The Phenolic Composition of South African Pinotage, Shiraz and Cabernet Sauvignon Wines *

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Phenolic compounds are major constituents of red wines and impact on certain wine-quality parameters. This study has aimed to increase the knowledge base on the phenolic composition of South African red wines by HPLC quantification of 39 individual phenols and 2 polymeric, phenolic groups in 260 South African wines of the cultivars Pinotage, Shiraz and Cabernet Sauvignon. Statistical analysis of the collected data revealed significant cultivar differences in the levels of many of the analysed compounds, as well as in the ratios in which specific anthocyanins are found in the wines. These data may be helpful in the cultivar authentication of wines of these cultivars. Discriminant analysis of the data showed statistically significant separations of cultivars, based on their polyphenol composition, and also how the use of data from a specific wine-producing area or vintage improved the possibilities for successful authentication. The data were collected from both pure cultivar wines and commercial wines, which may contain up to 15% of another cultivar wine. The collected data may therefore be further segmented into those obtained from pure cultivar wines and those obtained from blended wines in order to further enhance the accuracy of authentication of these respective groups of wines. The information obtained from this study opens several avenues for research on the impact of the noted cultivar differences on wine quality. Cultivar differences in the phenolic composition of young red wines also have important implications for the oenological management of oxidation reactions taking place during vinification and aging.

Phenolic compounds are major constituents of wine and have an impact on certain sensory properties such as colour, mouth-feel characteristics and taste (bitterness) (Gawel, 1998; Ribéreau-Gayon *et al.*, 2001). Manipulation of the levels of these compounds in wine through the application of viticultural and oenological practices may therefore be a useful tool to improve wine quality. However, knowledge is lacking on exactly which polyphenols are responsible for specific sensory observations and little is known about the polyphenol composition of South African red cultivar wines. Recently, a typical polyphenol profile or fingerprint was compiled for Pinotage (Rossouw & Marais, 2003). This cultivar was developed in South Africa and is an important local asset.

Different groups of phenolic compounds are found in wine, including phenolic acids, cinnamic acid esters, flavonols, flavan-3-ols and anthocyanins. The group known as phenolic acids can be further subdivided into benzoic and cinnamic acids. Table 1 shows the basic structures of some of these compounds with examples from each group and the sensory impacts these compounds may potentially have in wine.

Anthocyanins, the pigments responsible for red wine colour, are increasingly being used in the authentication of red cultivar wines (Revilla *et al.*, 2001; Burns *et al.*, 2002). The relative levels of these compounds in the grapes of different cultivars show clear distinctions and have been used to determine the parentage of grape cultivars (Castia *et al.*, 1992). The use of these relative ratios in wine authentication has, however, been debated in the scientific literature. Some opposing viewpoints exist as to the usefulness of some of these ratios as sole measures of cultivar authenticity (Burns *et al.*, 2002). It is possible that cultivar-associated differences in the levels of polyphenols, other than anthocyanins, could exist which could be a further aid in the more reliable authentication of wine cultivars. Polyphenol composition differences could contribute towards distinctive sensory characteristics expressed by wine from different cultivars.

The purpose of this study was to obtain a general overview of the polyphenol composition of South African red wines of the cultivars Pinotage, Shiraz and Cabernet Sauvignon, in order to alleviate to some extent the lack of knowledge that exists in this field. Such knowledge could be a useful tool both in wine-quality manipulation as well as in the cultivar authentication of South African red wines of these cultivars.

MATERIALS AND METHODS

Wines of the cultivars Pinotage (100 samples), Shiraz (76 samples) and Cabernet Sauvignon (84 samples) were obtained from the local 2002 Young Wine (117 samples) and Veritas (143 samples from vintages 1999 to 2002) wine shows. Some of the commercial Veritas wines may contain up to 15% of other red cultivar

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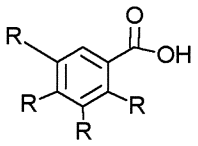
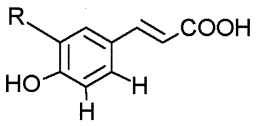
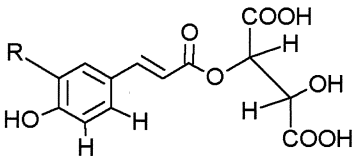
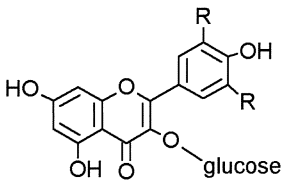
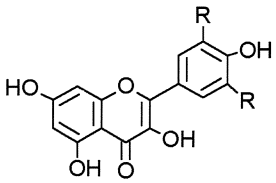
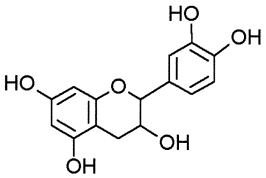
TABLE 1

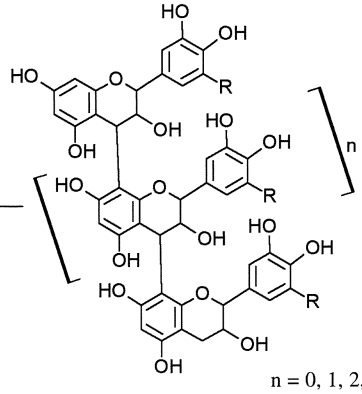
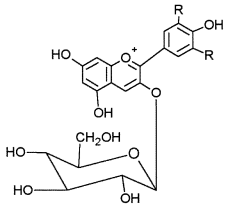
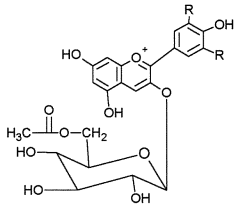
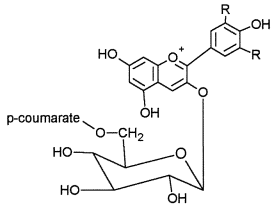
Examples of phenolic compounds found in wine: Their basic structure and potential sensory impact.

Name of phenolic compound group	Generalised structure R = OH or H or OCH_3	Examples	Sensory attribute potentially impacted
Phenolic acids			
Benzoic acids		Gallic acid, protocatechuic acid	Colour and taste (bitterness)
Cinnamic acids		Caffeic acid, *p*-coumaric acid	
Cinnamic acid esters			
		Caftaric acid, coutaric acid	Taste (bitterness)
Flavonols			
Flavonol glycosides		Quercetin-3-*O*-glucoside, Quercitrin.	Taste (bitterness) with weak astringency. Colour intensity and hue through co-pigmentation with anthocyanins
Flavonols		Quercetin, myricetin, kaempferol, isorhamnetin	
Flavan-3-ols			
monomers		(+)-Catechin, (-)-epicatechin.	Astringency and bitterness sensations. Impact on colour through co-polymerisation with anthocyanins

(Table 1 continues on next page)

TABLE 1 (continued)

Examples of phenolic compounds found in wine: Their basic structure and potential sensory impact.

