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Pigment profiles of grapes and of wines

by

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Introduction

Objective quality control of pigments and tannins in red wine production must be based upon adequate knowledge of the total phenolics initially extracted from the grape and of subsequent changes during ageing of the wine. The importance of these materials as quality factors in wine technology has long been recognised, and they have received much attention in oenological research. Nevertheless, the complexity of problems relating to their investigation has prevented any real advance on traditional methods for their control and evaluation in the industry, and the technology remains quite empirical in this regard.

Since 1953, the use of paper chromatography and related procedures has permitted detailed study, notably by P. Ribéreau-Gayon (1964), of the anthocyanins and accompanying acylated anthocyanins of wine grapes, and, to a lesser extent, of their fate in wines. These were regarded as being almost entirely responsible for the colour of grape extracts and although it was known that the anthocyanin content of wines decreased to very low levels during ageing (P. Ribéreau-Gayon 1964), the nature of residual pigments was not examined during this period.

It is noteworthy that the presence of polymeric pigments had been suggested many years earlier by the observation of non-dialysable pigment in wines (J. Ribéreau-Gayon and Peynaud 1935), but this simple procedure for their isolation was apparently never subsequently applied in oenological research nor, for that matter, in any investigations of plant pigment extracts. Berg (1963) noted that the low response of red wine colour to changes in pH indicated the additional presence of a pigment form other than anthocyanins which was unresponsive to pH changes.

The existance of a polymeric pigment fraction both in wine grapes and red wines has been recently demonstrated, and its importance in relation to that of the monomeric anthocyanins has been emphasised. Thus the major fraction of total extractible pigment from Shiraz grapes has been shown to be tannin pigment, although most of the colour of extracts was indeed due to the anthocyanin content (Somers 1967). Similar dark red tannins, described as condensed flavonoid pigments, were isolated from red wines in amounts far exceeding the levels of anthocyanins (Somers 1966 a).

Such materials, isolated by gel filtration in aqueous organic solvents, constituted the whole tannin fraction of grapes and of wines, there being no evidence, for example, of a yellow brown fraction such as wine tannins have been presumed to be (P. Ribéreau-Gayon 1964). It is now obvious that the tannin pigments are of prime importance in oenology, being the major fraction of total phenolics at all stages of the wine making process. Thus adequate description of the colour of grape extracts or of wines requires data relating to these polymers as well as to the monomeric pigments.

Use of the gel filtration method provides precisely this information in a simple and useful fashion, as it not only allows easy isolation of the tannin pigment fraction, but also provides a measure of the distribution of total pigments in terms of

the three general categories present, i. e. tannin pigments, acylated anthocyanins and anthocyanins.

This paper describes an automated procedure for rapid analysis of grape or wine pigments by gel filtration, and its use in the examination of the pigment composition of several grape varieties and varietal wines. The nature of colour extraction occurring in the winemaking process, and of subsequent changes in composition during ageing of wines have also been investigated. The elution curves obtained are presented as pigment profiles of the grape or wine materials.

Materials and Methods

Grapes were from the vine variety collection of the South Australian Department of Agriculture in the Barossa Valley of South Australia (1967 and 1968 seasons). The variety Shiraz (syn. Syrah) was also sampled from the river-irrigated Murray Valley district. Sugar contents, by refractometer, were 23—25%. Parts of bunches having 10—12 berries were taken and stored in polythene bags at 10° C until required.

Shiraz wines of vintages 1957—1967 were made in the Institute's experimental winery. Older Shiraz wines, and young varietal wines (1967, 1968) were provided by commercial wineries in the Barossa Valley.

