering the elements evaluated for Pinotage and the graphical representation shown in Figure 6.3, yielded a pattern of point-distribution in which it distinguished three groups, corresponding to the wine cellars within the three localities. Magnesium and zinc had the highest total canonical structure coefficients on the first canonical variable, which were most likely responsible for the discrimination between geographic origins in the direction of canonical variable one. Iron had the highest total canonical structure coefficient on the second canonical variable and was therefore most likely responsible for the discrimination between geographic origins in the direction of canonical variable two.

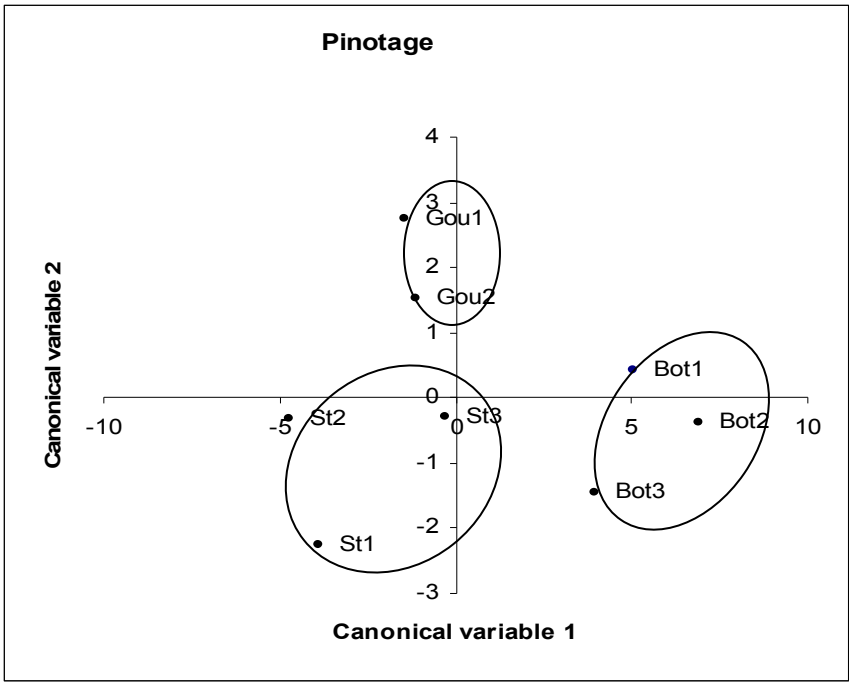


FIGURE 6.3

Plots of the first two canonical variables for Pinotage showing separation of wines from Bottelary (Bot), Stellenbosch (St) [Coastal] and Goudini [Rawsonville] (Gou) [Breede River Valley] areas based on magnesium, potassium, zinc and iron.

Merlot

Canonical variables one and two explained 74.56% of the total dispersion. Eigenvalues for the two functions were 16.1816 and 6.3011 respectively and the canonical correlations were 0.97 and 0.93, respectively. The total canonical structure coefficients of the two functions were sodium; Can. 1 = 0.00781 and Can. 2 = -0.341289. Barium; Can. 1 = 0.797452 and Can. 2 = 0.089579. Potassium; Can. 1 = -0.385510 and Can. 2 = 0.614726. Copper, Can. 1 = 0.515097 and Can. 2 = -0.318446. Phosphorous; Can. 1 = -0.387246 and Can. 2 = -0.456687. Zinc; Can. 1 = -0.256775 and Can. 2 = 0.529199. Iron; Can. 1 = 0.190121 and Can. 2 = -0.189791. Strontium; Can. 1 = 0.281895 and Can. 2 = -0.000647.

The discriminant analysis carried out considering the elements evaluated for Merlot, with graphical representation shown in Figure 6.4, yielded a pattern of point-distribution in which it distinguished four groups, corresponding to the wine cellars within the four localities. Barium and copper had the highest total canonical structure coefficients on the first canonical variable, which were most likely responsible for the discrimination between geographic origins in the direction of canonical variable one. Potassium and zinc had the highest total canonical structure coefficients on the second canonical variable and were therefore most likely responsible for the discrimination between geographic origins in the direction of canonical variable two.