Name of phenolic compound group	Generalised structure R = OH or H or OCH_3	Examples	Sensory attribute potentially impacted
Flavan-3-ols			
oligomers		Procyanidin B1, procyanidin B2	Astringency and bitterness sensations. Impact on colour through co-polymerisation with anthocyanins
polymers	n = 0, 1, 2, 3, 4, 5	Condensed tannins	
Anthocyanins			
Unacylated anthocyanins		Malvidin-3-*O*-glucoside, Peonidin-3-*O*-glucoside, Petunidin-3-*O*-glucoside, Delphinidin-3-*O*-glucoside, Cyanidin-3-*O*-glucoside	Colour intensity and hue of red wine. Impacts on mouth-feel of wine through co-polymerisation with other polyphenols
Acylated anthocyanins			
Acylated anthocyanins		Malvidin-3-*O*-glucoside-acetate, Peonidin-3-*O*-glucoside-acetate	Colour intensity and hue of red wine. Impacts on mouth-feel of wine through co-polymerisation with other polyphenols
p-Coumarylated anthocyanins		Malvidin-3-*O*-glucoside-*p*-coumarate, Peonidin-3-*O*-glucoside-*p*-coumarate	Colour intensity and hue of red wine. Impacts on mouth-feel of wine through co-polymerisation with other polyphenols

wines, according to the South African certification system. The samples were mostly representative of the wine-producing regions in South Africa; the only exception was that an insufficient number of Shiraz samples from the Olifantsriver wine region were obtained. The polyphenol composition of each of these wines was determined, using the HPLC method C described by Waterhouse *et al.* (1999). Compounds were separated on a PLRP-S, 5µ 100 Å, column of Polymer Labs using a Spectra System P2000 pump of Thermo Separations products with a vacuum degasser and AS1000 autosampler. Detection was achieved using a Spectra Systems UV6000LP diode array detector. Wines were stored at 0°C until analysis. Quantification of phenolic compounds was done using external standards, which were commercially obtained from Polyphenols and Extrasynthese. Anthocyanins were detected at 520 nm using malvidin-3-*O*-glucoside as external standard, hydroxycinnamic acids at 316 nm using caffeic acid, flavonols at 360 nm using rutin, flavanols at 280 nm using (+)-catechin and phenolic acids at 280 nm using gallic acid. Identities of individual polyphenols were confirmed by authentic standards. Authentic standards for the acetylated and coumarylated anthocyanins as well as for caftaric and coutaric acid were not commercially available. The identities of these

compounds were confirmed by their relative retention times and UV-visible absorption characteristics, using Singleton *et al.* (1978), Castia *et al.* (1992), Price *et al.* (1995), Waterhouse *et al.* (1999) and Peng *et al.* (2002) as references. Discriminant analysis of the analytical data was performed using SAS/STAT, Version 8 (SAS Institute, Inc., USA, 1999).

RESULTS AND DISCUSSION

Table 2 shows the mean concentrations of polyphenols determined in the Pinotage, Shiraz and Cabernet Sauvignon wines. Statistically significant differences were found between cultivars in the mean concentrations of certain polyphenols. Some of the most striking differences were significantly higher mean levels of the flavonols quercetin-glucoside (isoquercetin), quercitrin, quercetin and isorhamnetin in Shiraz wines, compared to Pinotage and Cabernet Sauvignon wines. Quercetin has been shown to elicit a bitter taste with weak astringency (Dadic & Belleau, 1973). These compounds have a yellow colour (Ribéreau-Gayon *et al.*, 2001) and may therefore have an impact on wine colour. They are also very effective copigments for anthocyanins, the pigments responsible for red wine colour. Wine colour intensity is increased and colour hue is altered by this copigmentation process (Boulton, 2001). It needs to be determined to what extent the observed higher levels of flavonols found in Shiraz wines impact on taste (bitterness), intensify colour or alter colour hue, compared to other cultivar wines that contain lower levels of flavonols.

Another important observation is the significantly higher levels of caffeic acid and caftaric acid found in Pinotage wines (Table 2). Cinnamic acids such as caffeic and its tartrate ester, caftaric acid, have been described as bitter and astringent (Dadic & Belleau, 1973; Ong & Nagel, 1978). However, Vérette *et al.* (1988) found neither caftaric acid nor caffeic acid to be bitter at concentrations higher than those found in the present study. Cinnamic acids can be expected to have little sensory impact in wine individually, due to the relatively low concentrations in which they occur. However, as a group together with benzoic acids they may well have more impact, due to a synergistic lowering in the taste threshold of such mixtures (Maga & Lorenz, 1973; Gawel, 1998). In Pinotage wine the individual concentrations of caffeic and caftaric acid may possibly be sufficiently high to have an impact on wine taste, but this still has to be assessed. The unidentified anthocyanin, which is denoted Pino in Table 2, could possibly be Pinotin A, identified by Schwarz *et al.* (2003). The Pino peak had similar HPLC elution characteristics to those described by Schwarz *et al.* (2004), for Pinotin A and its diode array spectrum agreed with the absorbance spectrum of Pinotin A as described by Schwarz & Winterhalter (2003). Pinotin A is a reaction product of malvidin-3-*O*-glucoside and caffeic acid. The mean concentration of Pino is significantly higher in Pinotage wines compared to wines of the other two cultivars (Table 2), which may be ascribed to the higher concentration of caffeic acid found in the wines from this cultivar. Pinotin A and other similar pyranoanthocyanins have a more orange or brick-red hue compared to the bluish-red hue of malvidin-3-*O*-glucoside and they are much less susceptible to pH shifts and retain their original colour over a much wider pH-range (Schwarz & Winterhalter, 2003). Due to these unique colour properties of Pinotin A, differences in its concentration in wine may have an impact on wine colour.

Cabernet Sauvignon wines contained higher levels of acetylated anthocyanins than the other two cultivars (Table 2). The only observed exception to this generalisation was peonidin-glucoside-acetate, which was highest in Shiraz wines. This may be due to the significantly higher mean level of peonidin-glucoside found in these wines. Shiraz wines also contained significantly higher levels of *p*-coumarylated anthocyanins, compared to the other two cultivars. Acylation of anthocyanins does change the colour hue expressed by these molecules in solution (Giusti & Wrolstad, 2003). The observed cultivar differences in acylation of anthocyanins may therefore possibly lead to differences in colour hue expressed by these cultivars.

