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ARTICLE *in* ANALYTICA CHIMICA ACTA · JUNE 2012

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Review

Analytical techniques for wine analysis: An African perspective; a review

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ARTICLE INFO

Article history:
Received 1 September 2011
Received in revised form
15 November 2011
Accepted 27 November 2011
Available online xxx

Keywords:
Grapes
Wine
Spectroscopy
Chromatography
Regulatory analysis

ABSTRACT

Analytical chemistry is playing an ever-increasingly important role in the global wine industry. Chemical analysis of wine is essential in ensuring product safety and conformity to regulatory laws governing the international market, as well as understanding the fundamental aspects of grape and wine production to improve manufacturing processes. Within this field, advanced instrumental analysis methods have been exploited more extensively in recent years. Important advances in instrumental analytical techniques have also found application in the wine industry. This review aims to highlight the most important developments in the field of instrumental wine and grape analysis in the African context. The focus of this overview is specifically on the application of advanced instrumental techniques, including spectroscopic and chromatographic methods. Recent developments in wine and grape analysis and their application in the African context are highlighted, and future trends are discussed in terms of their potential contribution to the industry.

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Contents

Abbreviations: AAS, atomic absorption spectroscopy; ABTS, 2,2′-azinobis(3-ethylbenzothialozinesulfonic acid); AOTF, acousto-optical tunable filter instrument; ATR, attenuated total reflection; BGE, background electrolyte; CAR, carboxen; CE, capillary electrophoresis; DAD, diode array detector; DPPH, 2,2-diphenyl-1-picrylhydrazyl radicals; DVB, divinylbenzene; ELSD, evaporative light scattering detection; ESI, electrospray ionisation; EU, European Union; FFAP, free fatty acid phase; FID, flame ionisation detector; FLD, fluorescence detector; FT-MIR, Fourier transform mid-infrared spectroscopy; FT-NIR, Fourier transform near-infrared spectroscopy; GC, gas chromatography; GC-O, gas chromatography-olfactometry; HILIC, hydrophilic interaction chromatography; HPLC, high performance liquid chromatography; HSSE, headspace sorptive extraction; HS-SPME, headspace solid phase micro-extraction; ICP-MS, inductively coupled plasma-mass spectrometry; IR, infrared; LDA, linear discriminant analysis; LLE, liquid–liquid extraction; MALDI, matrix assisted laser desorption ionisation; MIR, mid-infrared; MRM, multiple reaction monitoring; MS, mass spectrometry; NIR, near-infrared; NMR, nuclear magnetic resonance; OIV, international vine and wine office; OTTs, open tubular traps; PCA, principal component analysis; PCR, principal component regression; PDMS, polydimethylsiloxsane; PEG, polyethyleneglycol; PFPD, pulsed flame photometric detector; PLS, partial least squares regression; PSDVB, polystyrene-divinylbenzene; REA-PFGE, endonuclease analysis pulsed field gel electrophoresis; RI, refraction index; RMSEP, root mean square error of prediction; RP, reversed phase; SBSE, stir bar sorptive extraction; SEP, standard error of prediction; RPD, residual predictive deviation; SIM, selected ion monitoring; SIMCA, soft independent modelling of class analogy; SPDE, solid phase dynamic extraction; SPE, solid phase extraction; SPME, solid phase micro-extraction; TA, titratable acidity; TOF, time-of-flight; TSS, total soluble solids; UPLC, ultra-performance liquid chromatography; UV, ultraviolet; UV/Vis, ultraviolet/visible.

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doi:10.1016/j.aca.2011.11.064

Please cite this article in press as: A. de Villiers, et al., Analytical techniques for wine analysis: An African perspective; a review, Anal. Chim. Acta (2012), doi:10.1016/j.aca.2011.11.064

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1. Introduction

Mankind has been involved with winemaking since ancient times. Wine holds a special place in many countries and cultures and man could have encountered some of his earliest experiences in chemical reactions through the processes of fermentation and oxidation of wine. Historical records show the earliest winemaking activities in Mesopotamia and Caucasus by 6000 BC [1]. Colonization by the Romans of regions around the Mediterranean Sea resulted in the spread of the cultivation of the vine plant. Earliest records of winemaking on the African continent trace activities to the southern shores of the Mediterranean as early as 5000 BC and confirm ancient Egypt as the first winemaking region in Africa [1]. Much has been written about wine and ancient Egyptian civilisation; historical records show that it was served to noble men and pharaohs and stored in individual jars clearly marked with details of winemaker, vintage and vineyard. From Egypt, cultivation of the vine spread to other northern African regions with all the vineyards being close to the coast. The vine *Vitis vinifera* was introduced to the southern tip of the African continent by European explorers in the 17th century [1]. In 1655 Dutch settlers planted French vine cuttings on the lower slopes of Table Mountain in the Cape of Good Hope, South Africa. As early as the 18th century, Vin de Constance wines from the area now known as Constantia were amongst the worlds most sought after [2]. The early vineyard plantings accelerated with the French Huguenot settlements during the late 17th century and early 18th century in the Cape, and soon spread to the nearby region now known as Franschhoek.

The major African wine-producing regions are those with Mediterranean climate, typically with mild winters and dry, hot summers, in which the vine thrives. On the African continent, only very small regions located in Northern Africa and in the southern tip of the continent, the coastal areas of the Western Cape, fit this description. Today, the northern African countries Algeria, Morocco

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and Tunisia have established wine industries with important intra-African and African–European export components. Wine labelling laws are based on the French system of *Appelation d'Origine Controleé* and a strong influence of French wine grape cultivars like Cabernet Sauvignon, Syrah, Mourvedre, Carignan, Ugni blanc and Clairette is seen in plantings [1]. Muscat wines, that can be sweet or dry, are especially successful in Tunisia. Algeria annually produces some 600,000 hectolitres of wine and wine provinces Oran and Alger are renowned for red wine, whilst smaller quantities of rosé and white wine are also produced. Morocco has 15,000 hectare planted under vineyards, of which some 85% produces red wine, and the rest rosé and a pale white. Well-known Moroccan wine regions include Rabat, the coastal vineyards of Casablanca, Meknes and Fez.

South Africa is the principal wine-producing country in Southern Africa with some 60 appellations within the Wine of Origin scheme and a tiered system of wine regions, districts and wards [2]. Annual production of more than 100 million bottles places the country as the world's 7th largest wine producer. The area covered by South African vine plantings constitutes 1.3% of the world's vineyards [3]. Renowned wine regions include Constantia, Stellenbosch, Franschoek, Overberg and Robertson. Well-known white wine grape cultivars are Chenin blanc, Sauvignon blanc, Chardonnay, Muscat d'Alexandrie and Colombar, whilst red varieties include Cabernet sauvignon, Shiraz, Merlot and Pinotage [2]. The South African wine industry is dependent on exports and the wine quality is comparable with the world's best.

As in all areas of food and beverage production, the analysis of wine plays an essential role in the industry. Accurate analytical measurements are required at all stages of the winemaking process, starting in the vineyard, at the weighbridge where grapes are delivered, during the fermentation and maturation stages, at bottling and at certification (Fig. 1). These measurements are required for various reasons. In the first instance, analytical methods are used to provide information required by law for the production and marketing of these products. This includes regulatory analysis pertaining to the marketing and sale of these products in an increasingly competitive international market, which therefore has important financial implications. Secondly, from a research and development perspective, analysis is also used to shed light on more fundamental aspects such as the microbiological, genetic, physiological and chemical processes involved in grape and wine production and manufacturing. Whilst obviously important from a manufacturing perspective, this research also contributes to the

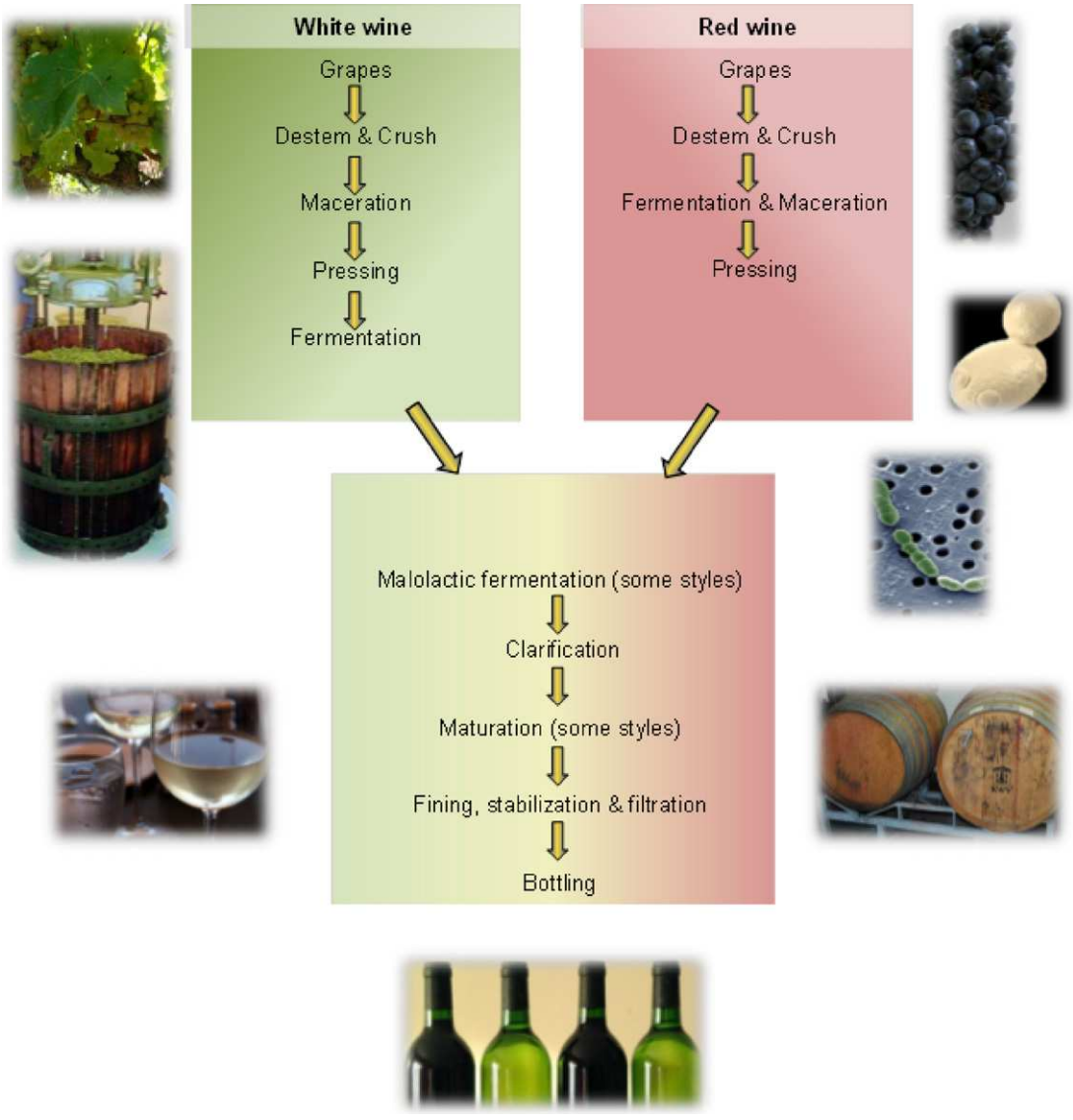

Fig. 1. Schematic illustration of the different steps involved in the winemaking processes of red and white wines. Adapted from [63].

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fundamental understanding of the chemical composition of natural products in general and the production of commodities useful for human consumption from these products.

Analysis of wine-related products involves an extremely wide variety of analytical techniques, reflecting the equally diverse goals of these analyses. The range of methods used for wine analysis mirrors to some extent the varied information relevant to wine producers and researchers. Techniques used vary between relatively simple wet-chemical methods and highly complex (and expensive) instrumental methods capable of detailed investigation of individual chemical constituents. Generally, the former types of methods are used for routine analysis aimed at demonstrating compliance with product legislation, since these methods are relatively cheap and may be performed in many laboratories. On the other hand, there is an increasing international trend of applying more advanced instrumentation for high-level research involving wine and derived products. The inherent inter-disciplinary nature of analytical research in this field has contributed to improving the quality of grape-derived products as well as new scientific knowledge.

Analysis of grape-derived products on the African continent to a large extent reflects current international trends: continuous development in analytical chemistry instrumentation and methods has resulted in the increased application of advanced spectroscopic and chromatographic methods. This review seeks to provide an overview of the analysis of wine, grapes, and their derived products as performed on the African continent. For the purposes of this review, literature reports including at least one author affiliated to an African institution are included. Furthermore, the focus is exclusively on the application of advanced instrumental analytical methods for grape and wine analysis. In the context used here, instrumental analytical methods refer primarily to spectroscopic, chromatographic and electrophoretic methods of analysis. Fig. 2 provides a graphical summary of the most important instrumental analytical techniques used for the analysis of grapes and wine in Africa.

2. Spectroscopic analysis of wines: global perspectives

Global production figures for 2008 recorded some 7800 million hectares under wine grapes and in excess of 240 million hectolitres of wine being produced [4]. These huge volumes make it clear that rapid, low-cost and environmentally friendly analytical methods are of critical importance to maintain sustainability of the international wine industry. This is particularly true on the African continent, where demands on existing natural resources, notably water and energy, are already high.

Spectroscopic methods applied for wine and grape analyses include a wide range of techniques, spanning atomic spectroscopic methods such as atomic absorption spectroscopy (AAS) [5] and inductively coupled plasma (ICP) and several molecular spectroscopic methods such as infrared- and ultraviolet/visible spectrophotometry, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Some of these technologies are extensively used in international wine research, but have not yet been exploited in Africa, and hence will not be covered in this review. For example, NMR is widely used globally for wine analysis, notably for authentication purposes [6]. In addition, recent developments in near-infrared (NIR) spectroscopy for remote sensing of vineyards [7] as well as development of portable devices for non-destructive monitoring of grape quality [8] have not yet found application in African wine research. Finally, whilst MS may be used directly for wine analysis [9], in the African content it has been used exclusively in hyphenated chromatographic and spectroscopic systems.