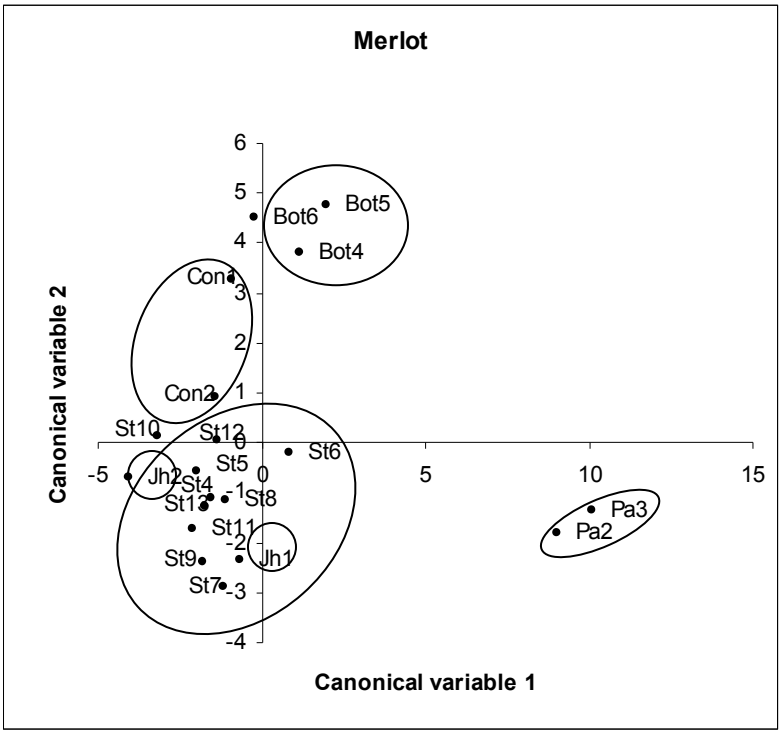


FIGURE 6.4

Plots of the first two canonical variables for Merlot, showing separation of wines from Bottelary [Kuilsriver] (Bot), Constantia [Cape Point] (Con), Jonkershoek (Jh), Stellenbosch [Faure] and Paarl (Pa) [Coastal] areas based on sodium, barium, potassium, copper, phosphorous, zinc, iron and strontium .

Shiraz

Total dispersion of 99.68% was defined in the first two canonical functions. Eigenvalues for the two functions were 569.5975 and 12.9652, respectively, and the canonical correlations were 0.99 and 0.96, respectively. The total canonical structure coefficients of the two functions were zinc; Can. 1 = 0.121767 and Can. 2 = 0.667445. Potassium; Can. 1 = 0.970903 and Can. 2 = 0.072978. Magnesium; Can. 1 = 0.581729 and Can. 2 = 0.807516 iron; Can. 1 = 0.580503 and Can. 2 = 0.605304. The discriminant analysis was carried out considering the elements evaluated for Shiraz, with the graphical representation shown in Figure 6.5, yielded a pattern of point-distribution in which it distinguished four groups, corresponding to the wine cellars within the four localities. Iron and potassium had the highest total canonical structure coefficients on the first canonical variable, which were most likely responsible for the discrimination between geographic origins in the direction of canonical variable one. Although magnesium showed the highest total canonical structure coefficient on the second canonical variable, the first Eigenvalue indicated that the discrimination was primarily on the first canonical variable.

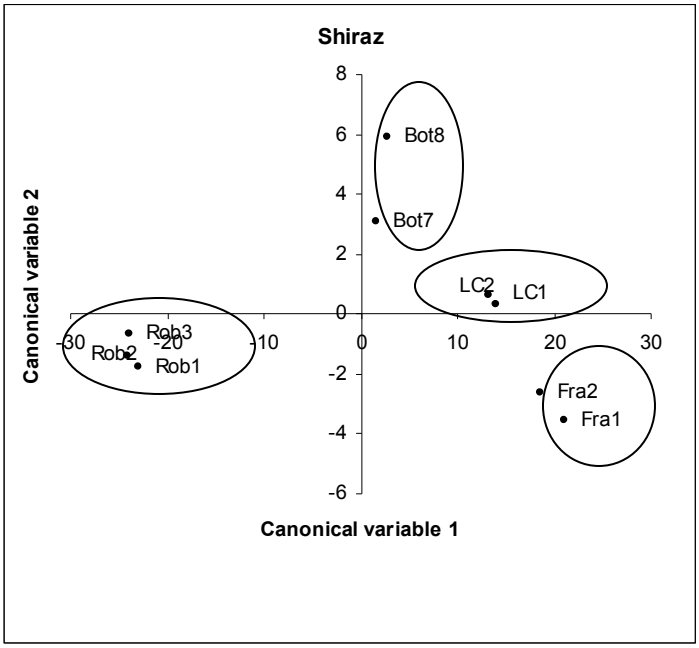


FIGURE 6.5

Plots of the first two canonical variables for Shiraz showing separation of wines from Bottelary [Kuilsriver] (Bot), Franschhoek (Fra) [Coastal], Le Chasseur [Rob] (LC) and Robertson [Ashton] (Rob) [Breede River Valley] areas based on of zinc, potassium, magnesium and iron.

Cabernet Sauvignon

Canonical variables one and two explained 99.70% of the total dispersion. Eigenvalues for the two functions were 27.2419 and 3.3529, respectively, and the canonical correlations were 0.98 and 0.87, respectively. The total canonical structure coefficients of the two functions were magnesium; Can. 1 = 0.941335 and Can. 2 = 0.088585. Iron; Can. 1 = 0.749908 and Can. 2 = 0.334620. Potassium; Can. 1 = 0.622417 and Can. 2 = 0.614181. The discriminant analysis was carried out considering the elements evaluated for Cabernet Sauvignon, with the graphical representation shown in Figure 6.6, yielded a pattern of point-distribution in which it distinguished five groups, corresponding to the wine cellars within the five localities. Magnesium and iron had the highest total canonical structure coefficients on the first canonical variable, which were most likely responsible for the discrimination between geographic origins in the direction of canonical variable one. Note that Stellenbosch cellars can only be distinguished by the second canonical variable, which was most likely due to the relatively high canonical structure coefficient of potassium.