Significant differences could also be seen between cultivar wines in their content of monomeric, dimeric and polymeric flavan-3-ols. Such differences may make a notable impact on the taste (bitterness), mouthfeel and colour of wines. The high molecular weight polymeric flavan-3-ols are more astringent than the smaller oligomers. However, astringency increases up to the heptamer level and then starts to decrease (Ribéreau-Gayon *et al.*, 2001). The monomers (e.g. catechin), dimers (e.g. procyanidin B1) and trimers have a more bitter taste than the polymeric forms (Arnold *et al.*, 1980; Peleg *et al.*, 1999). The ratio of smaller oligomeric to larger condensed procyanidins in a wine could therefore influence the perceived ratio of bitterness to astringency. The monomeric, oligomeric and polymeric flavan-3-ols also play an important role in the stabilisation of wine colour. Anthocyanins are not stable and may degrade under certain conditions, leading to a loss of colour and therefore wine quality (Romero & Bakker, 2000). However, pigments of young wines may be altered and stabilised during ageing by the reaction of anthocyanins with flavan-3-ols to yield complex, polymeric pigments (Peng *et al.*, 2002). This reaction leads to a change in wine colour from the purple red of young wines to the more brick red hue observed in older red wines (Romero & Bakker, 2000; Mateus & De Freitas, 2001). The ratio of anthocyanins to flavan-3-ols in any given wine could therefore have a marked impact on the final, stabilised colour of such a wine (Ribéreau-Gayon *et al.*, 2001). Differences in the phenolic composition of wines may have an influence on the rate at which important oxidation reactions take place in wine (Boulton, 2001). Cultivar differences in the phenolic composition of young wines will therefore have an influence on oenological practices, such as the management of oxygen concentration in wines during micro-oxygenation.

The concentrations of seven unknown compounds (denoted as hydroxycinnamate 15, flavanol 20, flavanol 33, flavonol 41, flavonol 42, flavonol 46 and flavonol 50), generally found in the analysed cultivar wines, are also shown in Table 2. The tentative designation of each of these compounds into their respective phenolic group was based on their absorption spectra as determined by diode-array detection during HPLC analysis. Although their identities are not known at this stage, they are major constituents of wine and, most importantly, their concentrations showed clear cultivar differences. Only the concentration of flavonol 41 did not show statistically significant differences between cultivars. Flavonol 50 is most probably a quercetin-glucuronide, based on the elution order of flavonols described by Price *et al.* (1995), who used a PLRP-S column, similar to that used in this study. Flavanol 20 may be procyanidin B3 or a dimer of catechin and gallocate-

TABLE 2

The mean concentrations of polyphenols in South African Pinotage, Shiraz and Cabernet Sauvignon wines.

Polyphenol (HPLC retention time in minutes)	Shiraz (76 wines)			Pinotage (100 wines)			Cabernet Sauvignon (84 wines)		
	Mean*1 (mg/L)	95% CL(-)	95% CL(+)	Mean*1 (mg/L)	95% CL(-)	95% CL(+)	Mean*1 (mg/L)	95% CL(-)	95% CL(+)
Gallic acid (7.2)	31.59^{b}	10.87	52.32	31.85^{b}	5.31	58.39	38.90^{a}	4.16	73.65
Protocatechuic acid (14.2)	1.34^{a}	-0.54	3.22	0.73^{b}	-0.45	1.91	1.19^{a}	-0.28	2.66
Procyanidin B1 (25.3)	39.64^{a}	24.07	55.21	40.48^{a}	20.68	60.29	36.16^{b}	17.37	54.96
(+)-Catechin (25.9)	50.40^{a}	28.87	71.93	41.82^{b}	21.22	62.43	54.59^{a}	16.56	92.61
(-)-Epicatechin (31.4)	40.58^{a}	11.31	69.84	29.94^{b}	7.58	52.29	34.13^{b}	8.96	59.30
Syringic acid (33.7)	2.10^{a}	-1.44	5.65	2.61^{a}	-0.17	5.39	2.49^{a}	-3.37	8.36
(-)-Epicatechin gallate (41.5)	8.82^{a}	5.25	12.38	6.67^{b}	1.37	11.97	9.31^{a}	6.42	12.20
Tryptophol (62.6)	3.56^{a}	0.13	6.99	2.43^{b}	-0.08	4.93	3.86^{a}	0.81	6.92
Polymeric phenols (76-82)	296.64^{a}	85.98	507.29	235.44^{b}	24.20	446.69	295.61^{a}	75.51	515.71
Quercetin-3-glucoside (49.5)	16.42^{a}	-3.69	36.54	7.93^{b}	-0.75	16.60	8.65^{b}	-5.31	22.62
Quercitrin (57.2)	18.27^{a}	7.55	29.00	11.19^{b}	6.49	15.90	12.17^{b}	6.79	17.55
Myricetin (67)	13.97^{a}	3.03	24.91	9.73^{b}	1.21	18.25	13.70^{a}	2.30	25.09
Quercetin (79.9)	31.60^{a}	2.32	60.87	14.06^{c}	2.44	25.67	23.01^{b}	4.70	41.32
Kaempferol (82.6)	3.60^{a}	0.84	6.37	2.58^{b}	0.62	4.54	3.56^{a}	0.92	6.20
Isorhamnetin (83.2)	6.54^{a}	1.14	11.94	2.32^{c}	0.79	3.86	3.46^{b}	1.43	5.48
Caftaric acid (22.7)	22.2^{b}	-4.30	48.70	35.47^{a}	-19.23	90.16	18.96^{b}	-1.92	39.84
Caffeic acid (29.3)	16.14^{b}	-0.65	32.93	37.96^{a}	-5.52	81.43	13.71^{b}	-0.76	28.18
Coutaric acid (30.8)	14.43^{a}	-2.43	31.29	11.16^{b}	-4.13	26.46	10.20^{b}	-0.95	21.35
p-Coumaric acid (43.3)	9.39^{ab}	-2.14	20.93	11.47^{a}	-0.40	23.35	7.36^{b}	-0.56	15.29
Delph-3-gluc (21.3)	8.62^{b}	-1.32	18.56	9.28^{ab}	-2.72	21.29	11.39^{a}	-6.16	28.94
Cyan-3-gluc (25.9)	1.24^{b}	0.50	1.97	1.31^{b}	0.16	2.46	1.54^{a}	-0.18	3.26
Pet-3-gluc (28.1)	13.86^{a}	-1.72	29.45	13.57^{a}	-3.03	30.17	11.38^{a}	-5.39	28.14
Peo-3-gluc (32.9)	8.98^{a}	-0.69	18.65	6.39^{b}	-2.40	15.18	6.13^{b}	-3.51	15.77
Malv-3-gluc (34.7)	107.41^{a}	-4.26	219.08	101.99^{a}	-14.58	218.56	97.50^{a}	-32.53	227.54
Delph-3-gluc-acetate (38.3)	3.10^{a}	-0.39	6.59	3.24^{a}	-0.97	7.45	4.00^{a}	-2.20	10.19
Vitisin A (39.4)	3.10^{b}	0.81	5.38	2.95^{b}	0.75	5.15	3.84^{a}	0.55	7.14
Pet-3-gluc-acetate (45.1)	5.02^{a}	0.24	9.80	4.68^{a}	-0.14	9.50	5.51^{a}	-0.63	11.65
Peo-3-gluc-acetate (50.3)	5.84^{a}	-1.80	13.48	3.48^{b}	-1.21	8.18	3.30^{b}	-1.61	8.21
Malv-3-gluc-acetate (51.5)	34.75^{ab}	-9.20	78.70	27.34^{b}	-7.38	62.06	38.33^{a}	-22.06	98.71
Delph-3-gluc-coum (56.4)	2.79^{a}	-0.02	5.61	1.77^{b}	0.17	3.37	1.52^{b}	-0.02	3.06
Pet-3-gluc-coum (61.9)	5.09^{a}	-0.75	10.92	2.77^{b}	0.08	5.46	2.70^{b}	0.09	5.31
Pino*2 (64.9)	1.46^{b}	0.03	2.90	2.59^{a}	-2.39	7.56	1.25^{b}	0.16	2.33
Peomalv-gluc-coum*3 (67.2)	24.54^{a}	-7.28	56.37	12.89^{b}	-4.38	30.16	11.81^{b}	-5.20	28.81
Polymeric pigments (76-82)	23.47^{a}	6.22	40.71	19.15^{b}	4.58	33.73	23.23^{a}	8.53	37.94
Hydroxycinnamate 15*4 (15.3)	8.32^{a}	1.86	14.77	5.35^{b}	0.87	9.82	4.27^{c}	0.37	8.18
Flavanol 20*4 (19.8)	36.09^{b}	18.05	54.13	23.72^{c}	9.91	37.52	43.12^{a}	22.31	63.93
Flavanol 33*4 (32.9)	37.82^{b}	2.21	73.43	22.57^{c}	-4.98	50.11	77.66^{a}	15.32	140.00
Flavonol 41*4 (40.7)	4.72^{a}	-0.82	10.26	4.23^{a}	-1.65	10.1	4.16^{a}	-1.82	10.14
Flavonol 42*4 (41.7)	18.27^{a}	0.96	35.58	12.80^{b}	-1.23	26.84	13.47^{ab}	-7.44	34.38
Flavonol 46*4 (46.4)	2.46^{b}	0.10	4.81	2.32^{b}	-0.25	4.89	3.66^{a}	0.38	6.94
Flavonol 50*4 (50.0)	24.01^{a}	8.73	39.29	12.11^{c}	4.11	20.11	19.55^{b}	3.69	35.40