Several features of spectroscopic techniques, particularly ultraviolet–visible (UV/Vis) spectrophotometry and infrared (IR) spectrometry, offer attractive features that make them ideally suited for handling very large volumes of the essential routine grape and wine analyses [10]. UV/Vis spectrophotometric methods are used extensively for determination of colour and phenolic compounds in grapes and wine [11,12], that have shown to be important drivers of preference amongst consumers [13]. For example, absorbance measurements at 280 nm are used for quantification of total phenolics and anthocyanins at 520 nm. Although the lack of specificity in these methods (compared to liquid chromatography) can result in overestimation of the phenolic content, spectrophotometric analysis nevertheless provides a rapid and inexpensive methodology particularly suited for high sample throughput [11,12]. Despite its utility, UV/Vis instrumentation has not seen much innovation in recent years.

Vibrational spectroscopy, both in the near- and mid-infrared regions, has recently received considerable attention in grape and wine analysis and the past two decades have seen a surge in quantitative and authentication applications in international wine industries [14–19]. Chemometrics is indispensable for interpretation of spectroscopic data and refers to a vast field of statistical and mathematical techniques that are used to extract relevant information from primary chemical or analytical measurements [20–22]. Typical problems addressed by spectroscopic data combined with chemometrics include multivariate calibration and classification [17,18,20], process monitoring [23,24], quality control and data display [21]. These applications address quantitative and qualitative challenges such as product authentication in grape and wine

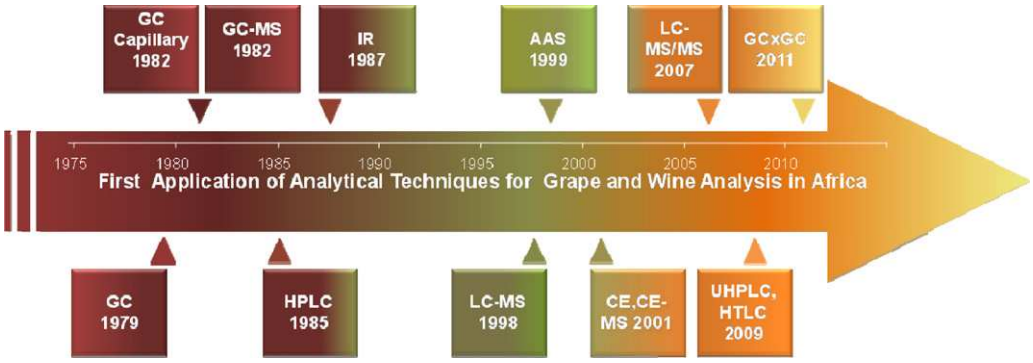

Fig. 2. Summary of the most important developments in instrumental analytical techniques applied to wine analysis in Africa since 1975. Arrows indicate the first published report of a particular method for wine analysis by African scientists. The relevant references for each application are: GC [49], capillary GC [56], GC–MS [56], HPLC [100], IR [32], LC–MS [152], AAS [43], CE, CE-MS [164], LC–MS/MS [159], GC × GC [79,80,83].

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analysis. Recent improvements in instrument hardware combined with powerful chemometric software packages, which are nowadays integrated with instrument software, undoubtedly made a significant contribution to these developments.

2.1. Vibrational spectroscopy in wine analysis

Vibrational spectroscopy offers several advantages and much has been written about these [14,25]. The technology is non-destructive and by nature of its indirect measurement, also reagentless, whilst no toxic waste is generated. Analysis time is in the seconds range and the technology can be fully automated, including processing and distribution of the analytical results. Very little sample preparation is required; mostly the only requirement is a filtration step to remove large particles from liquids and a degassing step for FT-MIR analysis that is achieved by simple vacuum filtration or sonication [26,27]. Drawbacks of the technology are the relatively high initial instrumentation cost, as well as the intensive calibration procedures that are a pre-requisite for implementation of the technology.

Vibrational spectroscopy is based on the measurement of the frequencies of the vibrations of covalent bonds in functional groups upon absorption of radiation in the near-infrared (NIR) and mid-infrared (MIR) region [28]. The NIR region is usually defined as ranging from 800 to 2500 nm, whilst the MIR region from 4000 to 400 cm^{-1} (2500–2.5 × 10^4 nm). In instrumentation, the exact wavelength range of these regions is customised to suit specific applications, and the visible region is combined with the NIR range in some spectometers. The main difference between the NIR and MIR regions is that absorption of MIR light by matter causes fundamental vibrations of covalent bonds, whereas absorption of NIR light results in overtones and combination bands [28]. The result is that MIR spectra show higher specificity than NIR spectra and MIR is therefore frequently preferred for quantitative applications. NIR light is not absorbed as well by matter as MIR light and is better suited for measuring whole fruits [29]. The measured frequencies in NIR and MIR spectra are processed through a series of mathematical procedures (which may include Fourier transformation) to calculate an absorbance spectrum. The latter, in turn, is correlated to the actual concentrations of the relevant components in the sample matrix through a calibration process that involves multivariate statistical procedures such as principal component analysis (PCA), principal component regression (PCR) and partial least squares (PLS) regression [20,30]. The application of FT-IR for the routine analysis of wine has recently received much attention [14,25].

The NIR spectrum of wine is dominated by two large absorption bands that correspond to O—H bonds around 1400 and 1900 nm, corresponding to water and ethanol, respectively [16]. The MIR spectrum is dominated by strong absorbance of water in the regions 1716–1543 and 3626–2970 cm^{-1}, whilst the region from 929 to 1600 cm^{-1} is referred to as the "fingerprint" area, and is particularly useful in molecular absorption spectroscopy since many different IR bands corresponding to the vibrations of the C—O, C—C, C—H and C—N bonds occur in this region [28]. The region from ~5000 to 3626 cm^{-1} does not contain much useful information. This area, as well as both water absorption areas, is frequently excluded in multivariate data analysis, due to the noise introduced in the IR spectra from these regions [26]. The utility of chemometric techniques for the design of PLS calibration sets was demonstrated with the use of PCA to identify the main sources of variation in a set of 329 South African wines [26]. The set included wines belonging to various styles: noble late and special late harvest wines (sugar levels ranging from 31 to 147 g L^{-1}), wooded and unwooded dry red and white wines, off-dry white wines and the young wines (sugar levels collectively ranging from 0.5 to 13 g L^{-1}). PC1 (that explained 96% of the variation) seemed to distinguish between samples based on sugar content (Fig. 3), whilst PC2 differentiated the samples based on the alcohol content. The PCA results clearly separated the different wine styles, illustrating the potential of FT-MIR spectroscopy to be used for style identification and verification.

The use of vibrational spectroscopy for quantification of wine compounds was first reported for filter-based NIR instruments where only a small number of wavelengths were available for measurements [31]. One of the early applications for wine analysis on a filter-based NIR instrument was the quantification of ethanol in wine [38]. Contemporary NIR instrumentation include, amongst others, acousto-optical tunable filter instruments (AOTF), photo diode array and Fourier transform (FT-NIR) interferometer systems [33]. Hyphenated instruments such as UV/Vis or Vis-NIR have also been used in wine and grape analysis [16]. Nowadays, the focus has moved from NIR spectroscopy to MIR spectroscopy for the routine analysis of wine, based on the more accurate determination of a wider range of compounds [34].

The marketing of Fourier transform mid-infrared (FT-MIR) instrumentation dedicated to routine wine analysis in 1998 (WineScan FT 120, Foss A/S, Denmark) provided a huge impetus to the implementation of infrared technology for routine wine analysis in analytical laboratories. The instrument is fitted with a Michelson interferometer and a 37 μm CaF_2 cuvette that is temperature controlled. Spectra are generated in transmission mode and sample volumes of ~30 mL are needed [35]. In terms of software, so-called 'global calibrations' for the quantification of a wide range of wine compounds and properties are available including levels of glucose, fructose, organic acids (tartaric acid, malic acid, acetic acid, lactic acid, gluconic acid, sorbic acid, citric acid), ethanol, density, CO_2, polyphenols, glycerol, pH, iron, copper, colour, ethanol, ethyl acetate and methanol. These parameters can be quantified in a single analysis for a wide range of wine styles and in the ranges normally found in grapes and wine [36]. Typical time of analysis, including sample preparation, is less than 1 min. Instrumentation with sample presentation modes in attenuated total reflection (ATR) have recently become available and have been used for routine analysis of wine [34]. A wide selection of materials is used for the sampling plates including diamond, Si, ZnSe and Ge. Advantages of FT-MIR ATR instruments include small sample volumes (less than 0.2 mL), samples are placed directly onto the ATR platform, much smaller physical dimensions than conventional laboratory instrumentation and lower cost, which makes it an attractive option for commercial laboratories [34]. Currently, analytical instruments suitable for multi-component analyses are available with impressive performance data in terms of accuracy, precision and speed of analysis.

Researchers at Stellenbosch University have focussed on the development of quantitative and qualitative applications using infrared spectroscopy in viticulture and oenology. This collaborative research combined expertise in the application of chemometric methods, primarily from Europe, with the African partners' expertise in winemaking and viticulture. This culminated in the formation of the Chemometrics Society of South Africa [22] and the first African–European conference on chemometrics *Data modelling in Biological Sciences and Industrial Processing*, held in Rabat, Morocco in 2010 [37]. The long-term ambition of this initiative is to strengthen ties between European and African countries in projects where chemometrics is the major focus areas.

Infrared spectroscopy has been applied to all stages of the wine production chain in South Africa, ranging from the vineyard to the bottled product. The utility of NIR spectroscopy in measuring important analytical compounds in South African wines was evaluated as early as 1987 [32] by Baumgarten, who used a filter NIR instrument to quantify ethanol in wine. Subsequently, the utility of FT-NIR in combination with chemometric techniques for

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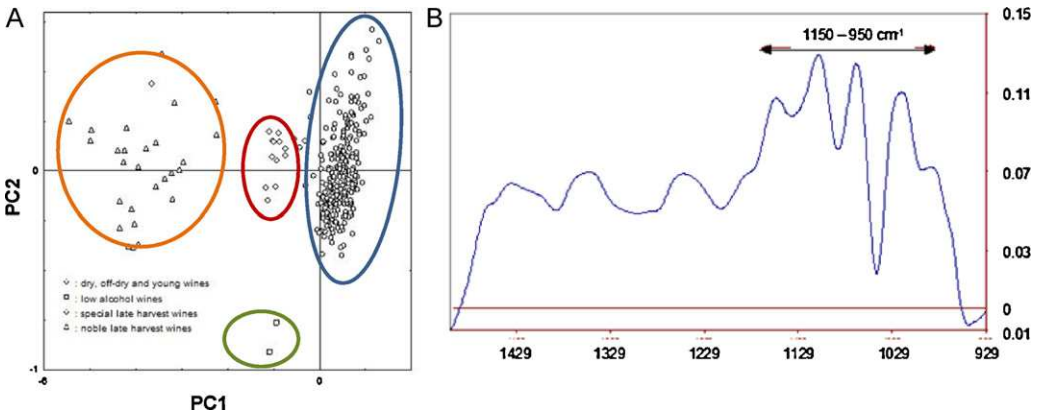

Fig. 3. (A) PCA score plot, PC1 versus PC2, based on of FT-MIR spectra of different wine styles: dry, off-dry and young wines (blue, circles); low alcohol wines (green, squares); special late harvest wines (red, diamonds); noble late harvest wines (orange, triangles). (B) PC1 loadings plot in the wavenumber region 1500–929 cm^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.). Reprinted with permission from [26].

quantitative and qualitative applications on South African wines was performed on Chardonnay fermented musts [38]. FT-NIR spectra were collected in the 100–2500 nm region, at a resolution of 2.5 nm using a 0.5 mm pathlength quartz cell. The percentage sugar and free amino nitrogen (FAN) values in the grape musts were determined, whilst FT-NIR and SIMCA (soft independent modelling of class analogy) was used to discriminate between Chardonnay samples ($n = 107$) in terms of their malolactic fermentation status and ethyl carbamate content.

Monitoring of grape quality in the vineyard during ripening and at harvest at the weighbridge was performed using FT-IR spectroscopy in the region 929–5011 cm^{-1} on a WineScan instrument [27]. PLS calibration models, using independent test set validation, were developed to quantify total soluble solids (TSS, expressed as °Brix), pH and titratable acidity (TA, expressed as $g\,L^{-1}$ tartaric acid). With this work the objective was to establish rapid, high-throughput and low-cost analytical methods for monitoring grape quality in an industrial South African cellar with an annual intake of some 105,000 tons of grapes and producing in excess of 75 million litres of wine [27]. FT-IR spectra of freshly pressed grapes ($n = 1170$) were collected in transmission mode over three vintages, 2005–2007. The average prediction error, referred to as standard error of prediction (SEP), was expressed in the same units as the reference measurement and calculated as described before [20]. The regression statistics obtained for TSS ($n = 647$ grape juice samples) were SEP = 0.34 °Brix, $r^2 = 0.99$ and a residual predictive deviation (RPD) value of 9. The prediction of pH had an average error of 0.04 units, $r^2 = 0.95$ and RPD value of 5. The models developed for TA, gave average prediction errors of 0.51 $g\,L^{-1}$, $r^2 = 0.96$ and a RPD value of 5. The RPD criterion was proposed to evaluate the calibration model [39]. An RPD value of less than three could be considered an indication that the calibration model is unsuitable for accurate quantification, a value between three and five indicates that the model is suitable for screening and a value greater than five indicates that the model is suitable for quantification.

FT-MIR spectroscopy has also been used as a tool to rapidly screen the fermentative properties of wine yeasts and to speed up the evaluation processes in the initial stages of yeast strain development programmes. This work was aimed at the isolation of yeast strains that produce elevated levels of glycerol [40]. The progress of the fermentations could clearly be seen in FT-MIR spectra obtained during the time course of the fermentations. PLS models for the quantification of volatile acidity, glycerol, ethanol, reducing sugar and glucose concentrations in fermented Chenin blanc and synthetic musts were derived from the FT-IR spectra of small-scale fermentations. The accuracy of quantification of volatile acidity in both wine and must was excellent, with root mean square error of prediction (RMSEP) values of 0.07 and 0.08 $g\,L^{-1}$, respectively. RMSEP in wine and musts for ethanol were 0.32% v/v and 0.31% v/v, and for glycerol 0.38 $g\,L^{-1}$ and 0.32 $g\,L^{-1}$. For glucose, the RMSEP values were 0.56 $g\,L^{-1}$ in Chenin blanc and 0.39 $g\,L^{-1}$ in synthetic must. These results showed that FT-IR spectroscopy could be used as a rapid low-cost screening method in biotechnological applications.