Pigment concentrates: The susceptibility of acylated anthocyanins to hydrolysis especially requires that the preparation of grape pigment extracts for analysis should be conducted quickly and at fairly low temperatures. Inconsistent results, consequent upon partial loss of this fraction during preparation, had been previously noted when basic lead acetate was used for precipitation of pigment from solution (Somers 1966 b). For the same reason, the use of the lead salt in concentration of wine pigments is also undesirable, particularly in dealing with young wines. The simple methods described here have given consistent results in gel column analysis, there being no evidence of any loss of acylated anthocyanins. Non-phenolic constituents were not detectable with the tannin fractions from grapes or wines, and their presence in the applied sample does not interfere with the measurement of later pigment fractions.

a) Grapes: The sample (usually 3—5 berries) was thawed and the skin removed, washed with water and dried on filter paper. Almost total extraction of pigment at room temperature was achieved in one hour by five or six successive macerations of the skins with methanolic 0.1% HCl (10 ml) in a test tube. The combined extracts were filtered on a Celite bed, evaporated near to dryness $< 35^{0}$ C and the residue treated with a 1.0 ml volume of 50% aqueous acetone HCl (1.5 ml conc. HCl/litre). After transfer to a small tube, the concentrate was clarified by brief centrifugtion.

b) Wines: A suitable wine aliquot was usually 10.0 ml, but volumes from 5.0 to 30.0 ml have been used, the choice depending on colour density and age of the wine. If necessary, the sample was first clarified by filtration with Celite filter aid. The sample was evaporated near to dryness $< 35^{0}$ C, the residue extracted thoroughly with methanolic 0.1% HCl (20—40 ml) and filtered with Celite. The final concentrate was obtained by evaporation of this extract $< 35^{0}$ C and solution of the residue in 50% aqueous acetone HCl (1.0 ml). No clarification was necessary.

Gel column analysis: A column 1.5 × 60 cm was prepared from Sephadex G-25 Fine in 50% aqueous acetone HCl. A narrow bed of glass micro beads upon glass fibre prevented movement of gel particles into the effluent line. Solvent

entry to and effluent exit from the column were by way of glass capillaries drawn to fit silicone rubber tubing of 1 mm i. d. After settling of the gel under gravity flow (about 20 ml/hour), the flow rate was increased to 45 ml/hour before first use by means of a peristaltic pump. The effluent line was connected to a 1 mm flow cell mounted in the sample beam of a recording spectrophotometer (Perkin Elmer 137 UV) fitted for continuous recording at a constant wavelength. The outlet from the flow cell led to a measuring cylinder for flow rate checks or to a fraction collector.

After application of the pigment concentrate (1.0 ml) to the gel bed by bulb pipette, elution with 50% aqueous acetone (HCl) was commenced at 45 ml/hour. The elution curve was recorded at a fixed wavelength within the range 495—540 nm (see below), and was complete within 2½ hours. After use, the column was washed by elution with about 150 ml solvent. Occasional back washing corrected the slight shrinkage of the gel and improved the flow rate, enabling the same column to be re-used many times without change in performance.

Elution and measurement of total pigments was possible within one hour at flow rates up to 120 ml/hour, with excellent separation of tannins from monomeric pigments in grape extracts and young wines. However, the measure of distribution of the latter pigments between acylated anthocyanins and anthocyanins was less satisfactory at the higher rates. Best results on the 1.5 × 60 cm gel column were obtained with flow rates from 20—50 ml hour. The elution curve was little altered by variation within this range, elution volumes of the three fractions being independent of flow rate. The preferred rate of 45 ml/hour allowed fairly rapid analysis and was convenient for quantitative assessment of fractions from the chart.

Choice of fixed wavelength: The wavelength setting of 540 nm was generally appropriate for monitoring the eluted pigments of grape extracts and of wines up to six months old. A preliminary separation was often necessary for correct choice of the wavelength at which to monitor the pigments from older wines. $\lambda_{max.}$ of the tannin pigment fraction of Shiraz wines, in the eluting solvent, decreases with age by about 3 nm/year up to 16 years, and initial wavelength settings were made accordingly. $\lambda_{max.}$ of residual anthocyanins does not decrease below about 530 nm, but because of their low level in aged wines and the broad nature of absorption at this level there is small error in using the same wavelength setting throughout the analysis.

Extinction values of pigment fractions: $E\,{}^{1\%}_{1\,cm}$ values of grape and wine tannin samples at the particular visible maxima were obtained after measuring optical densities in standard volumes of 50% aqueous acetone HCl, followed by evaporation and weighing of residue.

Estimations of total monomeric pigments (acylated anthocyanins and anthocyanins) were based upon a mean $E\,{}^{1\%}_{1\,cm}$ value of 200 in the eluting solvent. This figure was derived from the previously determined values for these two fractions in ethanolic 0.1% HCl (SOMERS 1966 b), using relative optical densities of many such fractions from six grape varieties in the two solvents.