CL = Confidence limit for individual measurements; gluc = glucoside; coum = coumarate; Delph = delphinidin; Cyan = cyanidin; Pet = petunidin; Peo = peonidin; Malv = malvidin.

*1 = Mean values in rows designated by the same letter superscript do not differ significantly ($p \leq 0.05$).

*2 = An anthocyanin tentatively identified as Pinotin A.

*3 = The sum of the peonidin-glucoside-coumarate and malvidin-glucoside-coumarate concentrations.

*4 = Unknown phenolic compounds with characteristic absorption spectra of either a hydroxycinnamate, a flavanol or a flavonol, respectively.

chin, and flavanol 33 may be epigallocatechin or procyanidin B4, based on the elution order of procyanidins under reversed-phase HPLC conditions (Dallas *et al.*, 1995; Pascual-Teresa *et al.*,1998).

Table 2 also shows the 95% confidence limits for individual measurements. These limits indicate the concentration range within which 95% of individual measurements of a specific polyphenol can be expected to fall for a specific cultivar. These confidence limits can be used as tools in cultivar authentication. For instance, Table 2 indicates the 95% confidence range for isorhamnetin in Shiraz, Pinotage and Cabernet Sauvignon wines to be 1.14 – 11.94, 0.79 – 3.86 and 1.43 – 5.48 mg/L, respectively. It would therefore cast doubt on the authenticity of a Pinotage or Cabernet Sauvignon wine, but not a Shiraz wine, that has an isorhamnetin level above 6 mg/L. Similarly, the confidence limits for each of the other analysed polyphenols can be used to determine whether a wine, stated to be of a specific cultivar, contains polyphenol levels that fall within expected limits. The use of these confidence limits as a measure of authenticity may, however, become complicated by the fact that a wine may fall within the determined confidence ranges for several polyphenol concentrations and for others not. The confidence limits depicted in Table 2 were calculated using data obtained from both pure cultivar wines (young wines) and commercial wines, which may contain up to 15% of another cultivar wine. These data are therefore applicable to both pure cultivar wines and blended wines. For increased accuracy, a wine known to be blended can also be authenticated using only the data collected from blended commercial wines.

Discriminant analysis can be used to analyse data of all the different polyphenols and determine whether discrimination between cultivars is possible (González-Neves *et al.*, 2001), and also determine the cultivar origin of an unknown wine. Discriminant analysis was therefore done on the individual analytical measurements from Table 2 (Fig. 1). Data on the concentrations of the seven unknown compounds (hydroxycinnamate 15, flavanols 20 and 33 and flavonols 41, 42, 46 and 50) were not used in this initial analysis. It is clear that this statistical analysis method was able to successfully discriminate between the three cultivars.

The canonical coefficient function loadings of the discriminant analysis (Table 3) indicated that the concentrations of caffeic acid, caftaric acid, petunidin-glucoside, coutaric acid, *p*-coumaric acid, delphinidin-glucoside, malvidin-glucoside-acetate and quercetin were most important in discriminating between cultivars using function 1. The concentrations of isorhamnetin, kaempferol, caffeic acid, petunidin-glucoside, peonidin-glucoside, malvidin-glucoside, delphinidin-glucoside and petunidin-glucoside-*p*-coumarate were most important to discriminate between cultivars using function 2. Function 1 was the most effective function in discriminating Pinotage wines from wines of the other two cultivars and function 2 in discriminating between Shiraz and Cabernet Sauvignon wines (Fig. 1). It is important to note that neither polymeric phenols nor polymeric pigments have high canonical coefficient function loadings and were therefore not very important in discriminating between wines of these three cultivars. The monomeric flavan-3-ols, catechin and epicatechin were much