FT-IR ATR spectroscopy was also evaluated for the differentiation of 11 *Brettanomyces bruxellensis* strains isolated from red wines [41]. The genetic diversity of the strains was determined by restriction endonuclease analysis pulsed field gel electrophoresis (REA-PFGE). Fingerprints of 11 *B. bruxellensis* strains were compared to the FT-IT ATR fingerprints of whole bacterial cells and with the FT-MIR spectra of experimental wines produced through contamination with these strains. Results showed the potential of FT-MIR ATR spectroscopy as a complementary method to molecular typing techniques.

A study towards authentication of South African young cultivar wines was performed using FT-MIR spectroscopy, gas chromatography and multivariate data analysis [42]. The volatile composition and FT-MIR spectra both contributed to the differentiation between the cultivar wines. The best discrimination model between the white cultivar wines, Chardonnay and Sauvignon blanc was based on FT-MIR spectra (98.3% correct classification) whilst a combination of spectra and volatile compounds (86.8% correct classification) was best to discriminate between the red wine cultivars, Pinotage, merlot, Shiraz and Cabernet Sauvignon.

2.2. Atomic spectroscopy

For the determination of mineral content of wines, atomic spectroscopic techniques are most often used. Application of flame atomic absorption spectroscopy (AAS) [43] and electrothermal AAS [44] for metal analysis in wine have been reported. Aside from regulatory analyses, geographical authenticity of wines may be established by a combination of multi-elemental analysis of wines and their provenance soils and multivariate statistical methods. For example, Coetzee et al. [45,46] described a fingerprinting technique for classification of South African wines according to geographical origin based upon elemental composition. The method uses the assumption that provenance soil is a primary contributor to the trace element composition of wines. A total of 40 elements were determined with inductively coupled plasma-mass spectrometry (ICP-MS), of which 20 carried geographic specific information and these were used in statistical methods. A very high success-rate

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was achieved for classification of these wines from three distinct geographical origins. In another study the elemental composition of wines and their provenance soils from four wine-producing regions of South Africa was also used to classify the wines and soils according to geographical origin. Principal component analysis was used to identify relevant variables, whilst a linear discriminant analysis (LDA) procedure of the identified variables showed a correlation between the elemental composition of the wines and their provenance soils. This relationship is an important pre-requisite for establishing a fingerprinting methodology [47]. Quadrupole-based ICP-MS was also used to determine the isotope ratios of $^{11}B/^{10}B$ and $^{87}Sr/^{86}Sr$ of wines and soils of four major South African wine-producing regions and to establish a fingerprint for origin verification of the wines. The $^{11}B/^{10}B$ ratios was used to discriminate between origins and together with the concentrations of selected elements, used as independent variables in linear discriminant analysis, yielded a highly successful method for classification of geographical origin. A good correlation between B and Sr isotope ratios and its provenance soil was found but the $^{87}Sr/^{86}Sr$ ratios showed limited potential as indicator of origin [48].

3. Chromatographic analytical methods

Despite the power of spectroscopic techniques for the high-throughput analysis of a wide variety of compounds in wine samples, many applications in grape and wine analysis require separation of individual chemical species. In many instances, spectroscopic methods do not provide the requisite selectivity and/or sensitivity for the analysis of specific compounds in the wine matrix. This is especially true for the complex organic fractions of wine, such as the volatile compounds, phenolics and influential trace-level constituents.

By far the most common chromatographic methods used for wine analysis are gas chromatography (GC) and high performance liquid chromatography (HPLC). The application of these and other separation methods vary between routine quantification of wine constituents, and the in-depth investigation of wine chemical composition. In the latter type of research, advances in instrumentation continue to be used to provide more detailed chemical information, especially using hyphenated techniques such as gas chromatography–mass spectrometry (GC–MS), liquid-chromatography–mass spectrometry (LC–MS) and advanced spectroscopic detection systems such as tandem MS instruments, NMR, etc. In fact, the continuous development of new methods has revolutionised our understanding of wine chemistry and ageing, and further developments in this field are essential for quality control purposes as well as for obtaining a more detailed knowledge of the chemistry of grapes and wine.

In the following sections, gas- and liquid-phase separations will be discussed separately in terms of their application to wine analysis in the African context. In much of the research reported here, sample preparation and advanced statistical analysis play important roles in conjunction with separation methods, and these aspects will also be addressed where relevant.

3.1. Gas-phase separations

Wine volatiles comprise of a diverse range of chemical molecules spanning few orders of magnitude in concentrations. To date more than 800 volatiles have been identified in wine. In terms of the analysis of these compounds, the vast majority of research focus has been on the determination of the base wine aroma compounds comprising the so-called major volatiles, which include the principal fermentation derived esters, alcohols and acids. Analysis of these compounds is routinely performed using generic GC methods combined with flame ionisation detection (FID) and more recently MS detection. On the other hand, for the analysis of specific odour impact compounds various dedicated extraction, separation and detection techniques have been described. Examples of these compounds include terpenes, volatile phenols, sulphur compounds, norisoprenoids, pyrazines, etc.

Modern developments in gas-phase separation technologies such as the progression from wide-bore packed columns to capillary columns have played a vital role in the expansion of analytical possibilities for wine analysis. Further important developments in sample pre-treatment procedures and more sensitive and selective GC detectors have been influential in extending the application of GC for analysis of wine volatiles.

3.1.1. Major volatiles

Early work on wine volatiles employed packed-column GC separation. For example, Van Wyk et al. [49] described for the first time the importance of isoamyl acetate in the distinctive fermentation bouquet of young Pinotage wines. Pinotage is a uniquely South African cultivar cross-bred from Hermitage (Cinsault) and Pinot noir in 1925. These authors reported a clear correlation between quantities of isoamyl acetate and the characteristic aroma attributes of young Pinotage wine, which decreased with ageing as the levels of this constituent declined. Houtman et al. [50] quantified two acetate and three ethyl esters in South African grape juice and wines to identify the most important factors influencing ester production during wine fermentation. No noticeable differences between grape cultivars were observed. In 1981 Marais et al. [51] used packed-column GC to quantify 16 major volatile constituents in Pinotage and Cabernet Sauvignon wines. The data were used in combination with discriminant analyses to differentiate the wines according to cultivar and geographical origin. The importance of isoamyl acetate levels in the differentiation of Pinotage wines was once again highlighted in this study.

With advent of capillary GC, the number of compounds that can be separated and quantified in a single analysis increased significantly. Of the vast variety of stationary phase coatings that is available for fused silica capillary columns, the preferred phases for separation of wine volatiles are polyethylene glycol (PEG) or 'WAX' phases. More recently nitroterephtalic acid modified PEG phases (free fatty acid phases, FFAP) have been used extensively due to the reduced peak tailing observed for polar analytes on these columns (especially relevant in the case of grape and wine volatiles). On the other hand, non-polar phases such as polydimethylsiloxane (PDMS) are preferred for the analysis of specific classes of apolar compounds such as terpenoids and volatile phenols [52,53], whilst dedicated phases such as the PDMS-based SPB-1 sulphur phase have been used for the analysis of sulphur compounds [54].

In combination with liquid–liquid extraction (LLE), typical routine capillary GC-FID methods enable the quantification of 20–50 acids, alcohols and esters. Freon was extensively used for the extraction of major volatiles in the past [51,52,55,56], although this has largely been replaced by more environmentally friendly solvents. For example, using diethyl ether LLE Louw et al. reported the levels of major fermentation derived aroma constituents in 925 young single cultivar South African wines [57]. These data were used to study the variation in volatile levels between cultivars and vintages, as well as to derive classification models for the identification of individual cultivars. Several other studies have used major volatile data to differentiate South African wines according to cultivar [58,59] and vintage [60]. Furthermore, major volatile data in combination with FT-MIR have been used to discriminate South African young cultivar wines according to grape variety using multivariate data analysis methods [42].

GC data has in recent years been employed extensively in biotechnology research related to grapes and wine [61,62].

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Intensive research has focussed on the importance of wine yeast on the flavour properties of wines and derived products [63–65]. For example, the effect of esterase activity [66,67] and branched-chain amino acid transaminase activity [68] on wine flavour profiles have been investigated. Yeast strain selection for wine and brandy production is also partially based on the volatile profiles of these products [69,70]. Furthermore, GC data are extensively used in metabolomic [71] and molecular biology [72] yeast research. Generic GC-FID data for major volatiles are typically used to relate volatile content to the biological aspect under investigation in these studies [66–69,71].

3.1.2. Other volatile compounds

In addition to the analysis of major volatiles, significant GC research in recent years has focussed on the determination of specific minor volatile constituents. These generally include impact odourants which are present at lower levels in the wine matrix, and therefore dedicated methods are required for their determination. Methods for trace-level compounds therefore also often require selective extraction- and pre-concentration techniques and/or selective detection strategies.

For example, Zietsman et al. [72] reported a method for the analysis of wine terpenoids in order to study the effect of co-expression of selected glucosidase and furanosidase genes in *Saccharomyces cerevisiae* to release free monoterpenoids. For the analysis of wines, a C18-based solid phase extraction (SPE) procedure was developed which allowed pre-concentration of the extract prior to analysis by GC-FID on a FFAP column.

Acrolein (2-propenal) is a toxic compound formed from 3-hydroxypropionaldehyde that has been implicated in the formation of bitterness in wines [73]. The determination of this compound is therefore of some importance, although its reactivity complicates the analysis [74]. For the analysis of acrolein in various matrices, derivatization is often employed, although methods for the analysis in wine using solid phase micro extraction (SPME) and sample enrichment probe (SEP) [75] extraction have been reported [74].

The volatile phenols 4-ethyl phenol, 4-ethyl guaiacol, 4-vinyl phenol and 4-vinyl guaiacol are known to originate from wood ageing, but elevated levels of these compounds are also associated with *Brettanomyces* spoilage. Smit et al. employed LLE using Freon 113 for the extraction of three volatile phenols in Weisser Riesling wines prior to their determination by GC–MS in scan mode [52]. This method was used to study the effect of expressing various phenolic acid decarboxylase genes in *S. cerevisiae*. The volatile phenols *o*- and *p*-cresol, phenol, ethyl guaicol, 2,6-dimethoxyphenol and guaicol together with other wood-derived volatiles including fufural derivatives and lactones were analysed in pot-still brandies by GC-FID on a WAX column [76–78].

Volatile thiols are influential aroma constituents, which may contribute positively or negatively to wine flavour. The analysis of these compounds is challenging due to their low levels of natural occurrence and their reactivity. Several highly volatile sulphur compounds such as methanethiol, dimethyldisulfide, dimethyltrisulfide and hydrogen sulphate are generally associated with off-flavours. The analysis of these compounds by large volume headspace injection using a programmed temperature vaporisation (PTV) injector and GC analysis in combination with selective pulsed flame photometric detection (PFPD) has been reported by Knoll et al. [54]. The sulphur compounds 4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-l-ol and 3-mercaptohexyl acetate contribute to varietal aroma of for example Sauvignon blanc wines. In order to study the production of these compounds, Swiegers et al. used stable isotope dilution analysis (SIDA) in combination with headspace (HS) SPME-GC-MS [64].

1,1,6-trimethyl-l,2-dihydronaphthalene (TDN) is a potent aroma compound in wine. This compound may be partially responsible for the typical bottle-aged kerosene character of aged Riesling wines and has an odour threshold value of 20 $\mu g\,L^{-1}$. TDN has been analysed by GC–MS in selected ion monitoring (SIM) mode following acid hydrolysis of the precursors isolated from wine by HPLC and thin layer chromatography (TLC). The glycosidic precursors of TDN in Riesling wines were structurally elucidated in these preparative fractions by means of NMR [53]. The determination of this compound by comprehensive two-dimensional gas chromatography in combination with time-of-flight MS detection (GC × GC-TOF-MS) in South African wines has also been reported [79,80].

The varietal aroma compounds in *Vitis vinifera cv.* Khamri grape juice, a native variety from Tunisia, were investigated by Souid et al. [81]. These included a number of higher alcohols, terpenes, acids, phenols and norisoprenoids. For the analysis of these diverse compounds, GC-FID and GC–MS were used, whilst gas chromatography-olfactometry (GC-O) was used to investigate the aroma profile of the juice. The authors fractionated the grape juice volatiles using SPE into free and bound fractions. The bound volatiles were enzymatically released prior to their analysis [81].

3.1.3. Sample preparation for wine volatile analysis

For the analysis of wine volatiles, sample preparation represents an especially important step in the analytical process. Effective extraction and pre-concentration of volatile constituents from the aqueous wine matrix is essential for their accurate qualitative and quantitative analysis. The choice of sample pre-treatment technique depends on the goals of the analysis. For the analysis of major volatiles, for example, LLE extraction is most often employed due to the relative simplicity and low cost of the technique. LLE using Freon as solvent was previously utilized extensively [51,52,55,56], although in recent years environmental concerns have largely resulted in the phasing out of its use. The use of diethyl ether in particular for the extraction of major volatiles has gained widespread application in recent years [42,49,50,57,65–69,71], although other solvent mixtures such as pentane/dichloromethane (2/1) have also been utilized [81].

On the other hand, important developments in sample preparation techniques have proved indispensable especially for the detection of low-level odour-active constituents, and have also allowed to significantly broaden the range of compounds that can be determined in a single analysis for untargeted methods. Sample pre-treatment methods which have gained widespread acceptance as powerful alternatives to conventional LLE for wine volatile analysis include SPE and various solventless sorptive extraction methods such as SPME and stir bar sorptive extraction (SBSE).