Chromatography: Fractions from the gel filtration of grape and wine pigments were compared by chromatography alongside known fractions of Shiraz grape pigment, using cellulose thin layer plates in n-butanol/acetic acid/water (6/1/2, BAW). Two dimensional cellulose thin layer chromatograms of tannin pigment fractions were made on 20 × 20 cm plates in 2% acetic acid and BAW.

Other wine analyses: Direct measures of the anthocyanin content of wines were obtained by the bisulphite decolorisation and pH methods (RIBÉREAU-GAYON and STONESTREET 1965). Total wine phenolics, based on a d-catechin standard, were determined by the Folin-Denis assay, using the procedure of SWAIN and HILLIS (1959).

As a measure of colour density in wines, the spectral curves 350—700 nm were run on a recording spectrophotometer using a 1 mm cell. The sum of the optical densities at the wavelengths of minimum and maximum absorbancies was used to express colour density, and the ratio $E_{min.}/E_{max.}$ for expression of colour tint (SUDRAUD 1958).

Response of tannin pigments to anthocyanin analysis: Wine tannin pigments were isolated from a 1964 Shiraz wine and then gel-filtered twice more to ensure absence of free anthocyanins. With the aid of a little methanol, a sample was dissolved in 12% aqueous ethanol saturated with potassium hydrogen tartrate to give a concentration of 800 mg/l. Analysis for anthocyanins by the bisulphite method showed an apparent level of 12 mg/l and, by the alternative pH method, 17 mg/l.

Results and Discussion

a) Grape varieties

The three fractions of Shiraz pigment (Fig. 1) have been previously examined in some detail (SOMERS 1966 b, 1967). The tannin pigment (fraction I) evidently consists

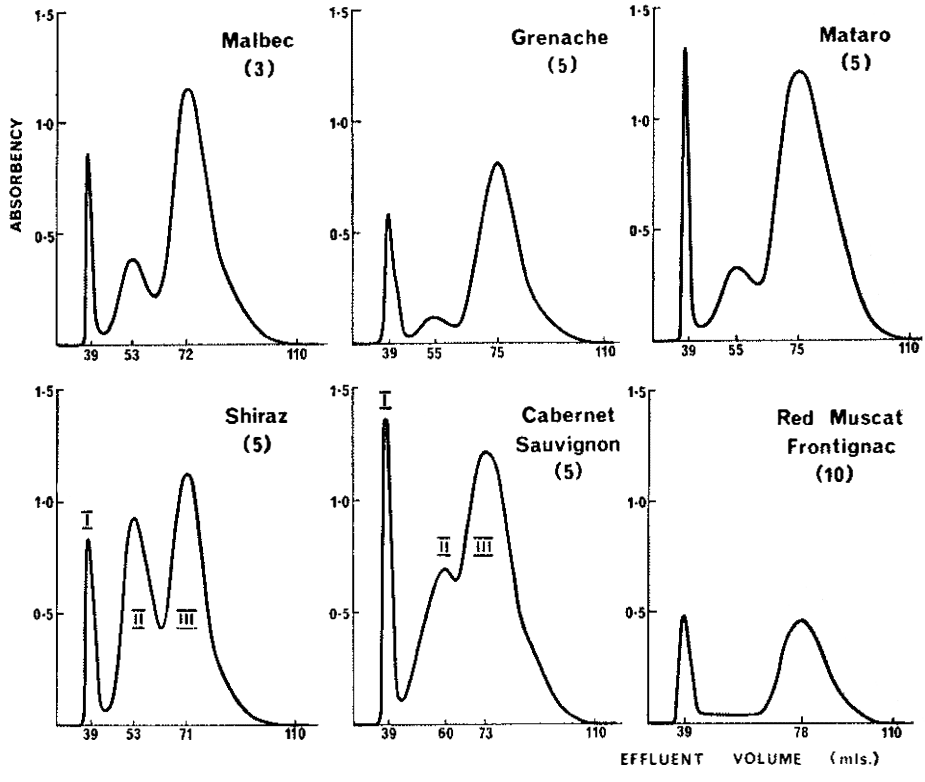

Fig. 1: Pigment profiles of wine grape varieties sampled from the Barossa Valley, 1967 season.