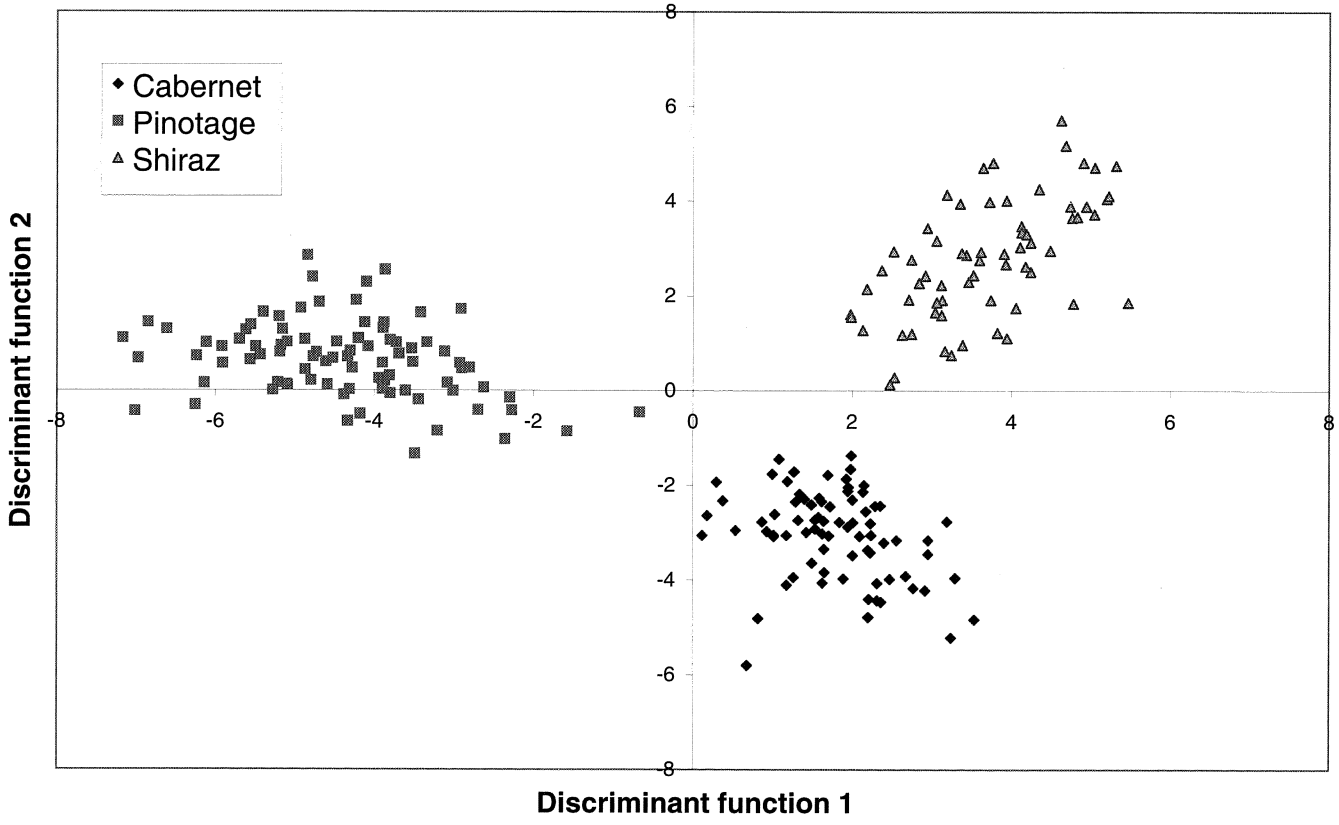


FIGURE 1

Discrimination between South African Pinotage, Shiraz and Cabernet Sauvignon wines (all vintages), based on polyphenol composition.

TABLE 3

Total sample standardised canonical coefficients.

	Unknown compounds excluded		Unknown compounds included	
Variable	**Function 1**	**Function 2**	**Function 1**	**Function 2**
Gallic acid	-0.21442	-0.27466	-0.26441	-0.16829
Protocatechuic acid	0.15563	0.32156	-0.06644	0.39210
ProcyanidinB1	-0.05230	0.38432	-0.00865	0.13689
(+)-Catechin	0.34961	-0.61117	0.22851	-0.13259
(-)-Epicatechin	- 0.32822	0.69458	- 0.17777	0.25738
Syringic acid	-0.19968	-0.18311	-0.03640	-0.28055
(-)-Epicatechin gallate	-0.02990	-0.13356	0.01169	-0.14418
Tryptophol	0.19673	-0.15379	0.02592	0.04399
Polymeric phenols	0.01667	-0.10857	-0.00124	-0.11176
Quercetin-3-glucoside	0.14522	0.55995	0.09362	0.42648
Quercitrin	0.50095	0.69480	0.20182	0.87895
Quercetin	0.76970	-0.50088	0.49439	-0.08197
Kaempferol	-0.65640	-1.32760	-0.68497	-1.12182
Isorhamnetin	0.00394	1.72695	-0.06057	1.30191
Caftaric acid	-2.69460	0.66404	-2.71933	-0.14378
Caffeic acid	-2.56582	0.83043	-2.59125	0.02529
Coutaric acid	1.42550	0.12897	1.39156	0.53063
p-Coumaric acid	1.03894	-0.07125	1.08776	0.22232
Delph-3-gluc	0.84126	-0.84837	0.50889	-0.06093
Cyan-3-gluc	-0.39321	-0.23998	-0.05830	-0.53468
Pet-3-gluc	-1.60198	0.92431	-1.28749	-0.10582
Peo-3-gluc	0.13459	0.95950	-0.41945	1.37930
Malv-3-gluc	0.26634	-0.85147	0.09770	-0.19711
Delph-3-gluc-acetate	0.60160	0.37033	0.80777	-0.00073
Vitisin A	0.06177	-0.15990	0.03718	-0.11448
Pet-3-gluc-acetate	-0.16934	-0.36059	-0.17356	-0.25060
Peo-3-gluc-acetate	0.51081	0.29636	0.23410	0.48712
Malv-3-gluc-acetate	-0.77428	-0.22902	-0.77703	-0.03573
Delph-3-gluc-coum	-0.01683	0.36452	-0.05848	0.27298
Pet-3-gluc-coum	0.59476	-1.09202	0.97913	-0.84873
Pino	-0.59546	0.07727	-0.46732	-0.14100
Malv-3-gluc-coum	-0.07554	0.52340	-0.19941	0.04955
Polymeric pigments	0.16967	0.01717	0.24823	0.05472
Myricetin	0.23337	0.04449	0.21856	0.14573
Flavanol 20			0.54234	-0.03038
Flavanol 33			0.73940	-1.14877
Flavonol 41			0.13600	-0.12715
Flavonol 42			-0.11661	-0.14872
Flavonol 46			0.13773	-0.11862
Flavonol 50			0.59207	-0.15221
Hydroxycinnamate 15			-0.17364	0.47050