3.1.3.1. Sorptive extraction techniques. Sorptive extraction techniques such as internally coated open tubular traps (OTTs), solid phase micro extraction (SPME) and stir bar sorptive extraction (SBSE) have been shown by several authors to be advantageous for the extraction of volatiles from complex matrices such as wine. Sorptive extraction is based on the partitioning of chemical constituents into a liquid stationary phase. This approach provides several benefits compared to conventional extraction methods such as LLE, including elimination of the use of (often toxic) solvents, higher sensitivity and easy automation. The most common phase used in sorptive extraction is PDMS due to its well-known advantages of high temperature stability and inertness. Note though that in the case of some phases used in SPME (for example PSDVB or Carboxen phases), analyte retention is due to adsorption rather than sorption.

OTTs involve the use of a tube coated with a thick layer (up to 12 μm) of PDMS. The application of OTTs in both headspace and immersion modes has been demonstrated. The sample is typically sucked or pumped through the trap until breakthrough occurs.

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The trapped analytes are subsequently eluted using a solvent, or thermally desorbed prior to GC analysis. Burger and Munro demonstrated the applicability of OTTs for wine analysis as early as 1986 [82]. OTT was used for the headspace extraction of volatiles in Gewürztraminer and Crouchen blanc wine, although no specific compounds were identified [82].

SPME involves the use of a fused silica microfiber coated with the extraction phase (a wide variety of sorbent or adsorbant phases and mixtures is nowadays commercially available). The fibre is fixed to the stainless steel plunger of a syringe, allowing easy exposure or retraction of the fibre. Depending on the nature of the analytes, headspace or immersion SPME is possible. Following extraction, the fibre is typically inserted in a hot split/splitless injector and exposed to introduce the analytes to the chromatographic column.

SPME, most often used in the headspace mode (HS-SPME) utilizing a variety of stationary phases has been shown to be ideally suited for the extraction of volatiles from wines. For example, Weldegergis et al. [80] used a carboxen/polydimethylsiloxane (CAR/PDMS) SPME fibre in the headspace mode for the extraction of volatiles from South African Pinotage wines prior to analysis by GC × GC. TOF-MS was used to identify a large number of volatile compounds, including major and minor constituents such as esters, alcohols, acids, aldehydes and ketones, acetals, terpenes, furans and lactones. Furthermore, volatile sulphur compounds as well as nitrogen containing constituents – notably methoxypyrazines – were also detected, clearly illustrating the utility of SPME when used in combination with highly sensitive detectors. More recently a similar methodology using HS-SPME-GC × GC-TOF-MS was used for the analysis of Pinotage wines submitted to malolactic fermentation [83]. In this case a DVB/CAR/PDMS fibre was used, although in general similar compounds were identified in both studies [79,83].

Significant research activity has focussed on developing novel phases for SPME. For example, Wan Ibrahim et al. developed a new sol–gel hybrid polydimethylsiloxane-2-hydroxymethyl-18-crown-6-coated fibre for the extraction of low levels of organophosphorous pesticides from a diverse number of fruits, including grapes [84].

SBSE, developed by Baltussen et al. in 1999 [85], involves the use of a magnetic stir bar that is encapsulated in a glass sleeve and coated with PDMS. The stir bar is introduced in the aqueous sample and sorptive extraction occurs whilst stirring. Extracted analytes are subsequently thermally desorbed for GC analysis. Similar to SPME, sampling can also be performed in the headspace, referred to as head space sorptive extraction (HSSE). Varying amounts of PDMS can be used in SBSE, typically ranging between ~50 and 200 μL. The higher amount of stationary phase is responsible for the higher sensitivity of SBSE compared to SPME. However, unlike SPME where a wider range of phases may be used, PDMS is currently the only commercially available phase for SBSE.

The application of SBSE in immersion mode for wine analysis was first demonstrated by the extraction of dicarboximide fungicides by Sandra et al. [86]. Thereafter several applications for the extraction of mostly major volatiles and semi-volatiles from wines were reported. Tredoux et al. utilized the technique, also in immersion mode, for the extraction of major volatiles, volatile phenolic compounds, furan-derivatives and some minor volatile constituents such as aldehydes, ketones and lactones. These volatile data were used to classify white and red South African wines according to cultivar [58]. Furthermore, the application of HSSE for the quantitative analysis of volatiles in young South African red and white wines has been demonstrated [87]. The compounds quantified comprised a number of major volatiles as well as some wood-derived compounds such as oak-lactones, vanillin and volatile phenols [87]. This validated HSSE method was also used in combination with multivariate statistical methods to classify South African wines according to cultivar [59]. Pinotage wines in particular were clearly differentiated by higher levels of isoamyl acetate and ethyl octanoate.

Solid phase dynamic extraction (SPDE) is an alternative sorptive extraction technique where the PDMS trapping phase is coated on the wall of the needle of a headspace sampling syringe. Analytes are sampled in the headspace, followed by thermal desorption and large volume injection. This technique was used by Malherbe et al. to investigate the volatile profiles of fermenting grape musts in problem fermentations. These authors reported the determination of a significant number of major volatiles, together with some minor constituents including several potentially odour-active esters, terpenes and norisoprenoids [88].

3.1.3.2. Solid phase extraction. SPE is based on the extraction of volatile compounds from aqueous solutions using a suitable stationary phase. For wine volatiles, C18 and polystyrene-divinylbenzene (PSDVB) phases are most commonly used. The high capacity of these cartridges imply that large pre-concentration factors may be achieved by SPE, whilst the careful selection of suitable rinsing- and eluting-solvents may be used to selectively extract certain classes of compounds.

A simple SPE method based on a C18 phase was used by Zietsman et al. [72] to extract and pre-concentrate free monoterpenes from wine prior to GC-FID analysis on a FFAP column. The procedure entailed rinsing the cartridge with water following sample loading, and subsequent elution of the volatiles using dichloromethane.

Souid et al. [81] reported an interesting SPE procedure based on a PSDVB phase for the fractionation of Tunisian grape juice volatiles. Free aroma compounds were eluted from the cartridge using dichloromethane, whereas the bound volatiles were eluted with ethyl acetate. This fraction was subsequently submitted to enzymatic hydrolysis followed by LLE with pentane/dichloromethane. These fractions were analysed by GC-FID, GC–MS and GC-O in order to in establish the aroma profile of the native Tunisian grape variety *V. vinifera cv.* Khamri [81].

SPE has also been used as alternative to SPME for the analysis of volatiles in South African wines by GC × GC-TOF-MS. These authors used an SPE method based on [89] to selectively remove the more polar major volatiles using a rinsing solvent consisting of 50% (v/v) methanol and 1% $NaHCO_3$. The authors demonstrated that this sample pre-treatment procedure proved much more suited for the analysis of apolar high-boiling compounds such as terpenes, volatile phenols, lactones and sulphur compounds [79].

3.1.3.3. Derivatization of wine constituents. Derivatization is often used to modify non-volatile or highly polar chemical compounds not otherwise amenable to GC analysis. For example, Jolly et al. used methylation [90] of fatty acids prior to their analysis by GC-FID [70] (note that underivatised fatty acids may nowadays also be analysed on FFAP columns).

Especially in metabolomics research derivatization prior to GC analysis is frequently applied [91]. For untargeted screening of wine or grape metabolites, including polar and high molecular weight compounds such as sugars, long chain fatty acids, amino acids etc., trimethylsilyl derivatization is often used. Grimplet et al. employed a trimethylsilyl derivatization protocol using N-methyl-N-trimethylsilyltriflouroacetamide together with trimethylchlorosilane as derivatization reactants for the determination of grape- and fermentation derived metabolites such as amino- and organic acids, phenolic compounds and sugars [92]. In related research, Ali et al. [93] recently investigated the stereochemistry of wine amino acids with the objective of establishing a method for wine age authentication. The time-dependent conversion of *L*-amino acids into the *D*-form follows first-order kinetics, with the result that the extent of enantiomerization may reveal the age of a wine. Amino acid enantiomers were determined by

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chiral GC–MS in selected ion monitoring (SIM) mode following ion exchange based sample clean-up and derivatization to yield the N-(O)-pentafluoropropionyl 2-propyl esters. Although the presence of *D*-enantiomers was established in aged wines, no correlation was evident between these stereochemical forms and product age.

3.1.4. Comprehensive two-dimensional gas chromatography

Whilst conventional capillary GC has proven to be an indispensable tool in the routine analysis of volatiles associated with wine aroma, these methods do show some limitations in terms of resolving power and dynamic range when complex mixtures such as wine are analysed. Comprehensive two-dimensional GC (GC × GC) has been shown to provide a powerful alternative method capable of providing much higher separating power. This is achieved by exploiting the use of two stationary phases to combine separations based on boiling point and polarity. In recent years, GC × GC has also been applied to wine analysis in Africa. Weldegergis et al. [80] used HS-SPME-GC × GC-TOF-MS for the detailed investigation of South African Pinotage volatiles. This approach allowed the identification of a much larger number of compounds compared to one-dimensional GC: 48 compounds were identified using standards, whilst a further 158 compounds were tentatively identified using a combination of linear retention index (RI) data and deconvoluted mass spectra obtained by TOF-MS. Compound classes identified included esters, alcohols, aldehydes, ketones, acids, acetals, furans and lactones, sulphur compounds, nitrogen compounds, terpenes, hydrocarbons and volatile phenols. Subsequently, the same group extended this research by using SPE pre-treatment in combination with GC × GC-TOF-MS analysis [79]. By removing the more polar major volatiles, the identification of trace-level, high-boiling apolar odourants such as terpenes, lactones and volatile phenols was facilitated. Fig. 4 presents and example of a contour plot obtained for the analysis of a South African Cabernet Sauvignon wine. 214 compounds were tentatively identified in this study, whilst an additional 62 compounds were positively identified using standards. Another recent report on GC × GC-TOF-MS demonstrated the applicability of this technique for semi-quantitative analysis of wine volatiles [83]. In this study, HS-SPME-GC × GC-TOF-MS was used to investigate the volatile composition of Pinotage wines submitted to malolactic fermentation using different lactic acid bacteria strains. Excellent differentiation was obtained using data obtained by GC × GC, which allowed identification of the volatile compounds responsible for the variation between the wines produced with the different starter cultures.

3.2. Liquid-based separations

Many wine and grape constituents are not amenable to gas-phase separations due to either limited volatility and/or thermal stability. For the separation of these compounds, liquid chromatography is the separation method of choice, although capillary electrophoresis (CE) has been applied as an alternative liquid-based separation technique. Aside from routine regulatory analyses (see Section 4.1), which are often performed using classical wet-chemistry methods, the application of HPLC for the analysis of a variety of wine constituents has been growing tremendously. Advances in columns and instrumentation have contributed to a significant increase in the number of non-volatile wine constituents which may be accurately quantified or identified using HPLC.

3.2.1. High performance liquid chromatography

HPLC methods for wine and grape analysis can roughly be divided into one of two types. In the first instance, routine methods are used for the quantitative analysis of wine constituents for regulatory purposes, as well as to monitor the production process. In this case, the emphasis is on simplicity, speed, robustness and quantitative accuracy, and as a rule simpler instrumental configurations such as HPLC with ultraviolet (UV) or fluorescence detectors are used for this purpose. These instruments are relatively cheap and robust, and therefore ideally suited for routine analyses in wine laboratories. On the other hand, for the detailed chemical investigation of complex, low-level constituents with the aim of investigating wine and grape chemistry and elucidating new constituents, advanced LC–MS methods are most often used. For each of these methods, but especially for the second type, sample preparation plays an essential role. In the following discussion, an overview of the application of HPLC for wine and grape analysis in Africa is presented, with the discussion structured according to chemical classes.

3.2.1.1. Organic acids and sugars. Organic acids affect the taste and mouth-feel of a wine, enhance colour stability, limit oxidation and together with ethanol, are largely responsible for the microbial and physicochemical stability of table wines [94,95]. As primary substrates during alcoholic fermentation, sugars are responsible for the formation of ethanol as well as a number of secondary products, and their concentrations are used to determine the endpoint of fermentation. The levels of glucose and fructose, the major hexoses present in grapes and must, are used to determine optimal grape ripeness. Whilst high-throughput methods (see Section 4.1) are typically used for the determination of acids and sugars in grapes and wine for regulatory purposes, more selective methods are required if an in-depth knowledge of the organic acid and sugar composition.

For this purpose, HPLC is the preferred chromatographic method. Use of standard reversed phase (RP) columns for organic acid analysis dictates aqueous mobile phases, whereas RP analysis following derivatization offers the advantages of better chromatographic performance and improved (more sensitive and selective) detection, although at the cost of increased method complexity. However, ion exclusion HPLC, where separation is achieved through a combination of ion exclusion and partitioning processes using dilute acidic mobile phases, is the method of choice for organic acid analysis using low-wavelength UV and refractive index (RI) detection [54,65,71,96,97]. These methods have the added advantage of simultaneously allowing the measurement of the major wine sugars (fructose and glucose) as well as glycerol and ethanol.

For sugar analysis, aminopropyl or equivalent polar-phase columns may be used in combination with water/acetonitrile mobile phases [97] in hydrophilic interaction chromatography (HILIC) mode. UV detection has been employed under these conditions, although more common is the use of RI or evaporative light scattering detection (ELSD). Compared to RI, ELSD offers increased sensitivity and gradient-compatibility, although this type of detector commonly produces non-linear calibration curves and gradient-dependant response [98].