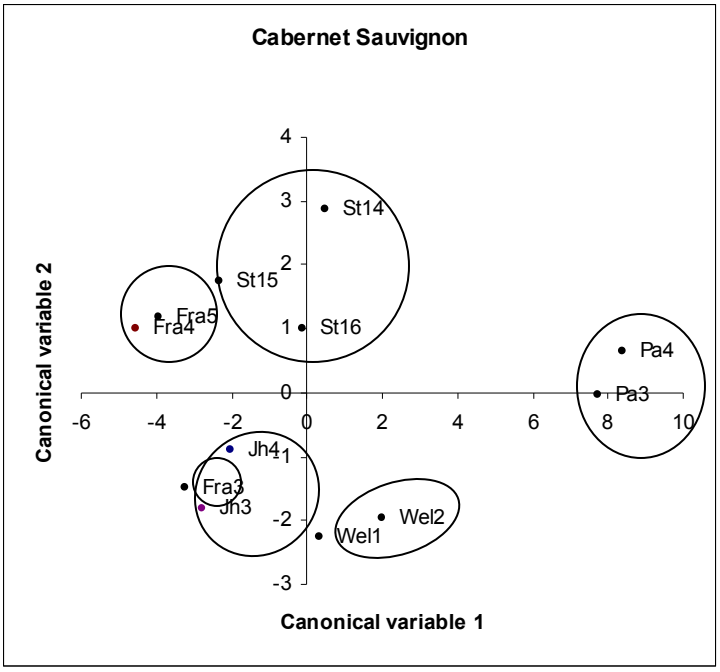


FIGURE 6.6

Plots of the first two canonical variables for Cabernet Sauvignon showing separation of wines from Paarl (Pa), Wellington (Wel), Stellenbosch [Faure] (St), Franschhoek (Fra) and Jonkershoek [Stell] (Jh) [Coastal] areas based on iron, potassium and magnesium.

Sauvignon blanc

Canonical variables one and two explained 99.95% of the total dispersion. Eigenvalues for the first canonical variable was 82.9891 and 3.997, respectively. The second canonical correlation was 0.99 and 0.89, respectively. The total canonical structure coefficients of the two functions were aluminium; Can. 1 = 0.572483 and Can. 2 = 0.791756. Boron; Can. 1 = 0.888081 and Can. 2 = -0.458201. Magnesium; Can. 1 = 0.968144 and Can. 2 = -0.089245.

The discriminant analysis was carried out considering the elements evaluated for Sauvignon blanc, with the graphical representation shown in Figure 6.7, yielded a pattern of point distribution in which it distinguished three groups, corresponding to the wine cellars within the three localities. Magnesium and boron had the highest total canonical structure coefficient on the first canonical variable, which were most likely responsible for the discrimination between geographic origins in the direction of canonical variable one.

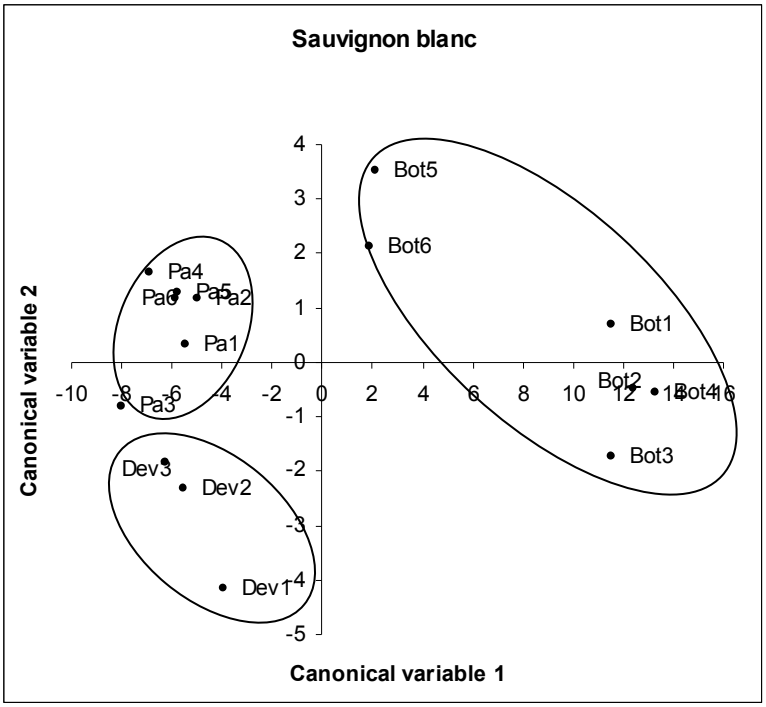


FIGURE 6.7

Plots of the first two canonical variables for Sauvignon blanc showing separation of wines from Bottelary [Kuilsriver] (Bot), Paarl (Pa) and Devon Valley [Stell] (Dev) [Coastal] areas based on aluminium, boron and magnesium.