The numbers of berries used in the analyses are shown in brackets. The analysis of Red Muscat Frontignac pigment was recorded at 530 nm and all others at 540 nm.

of a heterogeneous molecular mixture of polymeric phenolic pigments having as yet unknown constitution. Although darker in colour than the anthocyanins, with relatively much higher absorbance in the region 400–500 nm, the visible maximum of this fraction occurs at about the same wavelength, 535–540 nm in the chosen solvent medium, as found for the other two, an indication of their close inter-relationship. The acylated anthocyanins (fraction II) consist of up to twelve different p-coumaroyl and caffeoyl derivatives of the four anthocyanins of Shiraz – these derivatives are not separable by paper chromatography, some being also quite prone to hydrolysis during chromatography. Fraction III contains the parent anthocyanins, which are the 3-glucosides of delphinidin, petunidin, malvidin and peonidin. Although many colourless phenolics can be detected in fractions II and III, only the coloured materials are measured in the gel analysis.

The same four anthocyanins have been identified in other varieties of *Vitis vinifera* with several accompanying acylated derivatives (Ribéreau-Gayon 1959, 1964). There have been many similar reports by other workers since 1959, although often not all the anthocyanin pigments present have been identified. As expected from their genetic relationship, the red wine grape varieties of *V. vinifera* appear to contain identical anthocyanins and acylated derivatives. This was supported by chromatographic comparison of the varieties included in this investigation and also of many other varieties of *V. vinifera*.

However, varieties do differ widely in total amount and distribution of constituent pigments, and these variations are significant in oenology. Varietal differences in the relative amounts of anthocyanins present have been evident from one-dimensional paper chromatography of extracts (Rankine *et al.* 1958; Albach *et al.* 1959). It is now apparent that the assessment of such differences by paper chromatography, while extremely difficult to quantitate, is also inadequate, as such analysis takes no account of polymeric pigments, which constitute the large tannin fraction of red wine grapes.

Because of the chemical complexity of the total pigment and again because of the qualititive uniformity of anthocyanins within the species, quantitative differences in pigment composition are conveniently measured in terms of fractions.

The utility of the gel filtration method in this regard is illustrated by pigment profiles from samples of Shiraz, Cabernet Sauvignon, Malbec, Mataro, Grenache and Red Muscat Frontignac (Fig. 1). All tannin pigment fractions (I) were eluted in identical volumes, but consistent variations were found in the elution volumes of the monomeric pigments II and III, which most probably reflect small differences in composition of these fractions from each variety. Of particular note is the complete absence of acylated anthocyanins (II) from Red Muscat Frontignac, which is evident from the pigment profile, and also the relative displacement of peak II in Cabernet Sauvignon towards a higher elution volume. No detailed investigation of constituent pigments has been made in the present study, but chromatographic examination of fractions II and III from each variety did not show any obvious differences in anthocyanin composition within the respective fractions.

No discrete phenolics were detectable in two dimensional chromatograms of the fraction I components of any variety, most of the material remaining at the origin with slight uniform trailing to about R_f 0.5 in BAW. The nitrogen content of these tannin pigments, by micro Kjeldahl, was apparently zero ($< 0.5\%$). All fractions I were dark red on the column and in solution, with the exception again of the Muscat

Table 1

Visible spectral characteristics of grape tannin fractions, in 50% aqueous acetone (HCl)

Source of grape tannin	$\lambda_{max.}$ (nm)	$\lambda_{min.}$ (nm)	$E_{min.}/E_{max.}$	Mean $E^{1\%}_{1\,cm}$ at $\lambda_{max.}$
Shiraz	535—540	475—480	0.79	24
Cabernet Sauvignon	535—540	480—485	0.81	24
Malbec	540	475—480	0.66	26
Grenache	535—540	500—505	0.95	16
Mataro	540	480—485	0.74	23
Red Muscat Frontignac	500*	500*	1.00	9

* Point of inflexion.

variety in which this fraction was brown-red, having no visible $\lambda_{max.}$ Spectral characteristics and mean $E^{1\%}_{1\,cm}$ values of these grape tannin pigment fractions are listed in Table 1.