When using ion exclusion chromatography, direct injection of especially red wines results in co-elution and poor integration precision when using non-selective UV detection at 210 nm. One of the drawbacks of ion exchange methods is the limited scope for tuning the selectivity of organic acid analysis. Changing the pH does not greatly affect the retention of the earlier eluting compounds, and addition of organic solvent leads to an undesirable decrease in retention. To overcome these limitations, de Villiers et al. reported a sample clean-up procedure using SPE on PSDVB cartridges for the simultaneous analysis of organic acids and sugars in wine [97]. A low pH was used ensure retention of phenolic compounds (including phenolic acids) on the cartridge, whilst organic acids and sugars were eluted with 20 mM sulphuric acid. This procedure allowed

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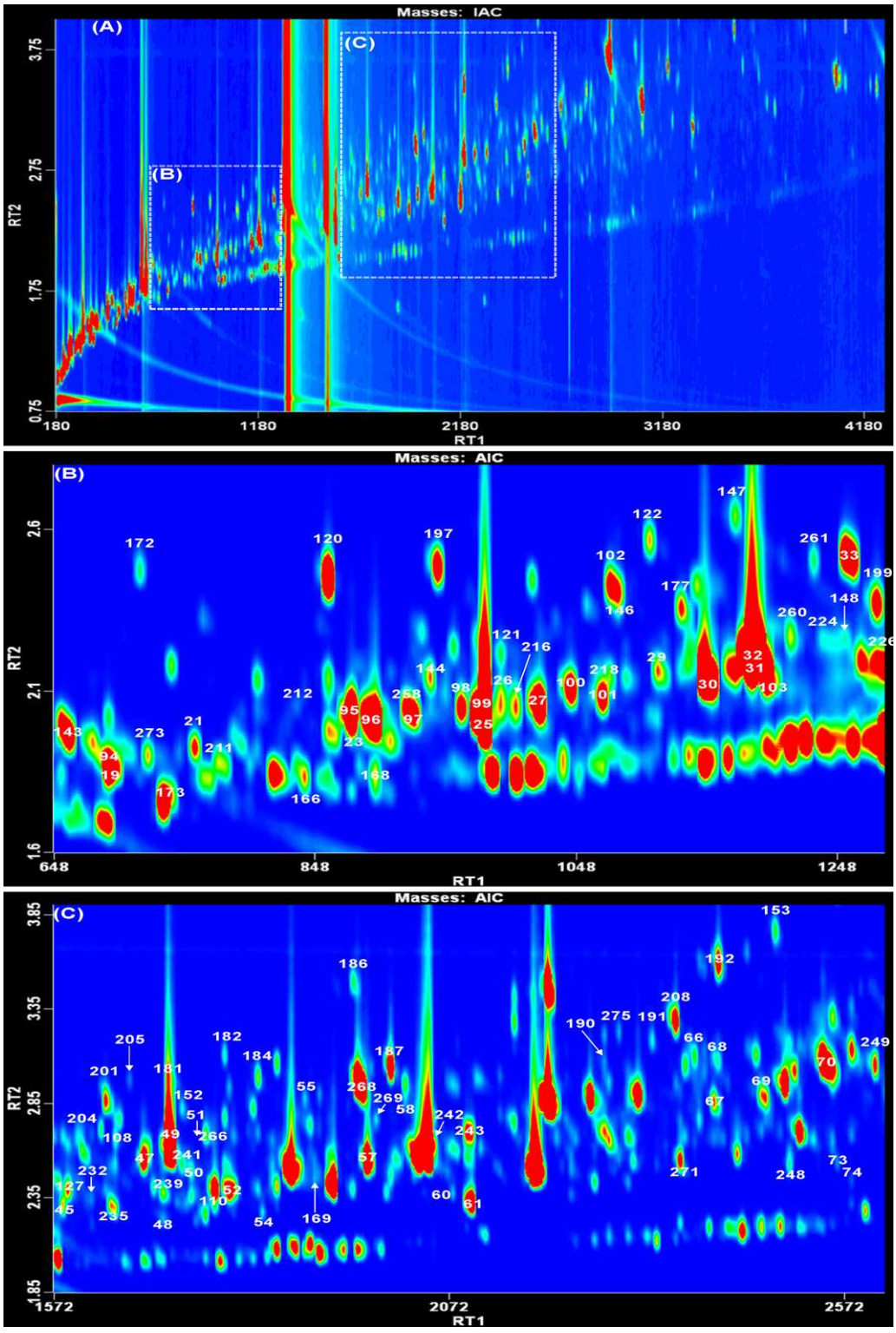

Fig. 4. (A) Example of a contour plot obtained for the SPE-GC × GC-TOF-MS analysis of a South African Cabernet Sauvignon wine. (B) and (C) present detailed portions of the contour plot to illustrate separation of volatile compounds. The column set used in these experiments consisted of a 30 m × 0.25 mm i.d. × 0.25 μm df Rxi – 5Sil MS primary column coupled to a 0.8 m × 0.18 mm i.d. × 0.18 μm df Rtx – PCB secondary column. Reprinted with permission from [79].

interference-free analysis of organic acids (by ion exclusion on an Aminex phase) and sugars (by HILIC-ELSD) in the same sample [97].

3.2.1.2. Biogenic amines. Biogenic amines are known to have physiologically detrimental effects if present in sufficient amounts, and are primarily formed by decarboxylation of amino acids–lactic acid bacteria are largely responsible for the production of bio-amines in wine. For a recent review on biogenic amines in wine covering the factors affecting biogenic amine formation, analytical and molecular methods used to detect biogenic amines and how to control their production in wine, the reader is referred to [99]. Analysis of amines in wine has been performed by GC and CE, although HPLC is more

commonly used, often in combination with the analysis of amino acids. A variety of derivatization reactions have been employed in combination with UV or fluorescence detection [99]. Cilliers and Van Wyk used an HPLC method with derivitization to quantify the histamine and tyramine content of South African wines [100]. Sample clean-up involved cation exchange on an Amberlite CG-120 cartridge, followed by derivitization with o-phtaldialdehyde prior to RP-LC analysis with fluorescence detection. The content of histamine and tyramine in 184 and 156 South African wines, respectively, were found to be similar to those reported for wines from other countries [100].

3.2.1.3. Chlorophylls and carotenoids. These photosynthetic pigments are of importance not only in the grapevine leaves, but also in grape berries as precursors for the production of norisoprenoids, which are known to be significant contributors to wine aroma. Lashbrooke et al. [101] reported and extensive study on the optimisation of a single-step extraction procedure for carotenoids and chlorophylls in grapevine leaf and berry tissue. Special attention was given to optimisation of extraction parameters in order to avoid degradation of the nine target analytes. Extracts were subsequently analysis by RP-LC with UV/Vis detection, which allowed the accurate monitoring of the carotenoids and chlorophylls in grape berries and leaves as a function of the different stages of ripening [101].

3.2.1.4. Phenolic compounds. Phenolic compounds are very influential constituents of grapes and wine. Phenolics affect organoleptic properties through their contribution to astringency, bitterness and colour. Furthermore, phenolics play an important role in the ageing of wines, as well as in grape browning. Finally, several important health benefits associated with modest consumption of especially red wine have been ascribed to the phenolic content of these products, which is partially responsible for the significant research activity in the field of wine phenolics.

In line with increased scientific interest in phenolic composition in wines in recent years [102], much African research into this influential class of wine constituents has also been performed. HPLC is most often employed to study wine and grape phenolics. RP-LC on C18 or equivalent stationary phases is virtually exclusively used for phenolic analysis. For quantification of the principal wine phenolics, UV detection is commonly employed, whilst the recent trend has been increasing application of MS for structural elucidation and quantification (the latter commonly using tandem MS) purposes. Due to the complexity of wine phenolics, extensive pre-fractionation is also often employed. In addition, in the past 5 years influential developments in HPLC, primarily in terms of the use of smaller particle-packed columns, elevated temperature and multidimensional separations, have also been exploited for these compounds. The application of these developments in HPLC to phenolic analysis has recently been reviewed [103].

Due to the variety and complexity of wine phenolics, a wide range of methods have been applied for their analysis. An extensive comparison of several of these methods for wine phenolic determination has been reported by De Beer et al. [104]. Techniques compared included liquid chromatographic methods (normal- and reversed phase HPLC), several selective chemical reactions for bulk determination of specific phenolic sub-classes (Folin-Ciocalteu, dimethylaminocinnamaldehyde, tannin, polymeric pigment and antioxidant assays) as well as cyclic voltammetry. Results obtained by each of these methods were compared for different classes of wine phenolics including monomeric phenols and anthocyanins, total phenol content, high molecular weight polymer and total polymer content. Significant correlation was observed for several of these methods for selected sub-classes [104]. The authors concluded that, due the complexity of wine phenolics, a combination of methods should be used for wine polyphenol analyses, with the specific methods selected dependant on the goals of the study (i.e. individual chemical constituents or total amounts of specific sub-classes).

Numerous literature reports provide quantitative data for phenolics in African wines. Goldberg and co-workers reported the concentrations of selected phenolic compounds in a large number of commercial red and white wines from across the globe [105,106]. Compounds were quantified by RP-LC with diode array detection (DAD) using UV spectral matching to confirm identity. For South African red wine cultivars, the levels of quercetin were found to be amongst the highest for the cultivars studied, whilst relatively high levels of p-coumaric acid were also found compared to wines from other countries [105]. In a related study, these authors used the same RP-LC-DAD method to determine the concentrations of selected phenolics (quercetin, *p*-coumaric acid, catechin and epicatechin, trans-resveratrol and polydatin) in 644 commercial white wines from all major wine-producing countries, including South Africa, and reported the highest levels of flavan-3-ols in Sauvignon blanc wines from this country [106]. Basha et al. reported the comparison of phenolic profiles of muscadine (*Vitis rotundifolia*) and *V. vinifera* grape wines from across the globe based on reversed phase HPLC analysis with UV detection at 280 nm [107]. Rossouw and Marais reported the levels of 39 phenolic compounds (including non-coloured phenolic compounds and anthocyanins) in 260 South African Pinotage, Shiraz, and Carbernet Sauvignon wines of four vintages [108]. The RP-LC method employed a PLRP-S polymeric column with diode array detection based on the work of Waterhouse et al. [109]. The authors were able to obtain a very good differentiation between the cultivars (independent of vintage) using discriminant analysis based on mean levels of the quantified phenolics [108]. de Villiers et al. reported data for non-coloured phenolic content in five red ($n = 55$) and three white ($n = 38$) South African cultivars [110]. White wines were directly injected, whilst a SPE method was used to remove interference from polymeric phenolics for red wines [97]. Quantitative data for 22 phenolics were used to classify the studied wines according to cultivar using multivariate statistical methods (Fig. 5). In fact, quantitative phenolic data obtained by HPLC have successfully been employed to differentiate South African wines according to both grape variety [108,110,111] and vintage [108] by multivariate statistical methods in various reports.

du Toit et al. utilized a LC-UV method on a monolithic column (Chromolith Performance RP-18) to quantify 21 non-coloured phenolics and anthocyanins, as well as polymeric pigments in South African red wines [13]. These data were used to ascertain the effect of micro-oxygenation on the levels of phenolics in the studied wines. In combination with sensory data, it was shown that micro-oxygenation may potentially be used to improve the quality of especially young red wines [13].

A monolithic column was also used by Liazid et al. [112] for the quantitative analysis of 13 non-coloured phenolic in grapes and derived products. A fast (14 min) method was developed utilizing a 100 mm Chromolith Performance RP-18 column operated at 2.5 mL min^{-1} in combination with UV and fluorescence detection. The method proved to be reproducible, and its application for the analysis of wine musts was demonstrated [112].

The stilbene content of wine, especially for the compound *trans*-resveratrol, has received extensive attention in literature due to the beneficial biological activity ascribed to this class of compounds [102]. Guebailia et al. reported for the first time the presence of a resveratrol tetramer, called hopeaphenol, in Merlot wines from Algeria [113]. In a more recent paper, the same group also reported for the first time the isolation and characterisation of the resveratrol dihydrodimer *cis*-ε-viniferin from Algerian wine [114]. These compounds were isolated from wine using a combination

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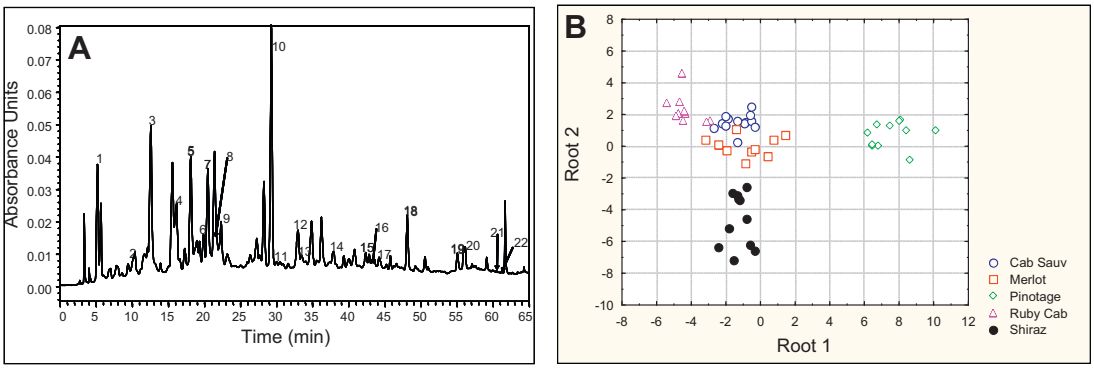

Fig. 5. (A) Typical HPLC-UV chromatogram obtained for the analysis of South African red wine, illustrating 22 phenolic compounds quantified; (B) scatter plot of the scores on the first two canonical roots obtained from the quantitative polyphenol data for red wines. Reprinted with permission from [110].

of column chromatography on a cation-exchange resin, centrifugal partition chromatography using a water/ethanol/ethyl acetate/hexane mobile phase and semi-preparative RP-LC on a C18 column. Following isolation, the compounds were characterised by MALDI-TOF-MS and ^{1}H NMR and 2D correlations [113,114]. Subsequently *trans*-resveratrol, *trans*-piceid, *trans*-ε-viniferin, pallidol, astilbin, hopeaphenol and *cis*-ε-viniferin were quantified in Algerian, Moroccan and Tunisian red and white wines using analytical RP-LC with UV detection. Wines from North Africa were found to contain high levels of resveratrol derivatives [113,114].