more notable contributors towards cultivar discrimination. The concentration of Pino, a compound most possibly identical to Pinotin A described by Shwarz *et al.* (2003), was a fairly strong distinguishing compound for Pinotage. This would be expected for Pinotin A in Pinotage wine due to the much higher concentration of the Pinotin A precursor, caffeic acid, found in this wine. Both caffeic acid and caftaric acid were strong distinguishing compounds in the direction of Pinotage. As could be expected from the genetic control of their concentrations in grapes (Castia *et al.*, 1992), nearly all of the anthocyanin concentrations were important distinguishing compounds. A notable exception to this generalisation is Vitisin A, which forms by a reaction of malvidin-glucoside with pyruvate during vinification and wine storage (Romero & Bakker, 2000). Of special note is the high importance of flavonols in discrimination between wines of these three cultivars as judged from their generally high canonical function loadings. Many of these compounds, especially isorhamnetin, are strongly distinguishing in the direction of Shiraz.

The estimated cross-validation discrimination error rate was calculated as 1.33%. This error rate could be minimized to 0% if the seven unknown compounds are also used in the analysis. The canonical coefficient function loadings for this analysis indicate relatively high values for some of these unknown compounds (Table 3). The inclusion of these unknown compounds as variables in the discriminant analysis increased the squared Mahalanobis distances between cultivars, which gives an indication of the degree of discrimination obtained (Table 4). The unknown flavanols 20 and 33 were mainly responsible for the improvement in the discrimination of Cabernet Sauvignon from the other cultivar wines, due to their much higher concentrations found in Cabernet Sauvignon wines.

Discriminant analysis could also distinguish between certain vintages for each cultivar individually. For example, Fig. 2 shows the graphical representation of the results for the Shiraz wines. The canonical coefficient function loadings of discriminant analysis indicated that for each cultivar different compounds were important to discriminate between vintages. It was not possible to discriminate between vintages when data from all three cultivars were combined (data not shown). The use of data from a specific vintage (Fig. 3) or wine-producing area (Fig. 4) improved the dis-

TABLE 4

Between-cultivar squared Mahalanobis distances from discriminant analysis.

Analysis on all samples. Unknown compounds not included.			
	Cabernet Sauvignon	Pinotage	Shiraz
Cabernet Sauvignon	0	51.62	37.37
Pinotage	51.62	0	69.86
Shiraz	37.37	69.86	0
Analysis on all samples. Unknown compounds included.			
	Cabernet Sauvignon	Pinotage	Shiraz
Cabernet Sauvignon	0	80.67	49.15
Pinotage	80.67	0	79.79
Shiraz	49.15	79.79	0
Analysis on 2002 samples only. Unknown compounds not included.			
	Cabernet Sauvignon	Pinotage	Shiraz
Cabernet Sauvignon	0	103.5	98.27
Pinotage	103.5	0	120.24
Shiraz	98.27	120.24	0
Analysis on Stellenbosch samples only. Unknown compounds not included.			
	Cabernet Sauvignon	Pinotage	Shiraz
Cabernet Sauvignon	0	82.85	79.88
Pinotage	82.85	0	129.24
Shiraz	79.88	129.24	0

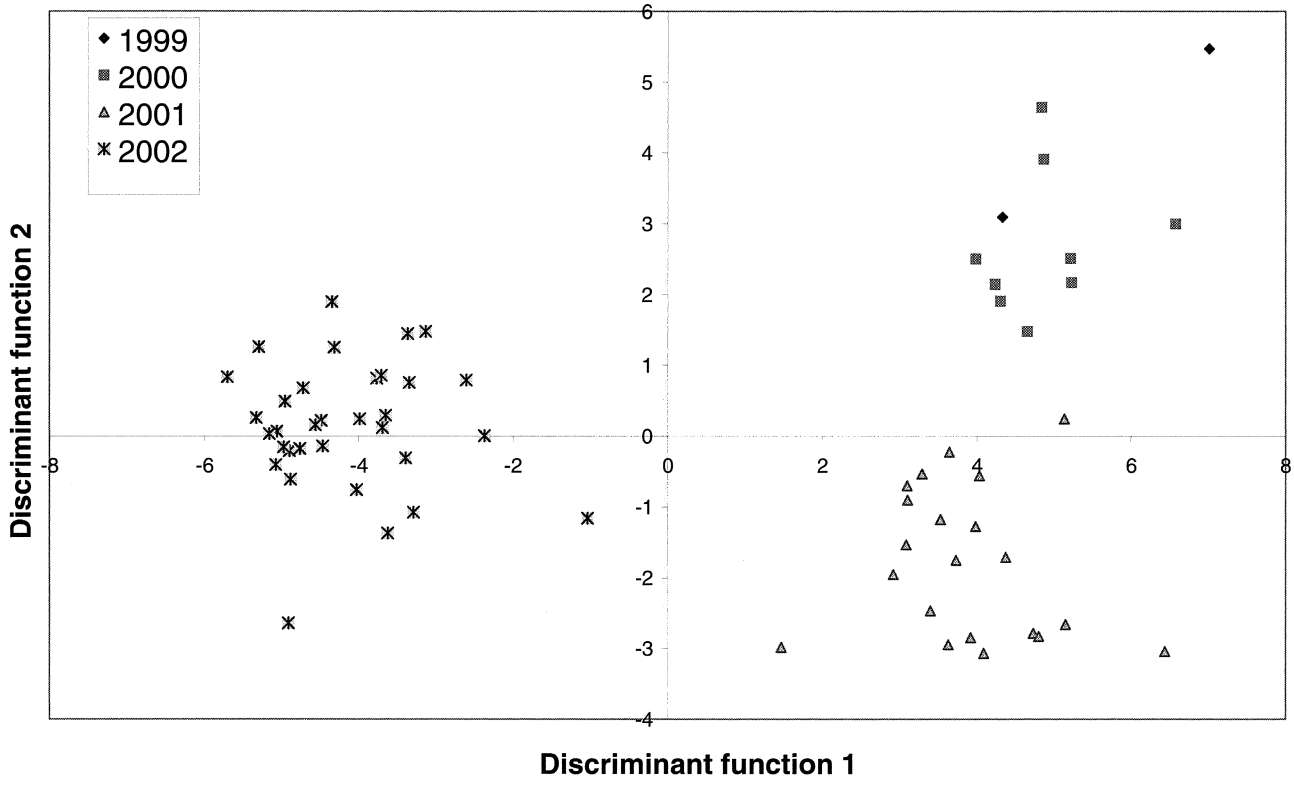


FIGURE 2

Discrimination between Shiraz wines from different vintages, based on polyphenol composition.