Gross differences in pigment composition are evident from the pigment profiles (Fig. 1), and quantitative data was derived by use of mean extinction values after measurement of curve areas. Although the varietal differences in relative content of acylated anthocyanins are quite clear, resolution of this fraction from the anthocyanins is not good enough to permit their separate measurement in every case. Total monomeric pigments (II and III) have therefore been considered together, using an estimated mean $E^{1\%}_{1\,cm}$ value of 200 in the eluting solvent. Table 2 presents the analyses of monomeric and polymeric pigments per berry from the profiles in Fig. 1, calculated by use of this value and of the appropriate mean extinction coefficient for the tannin pigment fraction (Table 1).

The tannin pigments are seen to represent a major part of the total phenolics in all varieties — the small contribution of this fraction to total colour is due to the much lower mean extinction coefficients compared with that of the anthocyanins. Although the anthocyanin level per berry varies quite widely in these varieties, the content of tannin pigments is remarkably uniform on this basis (Table 2).

Fresh berry weight figures are not available for these analyses, but subsequent berry weight measurements of such sample materials indicated that, on a berry weight basis (i. e. mg/g fresh weight), the levels of tannin pigments and also of total

Table 2

Pigment contents of *V. vinifera* grape varieties, sampled from the Barossa Valley

Grape variety	Amounts per berry (mg)	
	Tannin pigments	Total anthocyanins
Shiraz	2.1	2.6
Cabernet Sauvignon	3.3	3.1
Malbec	2.5	3.7
Grenache	2.6	1.5
Mataro	3.8	3.0
Red Muscat Frontignac	2.2	0.3

anthocyanins in Cabernet Sauvignon and in Malbec are more than twice those in any of the other wine grape varieties examined.

Excellent reproductions of pigment profiles were obtained in repeat analyses of the same extracts and, despite the small samples used, the experience with repeat analyses of different samples was that the profiles are all sufficiently distinctive to be regarded as characteristic of the grape varieties for this particular viticultural area. Sample variability in pigment composition of Shiraz is illustrated in Fig. 2, in which consecutive analyses of different samples from the same grape cluster are shown together.

Furthermore, the same distinguishing characteristics of varietal pigment profiles evident in the 1967 season were seen again on brief examination of similar samples from the 1968 season.

Regional effects: In contrast to the above, consistent differences were observed, in the same 1967 season, between Shiraz pigments from the Barossa and from the Murray Valley. Typical pigment profiles are shown in Fig. 3, in which the relatively higher content of acylated anthocyanins in Murray Valley Shiraz is evident.

The likely generality of this observation has eben supported by other measurements, in each season from 1964—1968, the earlier comparisons by paper chromatography being confirmed and more easily quantitated in recent years by gel column analysis. During this period numerous Shiraz samples from several irrigated vineyards in the Murray Valley, and also from several parts of the Barossa Valley and

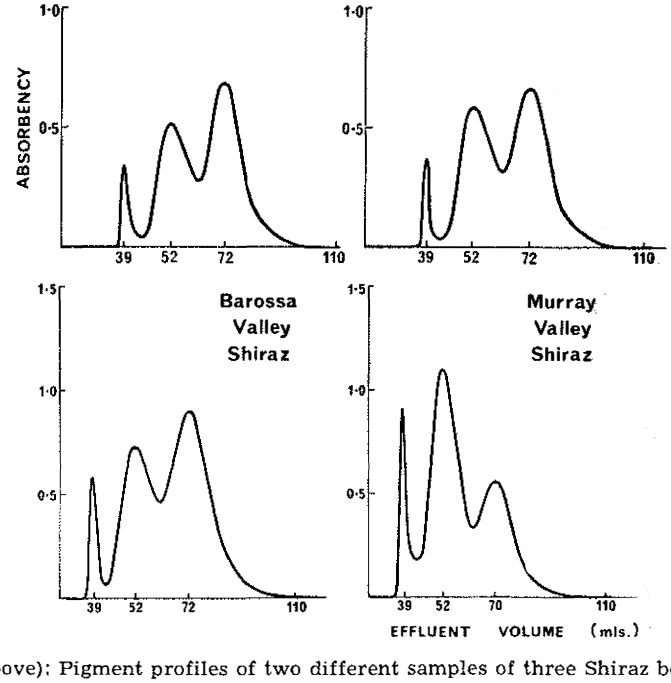

Fig. 2 (above): Pigment profiles of two different samples of three Shiraz berries from the same cluster.