The antioxidant properties of wines, and especially the importance of phenolic compounds in this regard [115,116], have been investigated extensively. The role of phenolic compounds in wine as antioxidants has been reviewed by de Beer et al. [117]. The same group also reported extensive data on the antioxidant capacity of South African wines. The free radical scavenging activity of South African red and white wines was determined using 2,2′-azinobis(3-ethylbenzothialozinesulfonic acid) radical cations (ABTS) and 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) [118]. In further work, de Beer et al. reported the *in vitro* inhibition of microsomal lipid peroxidation for the major South African red and white wine varieties [119]. Roginsky et al. [120] used similar methods in a study which concluded that the antioxidant activity of Californian red wines do not correlate with wine age. Several possible reasons for this rather unexpected observation were discussed [120]. Furthermore, the relationship between the content of individual phenolic compounds and the total antioxidant capacity of Pinotage wines was investigated [121]. Twenty-four individual phenolic compounds (comprising, flavanols, flavonols, anthocyanins and phenolic acids), as well as polymeric phenolics, were quantified using a RP-LC method on a polymeric column [122]. It was found that individual monomeric phenolics were responsible for only a small fraction (11–24%) of the total antioxidant activity of Pinotage wine. The remainder may be ascribed to unidentified compounds (including polymeric phenolics), as well as to synergetic effects involving phenolic compounds [121]. This same RP-LC method was also used in a study of the effect of oak maturation using different products on the phenolic composition, antioxidant activity and colour of Pinotage wines [123]. Whilst the phenolic content and colour of wines submitted to oak maturation using both traditional and alternative oak products were altered significantly, the total antioxidant activity was found to remain constant [123]. In another study by the same group, the effect of oxygenation on Pinotage phenolic content, colour and antioxidant activity was investigated [124]. The authors concluded that controlled minimal doses of oxygen should be used to avoid detrimental effects in terms of sensory properties and antioxidant activity.

Concerning grape phenolics, Youssef and El-Adawi [125] reported a study on the optimisation of the extraction of Egyptian red grape seed phenolics. RP-LC was used to quantify gallic acid, catechin and epicatechin in the extracts. In other work on proanthocyanidins, Hmamouchi et al. [126,127] investigated the phenolic content of four Moroccan varieties of *V. vinifera* leaves. 10 flavonoids were detected in these leaves [126], whilst the oligomeric proanthocyanidin content was found to consist of varying ratios of prodelphinidins to procyanidins [127].

Van Jaarsveld et al. in a series of papers reported the effect of different wood types, treatments and extraction media to induce rapid ageing of brandy [76–78]. As part of this study, 12 volatile constituents including influential volatile phenols were determined by GC-FID, whilst a further 10 phenolic compounds were quantified in the extracts by RP-LC with UV detection. These data were correlated with sensory data to determine the highest quality products and relate these to their chemical composition. In general, the best quality extracts contained higher levels of volatile and semi-volatile wood-derived compounds. Better quality products were also obtained using higher concentrations of ethanol as extraction medium [76] and toasted oak [78]. French oak was found to yield initially better quality products, although after 8 months ageing similar results were obtained for the American oak products, whilst the chemical composition of the products produced from each of these types of oak were found to vary [77].

3.2.1.5. Anthocyanins. Anthocyanins (anthocyanidin-glycosides) are phenolic compounds responsible for the colour of red grapes and wine. These compounds are also important for their contribution to the health benefits associated with wine consumption and the vital role they play in the ageing of red wines.

de Villiers et al. reported a method for the analysis of anthocyanins in five South African red wine cultivars using an RP-LC-UV-MS method [111]. Forty-four anthocyanins and derived products were identified using MS detection and retention times. Sixteen compounds were quantified as malvidin-3-O-glucoside equivalents using selective UV detection, and these data were used to differentiate between the different cultivars. This classification proved to be less effective than that obtained using non-coloured phenolic data [110], at least partially due the fact that a wide range of vintages was studies, and therefore the reduction in levels of free anthocyanins as a function of time affected the differentiation between cultivars.

Ghassempour et al. [128] reported a study on the extraction of anthocyanins from Iranian red grape skins by microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE). These authors used RP-LC on C18 columns in combination with UV and MS/MS detection for the quantification and identification of nine anthocyanins in the extracts. Both MAE and UAE were found to

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be suitable methods for the efficient extraction of grape anthocyanins. Choi et al. [129] reported the tentative identification of 19 anthocyanins in the skins of *Vitis coignetiae* Pulliat (meoru), a wild vine species native to Korea, by making use of RP-LC-MS/MS. These authors also reported the antioxidant activity of anthocyanins present in the skins.

de Villiers et al. reported an investigation into the reversed phase separation of anthocyanins and the factors affecting the efficiency of these separations [130]. The authors demonstrated that the relatively slow inter-conversion (on the same time-scale as the separation) between carbinol- and flavylium species in the mobile phase results in relatively broad peaks for anthocyanins under conventional RP-LC conditions [130]. It was further shown how and increase in analysis temperature and decrease of the stationary phase particle size may be used to significantly improve the analysis of red wine anthocyanins [130–132]. The benefits of this approach were exploited by using a 200 mm 1.7 μm phase operated at 50 °C in combination with positive mode ESI-MS for red wine analysis [133]. The authors report much improved separation under these conditions, and were able to identify 101 anthocyanins and 36 proanthocyanidins in a single analysis [133].

RP-LC methods based on polymeric C18 phases have been used extensively in the analysis of anthocyanins [121–124]. Oberholster et al. [134] used this approach for the quantification of non-coloured phenolics and anthocyanins in a study related to the mouth-feel of white wines produced with pomace contact and added anthocyanins. Ristic et al. [135] utilized reversed phase HPLC on Synergy Hydro-RP and polymeric (polystyrene-divinylbenzene) columns for the analysis of anthocyanins and tannins and flavonols in Australian Shiraz grape skins and seeds and wine to study the effect of shading on grape and wine composition. Several monomeric proanthocyanidins, anthocyanins and flavonols were quantified using these methods, whilst tannin sub-unit composition was studied using phloroglucinol acid-cleavage. Bindon et al. [136] used RP-LC on a Lichrospher 100 RP-18 column for anthocyanin determination in grape berries, whilst tannins were analysed on a polymeric RP phase according to Peng et al. [122]. Changes in anthocyanin composition of berries as a result of partial root zone drying were reported in this study [136].

Non-coloured phenolic compounds such as flavonols may also affect wine colour as a result of chemical reactions involving these compounds during wine ageing [137]. In order to elucidate the pigments formed in this manner, model solutions are often used in combination with advanced analytical methods such as preparative LC, LC–MS and NMR to study reaction products. Es-Safi et al. [138–143] have in this manner extensively studied the reactions involving (+)-catechin in model solution. Thus, using HPLC-DAD, LC–MS and NMR spectroscopy, this compound was shown during artificial ageing experiments to first produce colourless dimeric reaction products, followed by the formation of various xanthylium pigments [138,144]. These compounds were also successfully detected in red wine samples by RP-LC-ESI-MS. Furthermore, the reaction between (+)-catechin and glyoxylic acid (the latter produced from oxidation of tartaric acid in wine media), has been studied in model solution. RP-LC with DAD and ESI-MS detection was used to detect the derived products [139,141,145], whilst two-dimensional NMR methods such as COSY, TOCSY, ROESY, HSQC and HMBC techniques were used in the unambiguous structural elucidation of each of the reaction products following semi-preparative LC isolation [140]. An overview of the interactions between (+)-catechin and glyoxylic acid and their importance in terms of food organoleptic properties was reported by Es-Safi et al. [142].

The same group has extensively studied the reactions between flavonols and anthocyanins with the ultimate goal of elucidating some of the important reactions occurring during wine production and ageing (and in fact fruit-derived beverages in general). Thus the reaction between (epi)catechin and various aldehydes (acetaldehyde, glyoxylic acid, furfural and 5-(hydroxymethyl)furfural) in the presence of mavidin-3-O-glucoside were studied in model solution using a combination of LC-DAD- and LC–MS [146–149]. Both coloured and non-coloured products increasing in size up to tetramers were identified in the reaction mixtures. Further extension of this work involving (+)-catechin and cyanidin-3-O-glucoside in the presence of furfural and 5-(hydroxymethyl)furfural produced similar pigments, although the formation of coloured products were favoured compared to malvidin-3-O-glucoside. Various bridged oligomeric and polymeric products were identified. The eventual precipitation of some products reported may play a role in the reduction in astringency of red wines during ageing [150]. Taken together, this work points to the great diversity of products that may be formed during wine storage and ageing, and the effect that the derived products may have on the organoleptic properties of the product.

In addition, numerous other pathways exist for the transformation of grape-derived anthocyanins in the wine medium [133,137,151]. Fulcrand et al. [152] were the first to identify the pyranoanthocyanin products formed by reaction of anthocyanins with pyruvic acid using a combination of HPLC, MS and NMR. Es-Safi et al. have also investigated the reactions involving malvidin-3-O-glucoside in ethanolic solutions [153]. Using a combination of RP-LC-DAD, HPLC-ESI-MS and one- and two-dimensional NMR analysis of fractions collected by HPLC, these authors successfully characterised two new colourless products formed during storage of malvidin-3-O-glucoside in ethanol [153].

The phenolic content of Pinotage wines has received considerable attention. Characterisation of Pinotage non-coloured and anthocyanin phenolics has been utilized for purposes of classification according to cultivar [108,110,111] and vintage [108], whilst the antioxidant properties of this wine have been studied in depth [121,123,124]. In addition, this wine has received attention due to high levels of a 4-vinylcatechol adduct of malvidin-3-O-glucoside, referred to Pinotin A [154]. Pinotin A was isolated from Pinotage wines by Schwarz et al. by making used of a combination of SPE and high speed counter-current chromatography (HSCCC) [154]. The formation of this compound in wine has been ascribed to the chemical interaction of caffeic acid and malvidin-3-O-glucoside, the principal anthocyanin present in young wines [155]. Whilst not unique to Pinotage wines, the formation of Pinotin A is favoured in wines of this cultivar due to the high content of caffeic acid which is characteristic of Pinotage [156]. Quantitative analysis of this compound obtained by RP-LC analysis for 50 Pinotage wines of vintages 1996–2002 indicated that its levels increase up to ~4 years, whereafter polymerization or degradation reactions lead to a reduction concentration [156].

An interesting study involving anthocyanins was reported by Gargouri et al. [157], who investigated the binding-equilibrium and kinetics of the *V. vinifera* enzyme anthocyanidin reductase. A chromatographic method utilizing a size exclusion column was used to study the binding of the enzyme with NADPH, $NADP^+$ and catechin. In this method, the mobile phase contains a fixed amount of potential ligand. By injecting increasing amounts of the ligand with the enzyme, the amount of ligand bound to the enzyme may be deduced from the threshold value where the ligand peak provides zero net absorbance. From these experiments, the dissociation constants for anthocyanidin reductase and the studied ligands could be determined [157].

3.2.1.6. Miscellaneous. The inherent selectivity of liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has also been exploited for the trace-level determination of methoxypyrazines in South African wines [158]. Although these aroma constituents are normally analysed using GC, LC–MS/MS

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under optimal conditions was found to provide much lower limits of detection, allowing accurate quantification of methoxypyrazines in white and red varieties (including the first report on the presence of 3-isobutyl-2-methoxypyrazine in Pinotage wine samples). Multivariate data analysis showed no significant correlation between the levels of methoxypyrazines in 575 South African Sauvignon blanc wines as a function of vintage or geographical origin [158].

Du Toit et al. [159] reported a novel LC–MS/MS method suitable for the simultaneous determination of reduced and oxidised glutathione, an important compound in the oxidation of white wines, in grape juice and wine. The method employed an Atlantis C18 phase and positive electrospray ionisation, with detection performed in multiple reaction monitoring (MRM) mode, providing limits of detection in the region of 0.2–0.4 mg L^{-1}. This method was used to obtain information on the levels of reduced glutathione levels in South African white wines produced with different levels of oxygen (i.e. reductive, control and oxidative treatments) [159]. More recently, Du Toit and co-workers reported the development and validation of an ultra-performance liquid chromatographic (UPLC) method with multi-wavelength UV detection for the analysis of glutathione, catechin and caffeic acid in grape juice and wine [160]. Glutathione was derivatised with *para*-benzoquinone to allow its detection at 303 nm. The use of 2.1 mm internal diameter 1.7 μm RP columns operated at elevated pressures allowed for the development of a rapid analytical method ideally suited for routine analysis and providing significant reduction in solvent consumption [160].

LC–MS has also been used in wine-related proteomic research. For example, Rossouw et al. [161] utilized an isobaric tag for relative and absolute quantitation (iTRAQ)-based proteomic analysis of two different wine yeast strains at various times during fermentation of a synthetic wine must for the comparative transcriptomic and proteomic profiling of these strains.

Another interesting study utilizing LC–MS/MS [162] deals with the (indirect) identification of malvidin in potsherds from archeological sites in Armenia and Syria. Presence of malvidin was established following extraction from the ceramic vessels and SPE sample clean-up followed by alkaline hydrolysis of malvidin to produce syringic acid, which was then detected by RP-LC-MS/MS in MRM mode. The presence of malvidin in some of the potsherds provides supporting evidence for the hypothesis that wine was produced in the Near Eastern highlands around 4000 BC [162].

3.2.2. Capillary electrophoresis

Capillary electrophoresis (CE) has, since the early 1990s, received significant attention in the literature as an alternative liquid-based separation method to HPLC. The principal benefits of CE are the inherently high efficiency and speed as well as the versatility of the technique, as reflected in the alternative separation mechanisms offered by the various modes of CE. However, CE methods generally suffer from lower sensitivity and robustness compared to standard HPLC methods, and partially for these reasons the technique has primarily found application in certain niche-areas where CE provides clear benefits compared to HPLC (for example chiral separations). Reflecting these trends, CE has also found application in the analysis of grapes and wine, especially for the analysis of compounds not easily determined by HPLC.