Chardonnay

Canonical variables one and two explained 93.32% of the total dispersion. Eigenvalues for the two variables were 8.3059 and 3.9140, respectively, and the canonical correlations were 0.94 and 0.89, respectively. The total canonical structure coefficients of the two functions were boron; Can. 1 = -0.202207 and Can. 2 = 0.940302. Selenium; Can. 1 = 0.959823 and Can. 2 = 0.279584. Tin; Can. 1 = 0.118957 and Can 2 = -0.623062. The discriminant analysis was carried out considering the elements evaluated for Chardonnay, with the graphical representation shown in Figure 6.8, yielded a pattern of point-distribution in which it distinguished seven groups, corresponding to the wine cellars within the seven localities. Selenium had the highest total canonical structure coefficient on the first canonical variable, which was most likely responsible for the discrimination between geographic origins in the direction of canonical variable one. Boron had the highest total canonical structure coefficient on the second canonical variable, which was most likely responsible for the discrimination between geographic origins in the direction of canonical variable two.

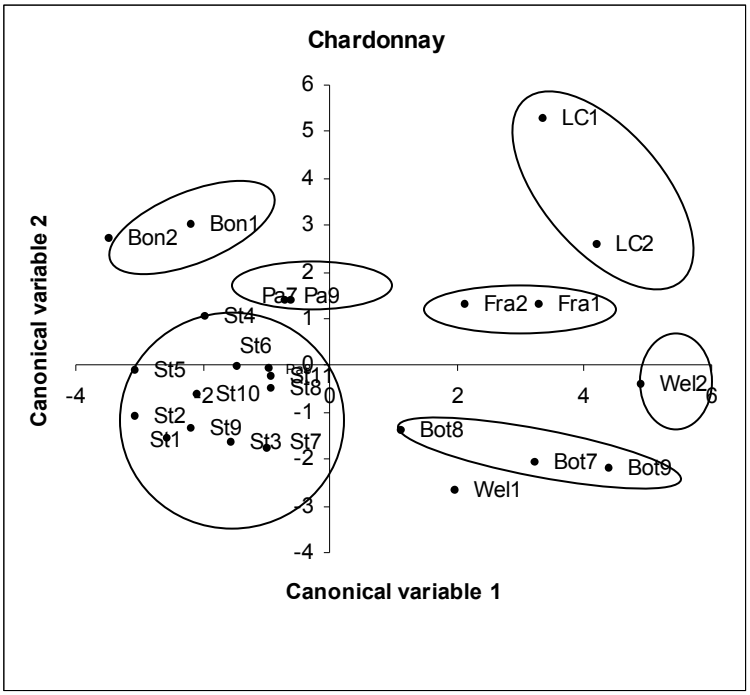


FIGURE 6.8

Plots of the first two canonical variables for Chardonnay showing separation of wines from Bonnievale (Bon), Le Chasseur (LC) [Breede River Valley], Bottelary (Bot), Franschhoek (Fra), Paarl (Pa), Stellenbosch [Faure] (St) and Wellington (Wel) [Coastal] areas based on boron, selenium and tin.

Chenin blanc

Because there were only two origins, there is only one canonical variable for this dataset. Eigenvalue for the variable was 2120.3194 and the canonical correlation was 0.99. The total canonical structure coefficients of this one function were magnesium; Can. 1 = 0.945342. Tin; Can. 1 = 0.225391. Barium; Can. 1 = 0.844440. For graphical representation, we have plotted canonical variable one against a constant one. This plot enables us to see the differentiation between Paarl and Stellenbosch. The discriminant analysis was carried out considering the elements evaluated for Chenin Blanc, with the graphical representation shown in Figure 6.9, yielded a pattern of point-distribution in which it distinguished two groups, corresponding to the wine cellars within the two localities. Magnesium and barium had the highest total canonical structure coefficient on the first canonical variable, which were most likely responsible for the discrimination between geographic origins in the direction of canonical variable one. Tin had a high *F*-value but was not included as a discriminating variable.

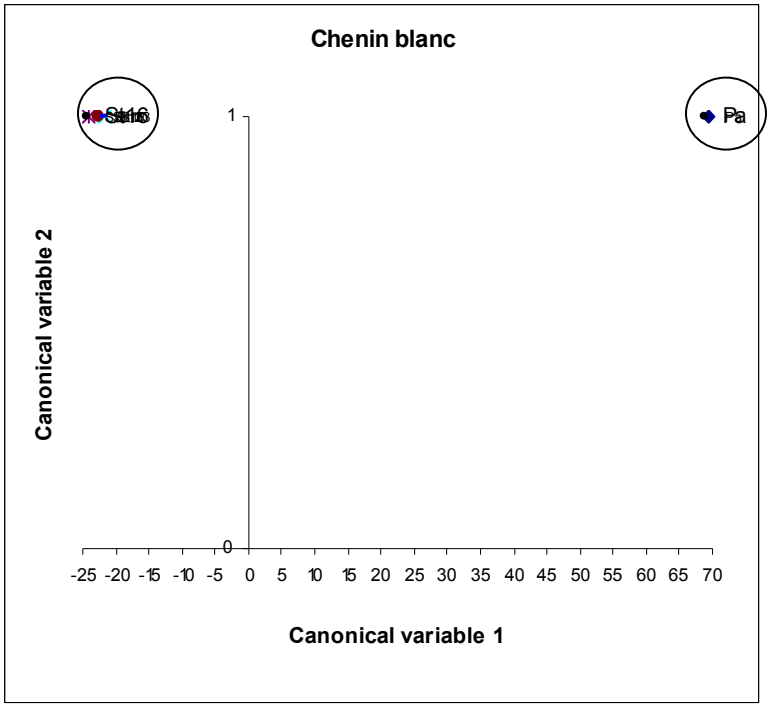


FIGURE 6.9

Plots of the first two canonical variables for Chenin blanc showing separation of wines from Paarl (Pa) and Stellenbosch (St) [Coastal] areas based on magnesium, tin and barium.