Fig. 3 (below): Pigment profiles of Shiraz grapes from the Barossa and Murray Valley regions.

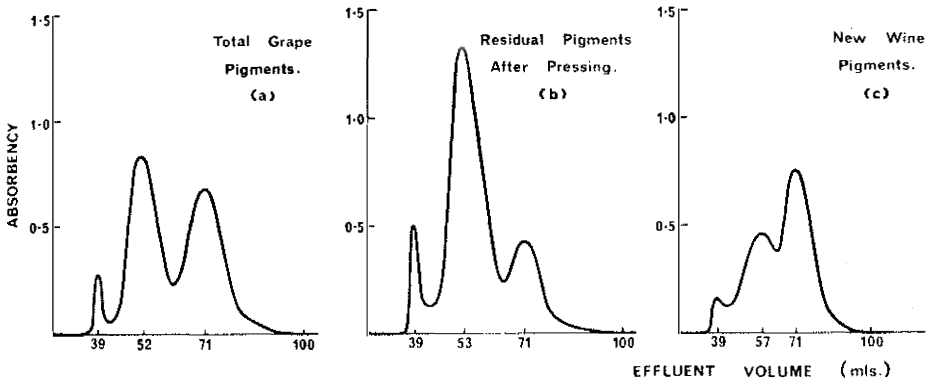

Fig. 4: The nature of colour extraction during fermentation on skins of Shiraz grapes from the Murray Valley is illustrated by pigment profiles.

other non-irrigated areas around Adelaide have been examined. Shiraz from the Murray Valley appears to contain consistently higher relative levels of acylated anthocyanins than does Shiraz grown in the local non-irrigated areas, notwithstanding some seasonal variations.

The Barossa and Murray Valleys provide quite different cultural regimes. Extensive irrigation is essential to viticulture in the Murray Valley because of the hot dry climate, and Shiraz yields are then about ten tons/acre. The Barossa Valley and other non-irrigated areas around Adelaide, some 200 miles distant from the Murray district, receive a higher rainfall and produce about two tons/acre without irrigation. Such Shiraz grapes are smaller and contain about 50% higher total pigments (as mg/g fresh weight) than those from the Murray Valley.

Pigment extraction during fermentation: It was realised that the composition of pigment obtained by exhaustive extraction of grape skins with solvents such as methanolic HCl might bear little relation to that actually appearing in a new wine. The quality of Shiraz pigment extracted during normal wine making procedure was therefore examined by gel column analysis of pigment remaining in the pressed skins, and also of that in the fermenting wine, immediately after pressing.

From the respective pigment profiles (Fig. 4), it is clear that the anthocyanin fraction is more efficiently extracted during the fermentation than are the other two. Residual pigments in the discarded skins (Fig. 4 b) were much richer in acylated anthocyanins than the intact skins (Fig. 4 a), this fraction being therefore much less prominent than the anthocyanins in the new wine (Fig. 4 c). This observations largely accounts for the relatively poor colour extraction from Shiraz experienced by wine makers in irrigated areas, as more than 50% of total colour in such Shiraz may be due to the less useful acylated anthocyanins (c. f. Fig. 3).

It is also notable that the tannin pigment fraction of the fermenting wine is quite small (Fig. 4 c), apparently because of the low extractibility of such materials from the grape. The $E^{1\%}_{1\,cm}$ values for this fraction and for subsequent tannin isolates during the fermentation were only about half that of the corresponding fraction of Shiraz grape pigment, there being also little change in the pigment profile during fermentation. These lower values are consistent with the presence of some grape skin tannin pigment, together with quantities of polymeric pro-anthocyanidin material arising by acid catalysed and oxidative condensation reactions following crushing

of the berries, the seeds being a likely source of such reactive phenolics. The tannin fractions respond strongly to the 'leuco-anthocyanidin reagent' (SWAIN and HILLIS 1959), and such pro-anthocyanidin material apparently constitutes the main tannin structure. The subsequent increase in mean extinction coefficient of the tannin fraction which occurs during ageing is thought to be due to anthocyanin interaction with the tannin matrix.

b) Young varietal wines

The initial composition of total wine pigments may therefore be somewhat different from that available in the wine grape because of preferential extraction of the anthocyanin fraction. However, the distribution between monomeric and polymeric forms is quite dynamic, moving irreversibly towards the polymeric pigment state during ageing of the wine.