The ionic nature of organic acids makes these compounds ideally amenable to CE analysis. The relative mobility difference between acids and other wine constituents is responsible for their separation. As a result, one of the principal advantages of CE for organic acid analysis is elimination of the requirement of sample preparation.

de Villiers et al. reported a CE method for the analysis of organic acids in South African wine. The inherent advantages of CE were exploited to allow the separation of the major organic acids in diluted wine. 2,6-pyridinedicarboxylic acid was used as background electrolyte (BGE) with indirect UV detection. The method was improved compared to previously reported procedures by the addition of ethelenediamine-tetracarboxylic acid (EDTA) to the BGE in order to suppress complexation of citric acid with trace metals present in the capillary. In addition, electrokinetic injection was utilized to avoid problems relating to with split peaks associated with pressure injection at high acid levels [163], which was ascribed to differences in the sample and buffer pH [96]. In addition to eliminating the need for sample preparation, the increased efficiency of

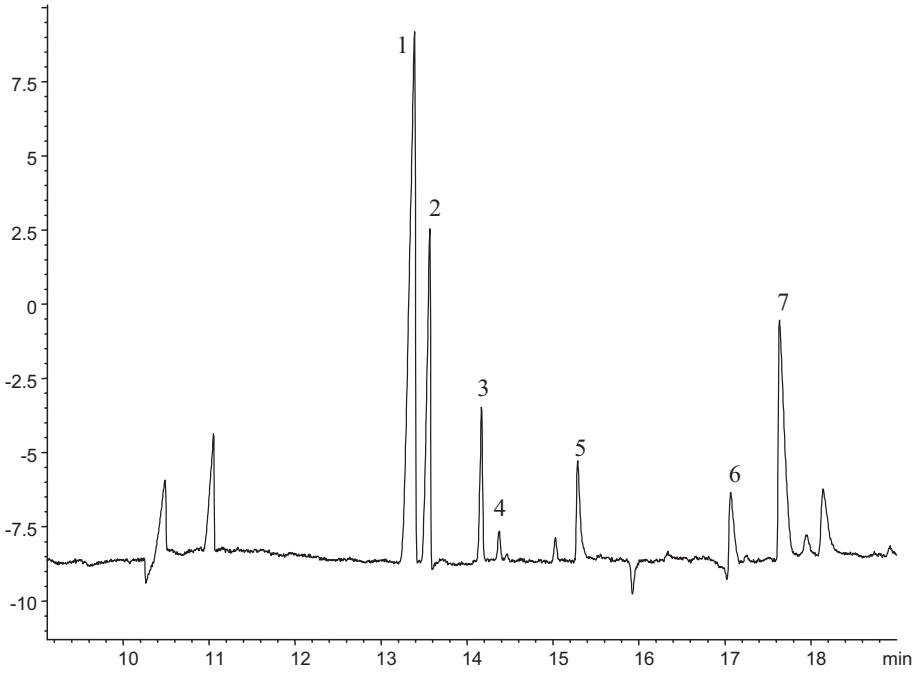

Fig. 6. CE analysis of organic acids in a diluted South African red wine. Background electrolyte: 7.5 mM PDC, 0.5 mM CTAB, 0.5 mM EDTA, pH 5.6. Capillary: 75 μm i.d., 111.3 cm L_{tot}. Peaks: 1 = formic acid (I.S.), 2 = tartaric acid, 3 = malic acid, 4 = citric acid, 5 = succinic acid, 6 = acetic acid, 7 = lactic acid. Reprinted with permission from [96].

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the method provided more reliable data than HPLC utilizing ion exchange [97] due to less co-elution of other wine constituent (especially problematic in the case of succinic acid in the HPLC method). An example of the analysis of a South African red wine using the developed method is presented in Fig. 6.

Capillary zone electrophoresis (CZE) has also been used for the analysis of phenolic compounds in South African wines. Vanhoenacker et al. [164] compared CZE-UV-ESI-MS with RP-LC-UV-ESI-MS for the analysis of monomeric phenolic compounds in diethyl ether extracts of red wines. These authors concluded the RP-LC remains the method of choice for phenolic analysis. CE-MS was found to suffer from poor sensitivity, rendering this technique insufficient for wine analysis. Furthermore, CE has also been applied to detect artificial colourants in red wine (see Section 4.2 for details) [165].

4. Regulatory analysis, food safety and quality assurance

In international commerce, laws are passed to regulate the quality, authenticity and health and safety of commodities. The global wine industry is possibly subject to more regulations than most because of the great diversity and complexity of its products. Regulations may cover aspects ranging from how grapes are grown to when and where wine is sold and consumed. In addition to appellation control regulations, national laws regulating this industry are enforced in most countries. Legislation often differs in terms of the additives and processes allowed in the winemaking process between different countries [166]. This has implications for importing/exporting wines between countries, as governed by the relevant trade agreements, and therefore also in terms of the analytical methods required to monitor this industry.

Regulatory laws are primarily concerned with quality, health and safety aspects and generally involve the chemical composition of wines. It should be noted that wine excellence cannot be guaranteed by either objective chemical analysis or the existence of a controlled appellations system. Although sensory evaluations are subjective, and therefore not strictly quantitative in nature, they nevertheless have greater significance than objective chemical analysis alone and play an important role in quality assurance systems (such as the one implemented in South Africa, for example) [94]. Controlled appellation systems largely (but not exclusively) make use of record keeping and inspections for ensuring compliance, whereas chemical analysis is the basis for ensuring conformity to national laws that regulate the wine industry in many countries. In the following discussion, analytical techniques employed in the African wine industry for regulatory, safety and quality assurance will be reviewed.

4.1. Regulatory analyses

Despite the extreme complexity of wine, only a few chemical compounds are typically regulated in wine legislation. Regulated wine parameters include alcohol content, reducing sugars, volatile acidity and sulphur dioxide. Analytical procedures for determining these regulated parameters are mostly official methods prescribed by the International Vine and Wine Office (OIV) and are frequently classical wet chemistry methods characterised by high robustness and precision and low cost (the latter is an important consideration in many wine laboratories). Alternative procedures utilizing modern, automated instrumental techniques, which provide high sample throughput, sensitivity, selectivity and precision, may also be applied for regulatory analyses. However, for these methods to be endorsed by the OIV, a systematic comparison with the official reference method is mandatory to ensure suitability [167,168]. In the following sections, a brief overview of the official methods used for regulatory purposes will be presented. Where relevant, more modern methods reported by African scientists will be discussed in more detail.

4.1.1. Alcohol content

The alcohol content of wine is an important parameter that is universally displayed on wine labels and which factors in the calculation of excise duty in commerce. The alcohol content of different types of wine is legislated in many countries. The determination of the wine alcohol therefore needs to be accurate and precise as tolerances in the order of 0.5–1.0% of the documented value are typically enforced. Procedures for the determination of wine alcohol content may be divided into methods that measure the physical characteristics of a solution (typically the distillate of a wine) and those based on the chemical properties of alcohol. Chemical methods include dichromate oxidation and enzymatic determination, whilst physical methods use specific gravity or boiling point depression. The official OIV method uses specific gravity for wine alcohol determination [167]. Instrumental methods such as HPLC and GC may also be used [169]. Fletcher and van Staden [170] described an automated sequential injection analysis technique utilizing dichromate oxidation and spectrophotometric detection for the determination of ethanol in distilled liquors. Recently, the suitability of rapid, multi-component non-specific NIR spectroscopic methods for alcohol has been demonstrated. These methods rely on extensive calibration protocols to ensure accuracy [14,15,40].

4.1.2. Volatile acidity

Volatile acidity is defined as the content of those wine acids which may readily be removed by steam distillation. Volatile acidity is an indicator of wine spoilage and is therefore regulated as a quality assurance parameter. Spoilage may result from bacterial action such as caused by acetic acid bacteria or spoilage yeasts, such as *Brettanomyces*. Since extrinsic factors may also play a role in development of volatile acids (for example in some dessert wines), specific legal limits are often dependent on the class or style of the wine [94,169]. The OIV prescribes steam distillation and titrimetry as the reference method for volatile acidity [167]. Enzymatic and flow injection methods as well as HPLC, GC and NIR spectroscopic methods have also been described for this purpose [14,15,168,169].

4.1.3. Sulphur dioxide

Sulphur dioxide is widely used in the wine industry as a chemical preservative and inhibitor of microbiological activity as well as an antioxidant to reduce chemical and enzymatic browning. Due to its negative sensory properties and adverse health effects, the sulphur dioxide content of wines is regulated. Sulphur dioxide can exist in inter-convertible free and bound states, the regulated levels of which vary depending on the type and style of wine as well as between bulk and bottled wines [94,169]. The official OIV analysis method involves oxidation of separated sulphur dioxide followed by titremetry. Free and total sulphur dioxide are separately determined in this way by entrainment at low temperature and high temperature, respectively. Titration with iodine may be used as a rapid alternative method, although this procedure is known to be inaccurate. Instrumental methods described for sulphur dioxide analysis include flow injection analysis, enzymatic analysis, HPLC, GC, potentiometry and polarography, ultraviolet and visible spectrophotometry, atomic absorption and fluorometric spectrometry as well as NIR spectroscopic methods [14,169].

4.1.4. Reducing sugars

The principal sugars utilized by yeast in alcoholic fermentation are glucose and fructose, referred to as reducing sugars as they are capable of reducing copper (as Cu(II)), a characteristic which is used in their analysis. The reducing sugar content is an

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important regulatory parameter that is used to classify wine styles [94]. Analytically, reducing sugars may be determined by chemical, enzymatic, flow injection analysis and HPLC [97] techniques, whilst GC may also be utilized following derivatization [168,171]. NIR spectroscopic methods for the determination of reducing sugars have also been described [14,15]. The official OIV method is based on the reduction of Cu(II) in boiling alkaline medium and determination of the remaining copper. In wine styles where the addition of sugar (usually sucrose) to the finished product is allowed, such as sparkling wines, these are subjected to a preliminary acid hydrolysis to convert disaccharides to their component reducing sugars [167]. Chaptalization (pre-fermentation addition of sugar) is typically illegal in warmer growing conditions such as encountered in Africa, where grapes usually develop adequate sugar levels. The addition of sucrose to the must can only be detected with stable isotope analysis since complete hydrolysis of sucrose at normal wine pH levels is expected in the finished product [94].

4.1.5. Heavy metals

Many minerals are found in wine and in most instances these reflect uptake characteristics of the rootstock and climatic influences on the rate of transpiration. Since heavy metals typically precipitate during fermentation, their elevated occurrence in finished wine is usually associated with contamination after fermentation [94]. Heavy metals are determined in wine with spectrophotometric and spectroscopic techniques. Due to the low maximum levels that are typically enforced for toxic elements, specialized techniques such as graphite furnace atomic absorption spectroscopy (AAS) (for Pb and Cd) and hydride generation AAS (for As and Hg) are prescribed by the OIV. Flame AAS methods are used for elements such as copper, iron and tin, for which relatively high maximum levels are typically enforced [167]. Onianwa et al. [43] successfully applied flame AAS for the determination of various metals, including lead and cadmium, in non-alcoholic wines using suitable sample mineralization and pre-concentration techniques. Inductively coupled plasma emission spectroscopy (using both optical and mass spectrometric detection) may also be used for multi-element analysis. Dessuy et al. [44] developed and validated a method for the determination of lead in wine using electrothermal AAS. The use of various chemical modifiers was investigated and palladium was found to produce optimal stabilization of lead during pyrolysis. The optimised procedure enabled the determination of lead in wine without any sample preparation with a limit of detection of 0.5 $\mu g\,L^{-1}$. Since this procedure is fully automated and sufficiently sensitive, it is suited for routine regulatory determination of lead in wines.

4.1.6. Preservatives

Antimicrobial agents are used to confer microbial stability to wine, the most frequently used being sulphur dioxide. Other preservatives such as sorbic acid, benzoic acid, dimethyl dicarbonate and natamycin are also allowed. Of these preservatives only sulphur dioxide and dimethyl dicarbonate possess reasonable wide-spectrum antimicrobial properties, whilst natamycin is prohibited in some countries (notably the EU). Dimethyl dicarbonate can effectively sterilize wine if used just before bottling. This compound decomposes rapidly to carbon dioxide and methanol and therefore produces no sensory defect or residue. However, it has low solubility and is corrosive and therefore requires expensive equipment for effective application. Sorbic acid and benzoic acid (or their sodium salts) generally have low effectiveness and produce negative sensory effects at higher concentrations. Their use is therefore subject to legislated maximum allowable concentrations [94]. Sorbic acid and benzoic acid may be determined with spectrophotometry, but are more often analysed in wine by HPLC with UV-visible detection [169]. For example, at the National Department of Agriculture in South Africa, sorbic acid in wine is determined by direct injection RP-LC-UV utilizing ion pairing or an acidic mobile phase to optimise chromatographic efficiency. UV detection at ~260 nm confers sufficient selectivity to the technique to yield detection limits in the low $mg\,L^{-1}$ range. Natamycin at its effective concentrations may also be determined in wine using HPLC with UV-visible detection, but for demonstration of compliance with EU standards, more sensitive methodologies are required. Alberts et al. [172] recently described a simple, robust and fast LC-ESI-MS/MS method for the determination of natamycin in wine. Sample preparation involved dilution followed by direct elution from aminopropyl SPE cartridges. The application of mutually supporting sample pre-treatment and chromatographic separations to eliminate matrix-related ion suppression enabled quantitative determination of natamycin in wine with external standard calibration. This critical benefit rendered the method suitable for routine analysis of large numbers of samples in support of the wine export industry to the EU. The method complied with EU standards in terms of sensitivity and selectivity for this application and was also used to study the degradation kinetics of natamycin in the wine matrix (an important aspect from a regulatory point of view).

4.1.7. Methanol

Methanol is usually present in wine in relatively small quantities and never accumulates to toxic levels using legitimate winemaking procedures. In humans methanol is oxidised to formaldehyde and formic acid, both of which are toxic to the central nervous system. As methanol is derived from the pectin content of the fermentable substrate, red wines typically evolve more methanol than white wines, and pectolytic enzymes added to the juice or wine to aid clarification may further increase methanol levels. The addition of distilled spirits to wine, such as in fortified wines, may also affect the methanol content [94,169]. Wine methanol content is therefore typically regulated by legislation. Methanol is usually determined by GC-FID following quantitative distillation of the wine to eliminate non-volatile constituents.

4.1.8. Wine authenticity

Establishing conformity with laws and regulations governing the wine industry is often dependent on the development of sophisticated analysis techniques. Because of the wide range of possible adulteration practices and the complexity of wine, these methods are often specifically designed for each type of adulteration [94]. For example, de Villiers et al. [165] developed HPLC and CE methods for the analysis of the artificial dyes brilliant blue and azorubine in red wines. Liquid–liquid extraction followed by ion-pair liquid chromatographic analysis allowed separation of these dyes from wine polyphenols to achieve detection limits in the parts per billion range with reliable UV-spectral identification. On the other hand CE analysis following SPE sample clean-up provided higher efficiency, reduced solvent consumption and faster analyses for the same analyses [165].