6.4.3 Stepwise and Canonical Discriminant Analysis (Combined Data)

The chemical data were analysed statistically according to region and grape cultivar. Good differentiation was achieved for certain regions and grape cultivars. In an attempt to improve the differentiation between wines according to region, a second procedure was conducted in which all the red and all the white cultivar data were combined to form two separate comprehensive data sets respectively. These two data sets were subjected to stepwise discriminant and canonical discriminant analysis. Results are shown in figures 6.10 and 6.11 respectively.

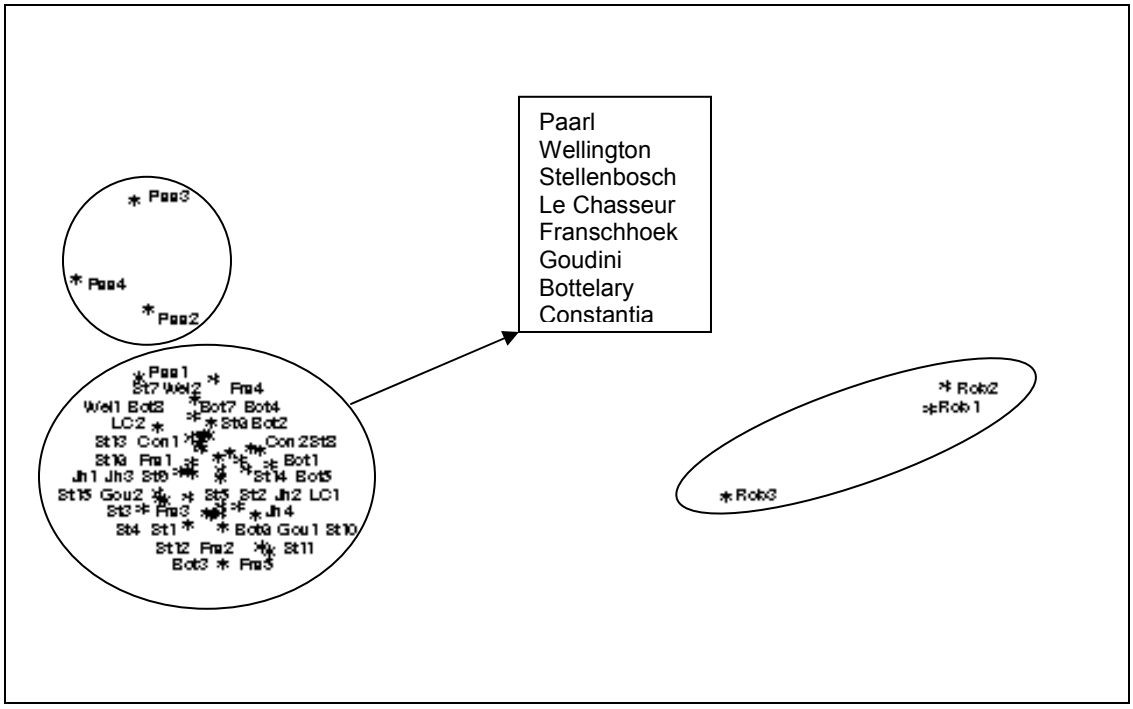


FIGURE 6.10

Plots of the first two canonical variables for combined red wine data, showing no differentiation of origin except for Robertson, based on magnesium, strontium, calcium, aluminium, silicon, antimony and phosphorous.