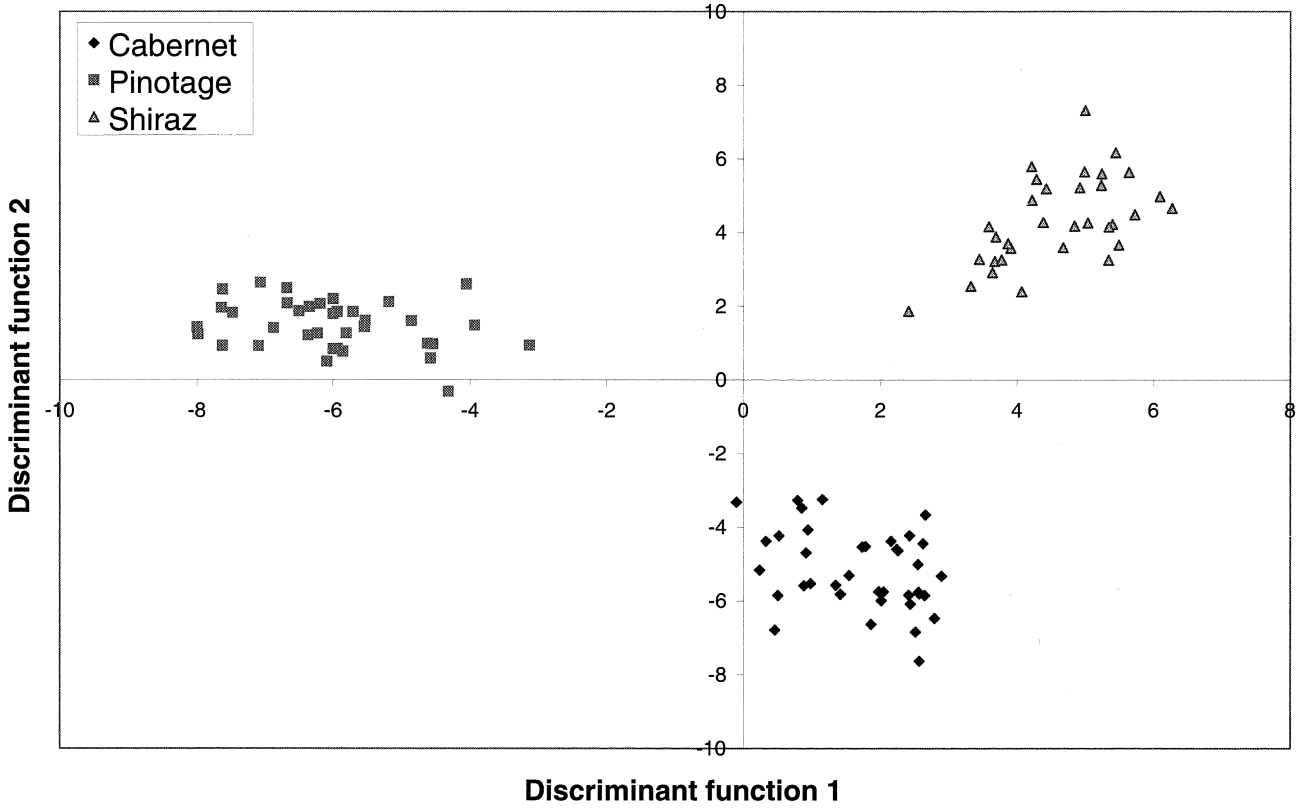


FIGURE 3

Discrimination between South African Pinotage, Shiraz and Cabernet Sauvignon wines from the 2002 season only, based on polyphenol composition.

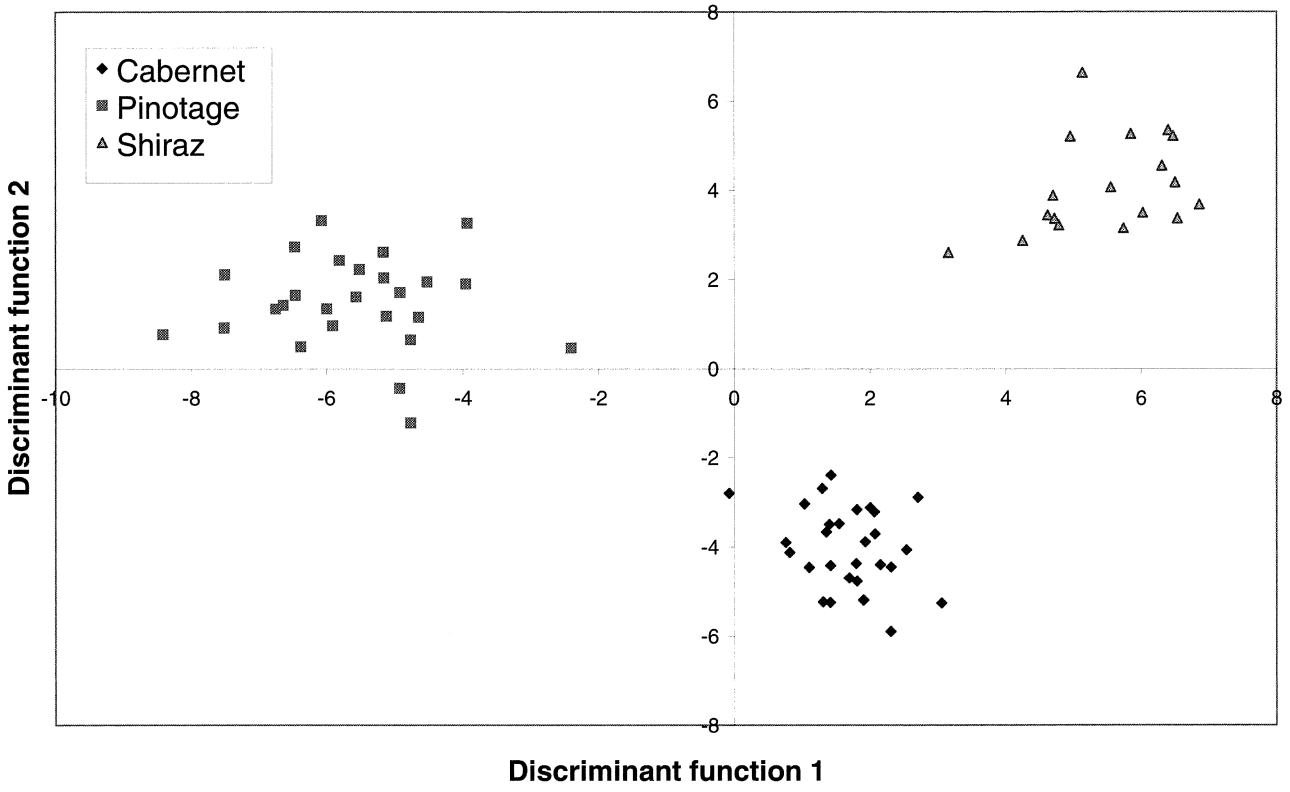


FIGURE 4

Discrimination between South African Pinotage, Shiraz and Cabernet Sauvignon wines from the Stellenbosch wine-producing area only, based on polyphenol composition.