4.2. Food safety

4.2.1. Pesticides

Synthetic organic pesticides are used for vineyard disease, pest and weed control. Integrated pest management schemes aim to limit the application of these treatments whilst increasing their effectiveness through the application of combined expertise in the fields of plant pathology, economic entomology, plant nutrition, weed control and soil science. However, programs to monitor wines for the presence of these substances are required for consumer health protection [94].

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Traditional pesticide residue analytical methods employ LLE or SPE for sample preparation prior to GC or HPLC analysis. Sandra et al. [173] described a multi-residue GC–MS method based on SBSE for the analysis of pesticide residues in aqueous foods and grapes. Solid samples were pre-extracted with methanol, diluted and extracted by SBSE followed by automated sample introduction by thermal desorption. Pesticide residues were identified using retention time locked GC–MS operated in full scan mode. Quantitation was performed using standard addition or isotope dilution, since matrix interferences affected analyte recoveries. The technique yielded detection limits in the sub-ppb range for over 350 pesticides [173]. The same approach was also used for the determination of dicarboximide fungicides in white wines, and provided detection limits in the low to sub-$\mu g\,L^{-1}$ range. For thermolabile congeners, the accuracy of the technique was verified by SBSE followed by liquid desorption and analysis with LC-APCI-MS [86]. The quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method is a novel sample extraction technique used effectively in multi-residue methods in combination with MS analysis techniques. Afify et al. [174] validated a method for the determination of 150 pesticide residues in grapes using QuEChERS in combination with LC-ESI-MS in the positive ionisation mode. Ionisation suppression effects were compensated with matrix matched calibration standards and recoveries of target pesticides ranged between 70% and 110%.

4.2.2. Toxins and mycotoxins

Ethyl carbamate is a carcinogen that is universally present in wines where it is mainly formed by the acid-catalysed reaction between ethanol and urea. Its concentration in liquor products is therefore regulated in several countries. Ethyl carbamate accumulates in wine over time and is also present in distilled products such as brandy. Since the presence and associated health risks of ethyl carbamate became known, measures have been instigated to reduce the risk of contamination, for example limiting vineyard nitrogen fertilization to reduce the formation of precursors (such as urea) [94,175].

Ethyl carbamate is usually determined with GC following SPE or LLE sample clean-up and pre-concentration. Waldner and Augustyn [176] used GC–MS to conduct a survey of the levels of ethyl carbamate in South African wines. Although red wines were found to accumulate more ethyl carbamate compared to white wines, low concentrations were mostly found. Alberts et al. [175] recently reported a novel RP SPE- normal phase LC-APCI-MS/MS method for the determination of ethyl carbamate in liquor products. This method offered good sensitivity and selectivity and its applicability for the analysis of wines, fortified wines and distilled spirits such as brandy was demonstrated. The authors reported levels of ethyl carbamate in South African products, as well as the factors responsible for its formation. Despite a clear correlation with age, all products contained low concentrations.

Ochratoxin A is a carcinogenic mycotoxin that is produced by several fungi. Since grapes are frequently contaminated, this compound also occurs in wine. Ochratoxin A is determined by RP-LC with FLD or MS detection. Confirmation of Ochratoxin A utilizing HPLC-FLD has been achieved by derivatization to the corresponding methyl ester products, whilst MS detection inherently lends itself to structurally specific confirmation. The low levels of occurrence of ochratoxin A in wine generally necessitate sample pre-concentration, which is most often performed using immunoaffinity columns.

Aboul-Enein et al. [177] described a LLE sample preparation procedure utilizing chloroform for the analysis of ochratoxin A in wine. Various surveys of the ochratoxin A content of South African wines found levels that were well below the suggested EU regulatory limit [178,179]. A study of the occurrence of ochratoxin A and identification of octratoxigenic microbiota in Tunisian vineyards found that *A. carbonarius* is the principal cause of contamination in Tunisian grapes. The potential for ochratoxin A contamination is highly variable and increases during grape ripening, whilst some grape varieties are more susceptible [180]. Selouane et al. [181] studied the effect of temperature, water activity and incubation time on growth and ochratoxin A production by fungi isolated from Moroccan grapes. Morocco has a warm, humid climate which is conducive to the development and growth of molds, and therefore the risk of mycotoxin contamination is relatively high. A review of the occurrence and legislation of mycotoxins in food and feed from Morocco reported relatively high levels of ochratoxin A in wines (red wines were particularly affected) [182].

5. Conclusions

This survey of the analysis of grape, wine and derived beverages performed in Africa allows several general conclusions to be drawn. Clearly, based on the number of references reported herein, extensive and increasing analytical research involving these products is performed on the African continent. The significant increase in the number of papers from African authors dealing with this topic, especially during the last decade, is evident from Fig. 7, which presents a concise summary of the reports cited in this review. It should be noted that much of the regulatory analyses, often performed using instrumental techniques, are rarely reported in the scientific literature, and therefore the amount of research performed on this topic arguably significantly exceeds an estimate based solely on the papers cited here.

In terms of the analytical methods employed in these studies, there has been since the 1970s a continuous trend in using more advanced analytical instrumentation to shed light on the chemical composition of these samples. This phenomenon may be ascribed first of all to concomitant developments in methods of instrumental analysis. Secondly, accurate analytical data play an important role in many spheres of research involving grapes and their derived products. As research questions become more detailed and challenging, more advanced chemical analysis methods are therefore required. Table 1 provides an overview of the most important milestones in analytical methods applied to wine analysis in the African context.

The past few years have seen a significant increase in the application of spectroscopic techniques in combination with multivariate data analysis methods for especially wine analysis. The papers cited show that IR spectroscopy has huge potential for rapid low-cost quantitative and qualitative applications throughout the production chain of winemaking. It is foreseen that some of the new

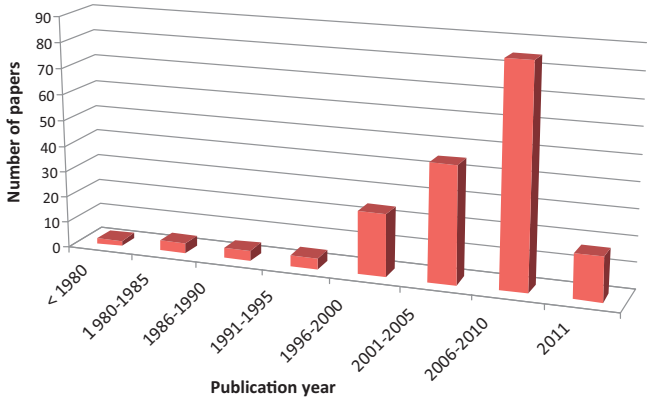

Fig. 7. Summary of the number of references in scientific literature dealing with the analysis of wine, where one or more authors are from an African institution.

Table 1
Summary of the most important milestones in instrumental chemical analytical methods applied to wine analysis in the African context.

Milestones	Selected references
Spectroscopy	
Quantitation of grape and wine compounds: Establishment of PLS-based algorithms for the quantification of important grape and wine quality parameters using NIR, FT-NIR and FT-MIR allows high-throughput quantification of wine constituents	[26,27,32,38]
Application in yeast breeding and identification studies: High-throughput screening of hybrid yeasts based on their fermentation profiles obtained with FT-MIR spectroscopy and chemometric techniques as well as identification of pure cultures of the spoilage yeast *B. bruxellensis*	[40,41]
Authentication studies: FT-MIR spectra of five important single cultivar wines were used to discriminate between cultivars	[42]
Gas-phase separations	
Capillary GC columns: The introduction of capillary GC columns resulted in a dramatic increase in chromatographic resolution and sensitivity compared to pack columns. Nowadays almost exclusively used for wine analysis	[42,56,57,81]
Gas chromatography–mass spectrometry (GC–MS): Coupling of MS with GC separation allows on-line identification of unknowns based on their mass spectra. MS also offers enhanced sensitivity, especially when operated in selected ion monitoring (SIM) mode	[52,53,64,81,93]
Gas chromatography-olfactometry (GC-O): GC-O combines the separation power of GC with the selectivity and sensitivity of the human nose to study odour-active compounds in wine	[82]
Solid phase extraction (SPE) in combination with GC: SPE is a selective sample preparation procedure which enables targeting of specific chemical classes by removal of interfering wine constituents and pre-concentration prior to GC analysis	[81,89]
Solid phase micro-extraction (SPME) in combination with GC: SPME is a solventless and sensitive sorptive sample preparation technique for GC which is used extensively in wine analysis; a wide selection of phases is available to tune selectivity	[64,79,80,83,84]
Stir bar sorptive extraction (SBSE) in combination with GC: Another sorptive extraction method, SBSE offers increased sensitivity compared to SPME due to larger amount of sorptive phase (PDMS); has been used as alternative to SPME for wine analysis	[58,60,83,87]
Comprehensive two-dimensional gas chromatography (GC × GC): GC × GC provides drastic improvement of chromatographic resolution and sensitivity due to the use of two orthogonal separation mechanisms; only recently been applied to wine	[79,80,83]
Liquid phase separation	
High performance liquid chromatography (HPLC): The application of automated high pressure instrumentation and columns has significantly improved the routine quantitative analysis of non-volatiles in the wine industry	[100,101,104,110,112,114]
Liquid chromatography–mass spectrometry (LC–MS): MS is a powerful structural elucidation tool and sensitive detector when used in combination with HPLC separation, which has found extensive application in wine analysis in recent years	[86,111,111,138–149,152,174]
Liquid chromatography tandem mass spectrometry (LC-MS^n): Tandem mass spectrometric techniques provide improved sensitivity and selectivity as well as improved structural elucidation performance, especially relevant for trace-level wine constituents	[131,158–160,172,175]
Capillary electrophoresis (CE) and CE-MS: CE provides several potential benefits compared to HPLC, primarily improved separation efficiency, although the technique has found limited application in wine analysis in Africa	[96,164,165]
Ultra high pressure liquid chromatography (UHPLC): A recent development in HPLC, where small particle-packed columns are operated at elevated pressures (>400 bar) for improved speed or efficiency; recently finding increasing application in wine analysis	[103,130–133,160,162]
High temperature liquid chromatography (HTLC): Elevated temperature is used primarily to provide shorter analysis times, often in combination with UHPLC; limited application to wine analysis to date	[103,130–133]

global trends in viticulture, particularly the use of remote sensing and portable spectrometers, will be increasingly used in monitoring grape quality in vineyards in Africa. The continent has substantial local expertise, as well as international collaborations, to exploit IR technology for the purpose of sustainability in agricultural production. In terms of product authentication, spectroscopic techniques, including NMR spectroscopy, which has to date seen little application in grape and wine analysis in Africa, will without doubt also be increasingly used in the future. Finally, the emerging technologies of IR and NMR imaging that frequently combines microspectrometry for in situ visualisation of chemical features in whole tissue, will feature more prominently in addressing more fundamental research problems of biological nature.

Also clear from the papers cited in this review, is the growing use of advanced chromatographic methods for wine and grape analysis. In terms of gas-phase separations, GC-FID remains a popular method for routine analysis of volatiles, although GC–MS is increasingly often being used both for quantitative and qualitative analyses in this field. This development may be linked to the obvious benefits of the technique in terms of sensitivity and identification power, as well as the fact that bench-top GC–MS instruments have become relatively affordable. Other important fields in gas-phase separations include sample preparation, alternative, more sensitive and selective detection strategies such as TOF-MS and tandem MS, and in recent years the application of GC × GC to wine analysis.

An overview of the application of HPLC for wine analysis in Africa highlights the importance of this technique, especially for purposes of routine analysis of a large number of non-volatile compounds. Data generated in this manner has been used broadly in studies involving wine chemistry and relating various manufacturing processes to wine chemical composition. In addition to the extensive use of HPLC for routine analyses, the technique has made a significant contribution to the detailed investigation of the complex chemistry of grapes and especially wine. For this type of research, LC–MS is increasingly being used for identification purposes of novel compounds (often in combination with preparative isolation and NMR) [183]. Moreover, a recent trend, also evident from the research performed in Africa, is the application of tandem mass spectrometry for the selective detection of trace-level compounds. CE shows promise for analysis of specific compounds where this technique provides benefits compared to HPLC. However, the relative complexity of the technique and limited availability of instrumentation and expertise in Africa means that HPLC will remain the chromatographic method of choice for the analysis of non-volatile constituents in wine.

Finally, the use of advanced (often multivariate) statistical methods in combination with analytical data for wine and grapes has developed significantly in the last decade [22]. This may partially be linked to the developments in analytical methodologies, as the increasing amount of information obtained using advanced spectroscopic and chromatographic techniques has highlighted in importance of extracting the information relevant for a particular experiment. Related to this is an increasing trend in the application of statistical analytical methods for the unsupervised

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analysis of grapes and wine. In these instances, the goal is not target analysis of a selected number of compounds, but rather to find differences between samples based on certain parameters, where the compounds differing are not known *a priori*. With the amount of information that may be obtained in a single analysis continuously increasing, this trend is expected to become more important in the future.

It should be noted that much of the research performed on grapes and wine is of an inherent inter-disciplinary nature [62]. Analytical techniques are extensively employed in various research areas related to the production of grapes and wine, including viticulture, soil science, horticulture, microbiology, biotechnology, etc. This aspect has also served to drive developments in the field of wine and grape analysis. Therefore, a large part of the wine research performed on the continent critically hinges on analytical techniques to obtain quantitative data for a wide range of compounds.

Clearly, analytical chemistry can be seen to play an important part in ongoing research aimed at improved understanding of wine production, with the ultimate goal of producing better products. In view of the chemical complexity of grapes and their derived products, these trends are expected to increase further in future, and in this manner analytical methods will continue to play an influential role in the understanding of the chemical composition of grapes, wine and their derived products on the African continent.

Acknowledgements

Financial support was provided by the South African National Research Foundation (NRF) and Winetech.

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