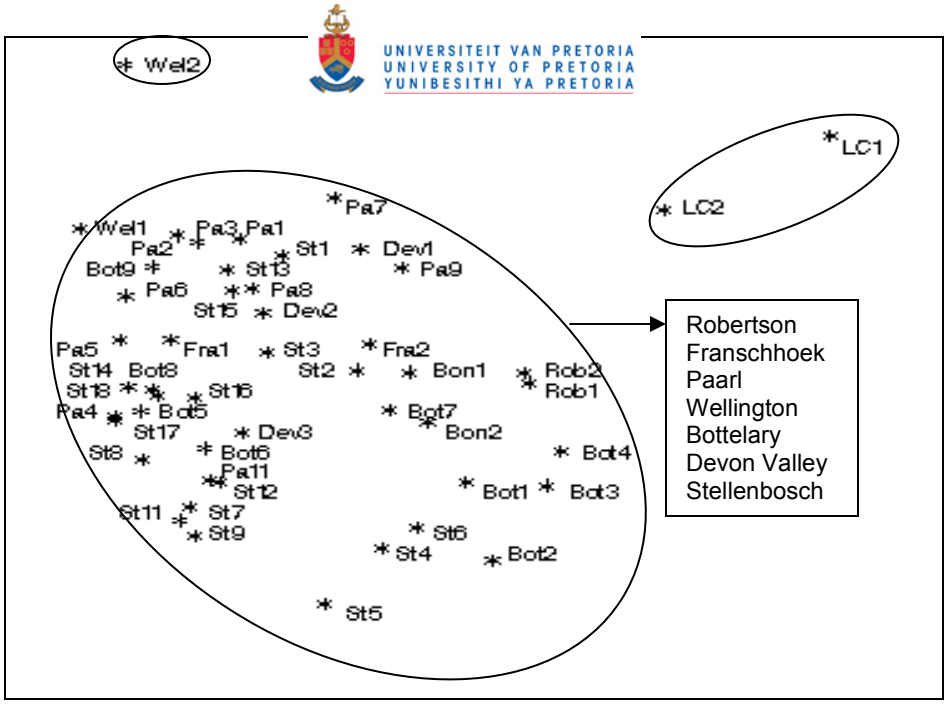


FIGURE 6.11

Plots of the first two canonical variables for combined white wine data, showing no differentiation of origin except for Le Grand Chasseur, based on boron, copper, selenium and antimony.

To summarise, the results that emerged from the canonical discriminant analysis (Region and Cultivar) to a certain extent indicate the possibility of separating wines from different geographic origins, using a selected subset of variables. Results obtained from canonical discriminant analysis procedure using combined red and white wine data yielded poor differentiation between geographic origins, with the exception of Robertson and Le Grand Chasseur. Details regarding the statistical analyses for the combined samples are available in Addendum L on the attached CD-ROM.

Differentiation between wine regions were also attempted, using linear discriminant analysis on the same set of variables (Region and Cultivar) as for canonical discriminant analysis. Results of linear discriminant analyses are tabled in section 6.4.4 below.

6.4.4. Linear Discrim

Refer to Tables 6.6 and 6.7 for linear discriminant analysis' results.

TABLE 6.6

Percentage correctly classified Cabernet Sauvignon with variables magnesium, iron, potassium; Shiraz with variables sodium, aluminium, potassium, magnesium; Merlot with variables sodium, barium, copper, potassium, phosphorous, zinc, iron, strontium and Pinotage with variables magnesium, potassium, zinc and iron.

Grape variety	Origin	n	Classification success
Shiraz	[1]Robertson (BRV)	3	3/3
	Le Chasseur (BRV)	2	0/2
	Franschhoek (Coastal)	2	2/2
	Bottelary (Coastal)	2	0/2
	Total correct classification (%)		**55%**
Merlot	Constantia (Coastal)	2	1/2
	Jonkershoek (Coastal)	2	0/2
	Bottelary (Coastal)	3	3/3
	[2]Paarl (Coastal)	2	2/2
	[3]Stellenbosch (Coastal)	10	7/10
	Total correct classification (%)		**68%**
Pinotage	Goudini (BRV)	2	0/2
	[3]Stellenbosch (Coastal)	3	2/3
	Bottelary (Coastal)	3	1/3
	Total correct classification (%)		**38%**
Cabernet Sauvignon	Franschhoek (Coastal)	3	2/3
	Paarl (Coastal)	2	1/2
	Stellenbosch (Coastal)	3	2/3
	*Jonkershoek (Coastal)	2	2/2
	Wellington (Coastal)	2	2/2
	Total correct classification (%)		**75%**

n=Number of wine samples for each geographic origin; [1] Robertson: Includes Ashton; [2]Paarl: Includes Voor-Paardeberg; [3]Stellenbosch: Includes Simonsberg-Stellenbosch; *Jonkershoek, which is part of Stellenbosch, was considered a separate origin (Ward) due to its topography; BRV = Breede River Valley.

TABLE 6.7

Percentage correctly classified Chardonnay with variables selenium, boron, tin; Chenin blanc with variables tin, magnesium, barium and Sauvignon blanc with variables boron, aluminium and magnesium.

Grape variety	Origin	n	Classification success
Chardonnay	Bonnievale (BRV)	2	2/2
	Le Chasseur (BRV)	2	1/2
	Bottelary (Coastal)	3	2/3
	Franschhoek (Coastal)	2	0/2
	[1]Paarl (Coastal)	3	3/3
	[2]Stellenbosch (Coastal)	11	9/11
	Wellington (Coastal)	2	0/2
	Total correct classification (%)		**68%**
Chenin blanc	Paarl (Coastal)	2	2/2
	[2]Stellenbosch (Coastal)	6	6/6
	Total correct classification (%)		**100%**
Sauvignon blanc	Bottelary (Coastal)	6	6/6
	Devon Valley (Coastal)	3	3/3
	Paarl (Coastal)	6	5/6
	Total correct classification (%)		**93%**

n= Number of wine samples for each geographic origin; [1]Paarl: Includes Voor-Paardeberg; [2]Stellenbosch: Includes Simonsberg-Stellenbosch; BRV = Breede River Valley

Although differentiation accuracies using linear discriminant analysis were poor between certain geographic origins, linear discriminant analysis has nevertheless shown the possibility of separating geographic origins. Detailed information on the statistical analyses for all samples is available in addenda F and G on the attached CD-ROM.