TABLE 5

Anthocyanins in South African Pinotage, Shiraz and Cabernet Sauvignon wines.

	% Fraction of total anthocyanins								
	Shiraz (76 wines)			Pinotage (100 wines)			Cabernet Sauvignon (84 wines)		
Anthocyanin	**Mean***	**95% CL(-)**	**95% CL(+)**	**Mean***	**95% CL(-)**	**95% CL(+)**	**Mean***	**95% CL(-)**	**95% CL(+)**
Delphinidin-3-glucoside	4.35^{c}	1.78	6.91	5.53^{b}	0.95	10.11	6.34^{a}	2.86	9.83
Cyanidin-3-glucoside	0.85^{b}	-0.67	2.37	0.97^{b}	-0.17	2.08	1.31^{a}	-0.69	3.31
Petunidin-3-glucoside	6.80^{b}	4.31	9.28	7.94^{a}	5.15	10.24	6.31^{b}	3.68	8.93
Peonidin-3-glucoside	4.60^{a}	2.58	6.63	3.76^{b}	1.64	5.71	3.51^{b}	1.37	5.64
Malvidin-3-glucoside	53.21^{b}	44.65	61.78	59.67^{a}	51.28	64.57	54.44^{b}	46.94	61.97
Peonidin-3-glucoside-acetate	2.77^{a}	1.31	4.24	2.00^{b}	0.90	3.03	1.98^{b}	0.50	3.46
Malvidin-3-glucoside-acetate	16.17^{b}	8.09	24.25	15.69^{b}	8.64	21.88	19.54^{a}	10.11	29.00
Unacylated anthocyanins	69.81^{c}	58.44	81.18	75.62^{a}	67.57	83.67	71.92^{b}	63.09	80.72
Acetylated anthocyanins	18.94^{b}	9.89	28.00	17.23^{c}	10.15	24.31	21.52^{a}	12.24	30.82
Coumarylated anthocyanins	11.25^{a}	6.00	16.49	7.37^{b}	4.25	10.04	6.57^{b}	4.45	8.68
Acylated anthocyanins	30.19^{a}	18.82	41.56	24.38^{c}	16.33	32.43	28.08^{b}	19.28	36.91
Anthocyanin ratio									
Acetylated/Coumarylated anthocyanins	1.77^{c}	0.64	2.89	2.73^{b}	-1.73	7.19	3.39^{a}	1.44	5.35

CL = Confidence limit for individual measurements.

* = Mean values in rows designated by the same letter superscript do not differ significantly ($p \leq 0.05$).

Total anthocyanins = Sum of the nine main anthocyanin concentrations.

Acylated anthocyanins = Sum of the acetylated and coumarylated derivatives of peonidin- and malvidin-glucoside.

Coumarylated anthocyanins = Sum of the coumarylated derivatives of peonidin- and malvidin-glucoside.

crimination between cultivars, observed as an increase in the squared Mahalanobis distances between cultivars (Table 4), and lowered the error rate of the analysis. This approach could be advantageous in the authentication of wine cultivars. Practically speaking, this would mean that it is best to compare a wine under scrutiny with wines from the same vintage and area to confirm its cultivar identity.

The relative concentration ratios in which anthocyanins are found in different cultivar wines, especially the ratio of acetylated to *p*-coumarylated anthocyanins, are increasingly being used in cultivar authentication (Revilla *et al.*, 2001; Burns *et al.* 2002). Table 5 shows the mean relative anthocyanin concentration ratios of the wines, expressed as % fraction of the total anthocyanins, and also the 95% confidence limits for individual ratio determinations. These limits indicated the range within which 95% of individual ratio calculations of a specific anthocyanin ratio can be expected to fall for a specific cultivar. They can be used to determine whether a stated cultivar is authentic or not. Only the concentrations of the nine main wine anthocyanins, namely the five mono-glucosides and the acetylated and coumarylated derivatives of peonidin- and malvidin-glucoside, were used in calculating the different anthocyanin ratios. The total anthocyanin concentration was therefore the sum of the concentrations of the nine main anthocyanins. The total acylated anthocyanins were calculated as the sum of the acetylated and coumarylated derivatives of peonidin- and malvidin-glucoside. The ratios of acetylated to coumarylated anthocyanins are also shown in Table 5. The data clearly indicate significant differences between cultivar wines with respect to their anthocyanin ratios. Pinotage wines, for example, contained significantly lower levels of total acylated anthocyanins than Shiraz or Cabernet Sauvignon wines. This is not surprising when considering the heritage (Pinot noir x Cinsaut noir) of this cultivar, since Pinot Noir wines contain no acylated anthocyanins (Mazza *et al.*, 1999).

CONCLUSIONS

A large amount of data has been collected on the phenolic composition of South African red wines of the cultivars Pinotage, Shiraz and Cabernet Sauvignon. Significant differences were found between these cultivars in the mean levels of certain phenolic compounds, including some unknown compounds. Due to the fact that these unknown compounds are helpful in discriminating between cultivars and that they are found at relatively high concentrations in wine, it is necessary to determine their identities. The data obtained in this study will be helpful in future as a tool in the cultivar authentication of wines from these cultivars. Even though the data were collected from both pure cultivar wines and commercial wines, which may contain up to 15% of another cultivar wine, good discrimination between cultivars could be obtained based on phenolic composition. The collected data may therefore be further segmented into that obtained from pure cultivar wines and that obtained from blended wines in order to further enhance the accuracy of authentication of these respective groups of wines. In addition, more wines from different vintages and wine-producing areas should be analysed in order to broaden the database and to make statistical analyses, applicable to specific vintages and areas, possible.

It is essential to determine the sensory impact of the observed differences in polyphenol composition between cultivars. The results of such investigations would facilitate the prediction of facets of wine quality, such as mouth-feel or taste characteristics of specific cultivar wines on the basis of their polyphenol composition. This approach will facilitate the monitoring of the impact of specific viticultural and oenological practices on wine quality. Cultivar differences in the phenolic composition of young red wines also have important implications for the oenological management of oxidation reactions taking place during vinification and aging.

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