6.5. PREVIEW OF CHAPTER 7

Chapter 7 focuses on a general discussion of the results. The strength of the study as well as the limitations and weaknesses of the study are noted. Recommendations are made and conclusions are drawn.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1. GENERAL DISCUSSION

The aim of this study was to differentiate between the geographic origins of wines produced in the Western Cape based on their element composition. Determination of differentiating variables firstly required quantification of the element content of single-variety wines by means of ICP-AES. Secondly, the applications of pattern recognition techniques to establish patterns or clusters in the data were applied. Thirdly, the application of stepwise discriminant analysis was used to establish a subset of variables and lastly, the determination of the classification success for different geographical origins (regions, districts and wards), using canonical discriminant analysis and linear discriminant analysis were implemented.

The original twenty-two elements measured for red wine samples included Na, Mg, K, Ca, Sr, Ba, Ti, Mo, Mn, Fe, Pd, Cu, Zn, Pb, Sn, Sb, As, Se, P, Si, Al and B. After a visual examination of the data, six variables, namely Ti, As, Mo, Pd, Pb and Sn were disregarded due to values below the detection limits. The original twenty-three elements measured for white wine samples included Na, Mg, K, Ca, Ti, Mn, Cu, Zn, B, Al, Si, P, As, Se, Sb, Sn, Pd, Mo, Sr, Ba, La, Pb and Fe. After a visual examination of the data, seven variables, namely Na, Ti, As, Pd, Mo, La and Pb were disregarded due to values below the detection limits (sodium was not quantified in white wines). Simple inspection of the element concentrations could not be used to differentiate the origin, however, multivariate analyses were able to detect similarities among wines according to origin for each grape variety.

Thirteen discriminant elements or variables which had the most effective discriminatory powers or highest *F*-values and which provided the best combinations for subsequent analysis were selected using stepwise discriminant analysis. The selected variables for all seven grape varieties were subjected to

CDA and LDA, to establish whether discrimination between wine regions was possible. The selected variables include Se, B and Sn for Chardonnay; Mg, Sn and Ba for Chenin blanc; B, Al and Mg for Sauvignon blanc; Mg, K and Fe for Cabernet Sauvignon; Na, Ba, Cu, K, P, Zn, Fe and Sr for Merlot; Mg, K, Zn and Fe for Pinotage and Na, Al, K and Mg for Shiraz. By applying CDA, wines from the Breede River Valley and Coastal region and wines with-in both regions could be differentiated using K, Mg, Na, Fe, Zn, P, Ba, Sr, B, Al, Sn, Se and Cu. In addition, stepwise discriminant analysis was performed in which all the red and all the white wine data were combined to form two separate comprehensive data sets respectively. Magnesium, Sr, Ca, Al, Si, Sb and P were selected as discriminant variables for red wine and B, Cu, Se and Sb were selected as discriminant variables for white wine.

Frías *et al.* (2003) stated that a number of authors list K, Mg, Mn, Na, Ca, Li, Rb, Cr, Fe, Zn, Ag, P, Co, Cs, Ba, Sr, B, Ti and Al as useful elements for wine origin differentiation. It has been shown that K and Mg were used in five previous studies, Na in four studies, Fe and Zn in three studies, P in two studies, while Ba, Sr and Al were used in one study. Thus, in addition to the elements listed by Frías *et al.*, discriminant analyses resulting from this study indicated that Se, Sn, Zn and Cu could also be used in differentiating between geographical origins. In this study, ten of the above-mentioned elements correspond with Frías' findings. Boron, Mg, Ba, Sn, Al and Se are especially valuable for discriminating between South Africa geographical origins in white wine. Magnesium, Zn, Fe, Ba, Cu, K, Na, Sr and Al played the dominant roles in discriminating between South African geographical origins in red wine. In soil, zinc is tightly adsorbed to magnesium and copper is especially plentiful in acidic, sandy soils. Magnesium, Al and Ba were used in the statistical analyses for both red and white wine samples as discriminatory variables. These conclusions were drawn from the significantly higher total canonical structure coefficients of the variables.

The two-dimensional plots of the canonical variables (Figures 6.3 – 6.9, Chapter 6) clearly indicate separation among wine regions, districts and wards for Pinotage, Merlot, Shiraz, Cabernet Sauvignon, Sauvignon blanc, Chardonnay and Chenin blanc grape varieties from certain origins. These results indicate that normal groups were formed using the selected subset of variables. It can therefore be accepted that differentiation between wine regions, districts and wards is possible by applying CDA to the selected subset of elements. This also verifies that effective classification can be achieved by means of the visual display and observation of data. The discrimination between wine regions was confirmed using LDA on the same set of variables as for CDA.

The elements Mg, K, Fe, Zn, Ba, Sn, B, Al, Se, Cu, P, Na and Sr were found to be highly discriminative for both CDA and LDA. Organic fertilisers may cause a fluctuation in the content of Na in wines (Maarse *et al.,* 1987; Latorre *et al.,* 1994) however, the high *F*-values of Na exclude the influence of individual variations in fertilising practices, but include the possibility of regional differences. In the long term, sodium may therefore be less suitable for differentiation purposes. Maarse *et al.* (1987) mention the influence of processing conditions on the Fe content of wines. The high *F*-values of Fe fail to indicate a significant variation within regions. These high *F*-values could be ascribed to processing conditions, for example; steel pipes and tanks versus plastic pipes and tanks. However, one cannot expect to find major regional differences in wine processing techniques, which could influence the Fe content of these wines. Consequently, the differences observed in *F*-values for this element, may be ascribed to variations in soil conditions. Similarly, the high *F*-values for K are not indicative of wine processing techniques, but rather indicate a source of variation in soil type (J. Wooldridge, personal communication, 2005).

This investigation has established that different grape cultivars originating from the same origin can be classified by different sets of elements in the discriminate functions. However, the fundamental concept of the wine fingerprinting procedure

is the assumption that one set of elements (variables) is used to classify wines according to region (Coetzee *et al,* 2005). The findings in this study indicate however, that different sets of variables were used in wine classification. A possible explanation for selecting different sets of discriminant elements by stepwise discriminant analysis can firstly be attributed to the origin of the soil-associated elements. South African wine farms, in contrast to European wine farms, are often larger, especially farms in the Breede River Valley and Swartland regions and secondly, the South African wine-producing region of the Western Cape is renowned for its diversity of soil-types within one wine farm (K. Conradie, personal communication, 2008).

A further explanation for selecting different sets of discriminant variables for origin differentiation can be ascribed to the fact that there is no direct correlation between element concentrations in wine and provenance soil (Serapinas *et al.,* 2008). Serapinas *et al.* have indicated that soil-plant interactions are highly complex. Rootstocks have an influence on the vine mineral content and therefore a definite influence on the up-take capability and utilisation of the scion cultivar on soil-available nutrients (K. Hunter, personal communication, 2008). This influence depends largely on the interaction between the rootstock and the root-system development, which is related to the type of soil, the soil minerals and water availability (A Deloire, personal communication, 2008). In other words, different grape cultivars growing in the same soil-type, but with different rootstocks, can utilise different nutrients and nutrient concentrations from the soil.

This study has illustrated the manner in which a small number of variables related to chemical composition of wines of different varieties, establishes a link between chemical composition and the geographic origin of wine. The fact that such a differentiation is possible, despite varying viticultural practices and winemaking processes, indicates that even though these two contributors are important, they do not necessarily influence wine origin differentiation.

7.2. STRENGTHS OF THE STUDY

- The accuracy, reliability and suitability of ICP-AES for multi-element determination ensure a widely available range of elements and the efficient processing of a large number of data with adequate precision.

- The development of a national data base of typical element concentrations and compositions of wine for different wine regions, districts and wards. The data base could facilitate authentication studies and render it less complicated to prove that a wine was indeed grown and produced in a specific region or district.

- Determination of the element content can provide meaningful criteria for wine origin differentiation.

7.3. LIMITATIONS AND WEAKNESSES OF THE STUDY

The results should be considered as preliminary due to the small number of samples analysed for certain regions, districts and wards. The data sets available for certain regions may therefore imply that the samples were not fully representative, which appears to have been inadequate to provide representative statistical data of the element content of wine in this study. The reason for this limitation can be attributed to the fact that element profiles were reliant on a small number of wines (for only two to three vintages from each winery could be collected) and the lack of availability of specific vintages and single grape variety wines. A more comprehensive number of wine samples may establish which elements are influenced by vine growth, soil type, grape variety, wine making processes and which elements are not influenced at all. It is also not clear whether the elements considered, although significant by the classification methods, provide indications of the structure of the population of the region of origin or only the random samples, which were taken. These results, however, indicate that under the conditions prevalent at the time of the study and with the

relatively small number of samples, differentiation between Western Cape wines according to geographic origin was possible using element concentrations. Although the discriminating elements may only be valid for the test set under study, examination of the data does allow a certain number of elements to be identified as common variables.

Nevertheless, with a more comprehensive element database and with the inclusion of additional elements that correlate with each region, district and ward, ICP-AES data could be used to establish element profiles or key identification variables.

7.4. RECOMMENDATIONS

Inductively coupled plasma spectroscopy ought to be applied to more wine samples to establish concentration levels of selected elements in wine over three to four consecutive vintages to establish more representitive and reliable data. It would also be valuable to establish the degree of attenuation of elements in young wines and matured bottled wines.

It would be valuable to both the producers and authorities to extend the evaluation of the element content of South African wine, by obtaining completely new and more representative samples from additional locations and to establish whether the results of this study can be repeated or improved on. In addition, the determination of the element content of wine needs to be applied to wines from other regions apart from the Western Cape Province to comprehensively evaluate the statistical procedure. It would also be useful to establish whether the method can be extended to cases, where uncertainties arise as to the grape variety (or blend) specified on the wine bottle.

7.5 CONCLUSION

ICP-AES proved to be suitable for direct and rapid determination of element concentrations under standard conditions and without modification of the ICP-

AES. Multi-element determination of wine samples using ICP-AES, accompanied by appropriate statistical analysis was found to be an effective method for differentiating between wine regions, districts and wards. It also demonstrated how a combination of advanced instrumentation and sophisticated data analysis techniques could be applied in wine research.