Such changes are apparent even in young wines. Spectral measurements of a Barossa Valley Shiraz wine have shown an approximate 15% increase in colour density during the first two years after vintage, which was due entirely to increase in E_{min} (in the region 420–460 nm) – $E_{max.}$ remained constant though shifting from 540 to 525 nm during this period. This colour stability was found to be accompanied by continuous change in the pigment profile, with increasing predominance of the

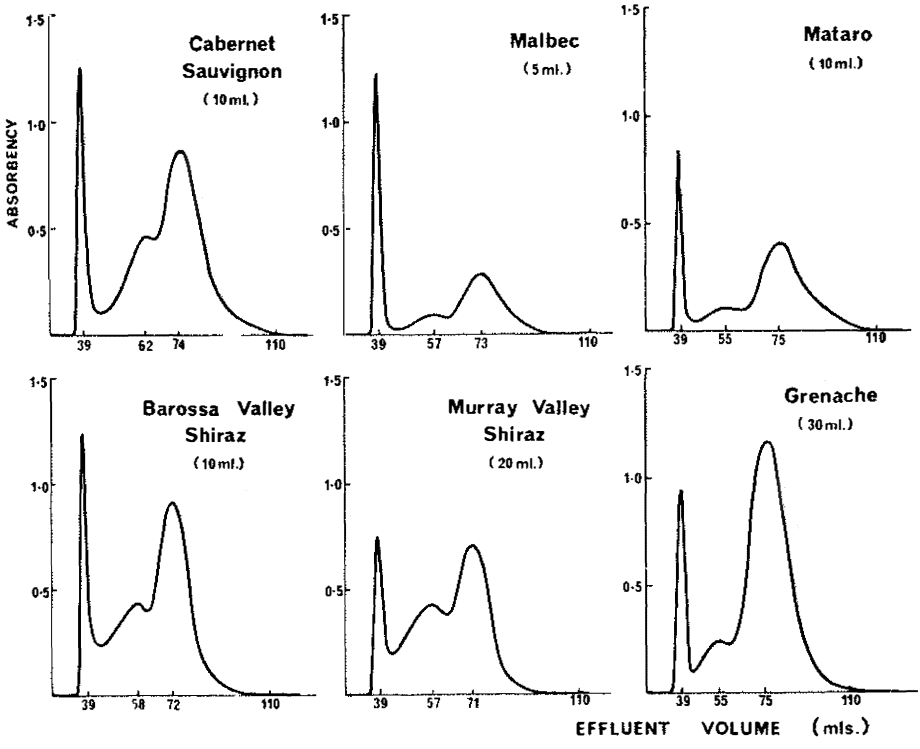

Fig. 5: Pigment profiles of young varietal wines from the Barossa Valley, and of a young Shiraz wine from the Murray Valley (wines aged 4—5 months).
The aliquots of wines used in the analyses are shown in brackets. All analyses were recorded at 540 nm.

Table 3

Visible spectral characteristics of wine tannin fractions from young varietal wines, in 50% aqueous acetone (HCl) (wines aged 4—5 months)

Source of wine tannin	$\lambda_{max.}$ (nm)	$\lambda_{min.}$ (nm)	$E_{min.}/E_{max.}$	Mean $E^{1\%}_{1\,cm}$ at $\lambda_{max.}$
Shiraz (Barossa Valley)	542	420	0.54	22
Shiraz (Murray Valley)	542	425	0.51	18
Cabernet Sauvignon	540	420	0.48	16
Malbec	545	425	0.52	20
Mataro	542	420	0.63	12
Grenache	540	420	0.56	10

tannin pigment fraction and decreasing anthocyanin levels in the wine. The pigment profile is thus a measure of the "chemical age" or state of maturation of the wine.

Profiles of a number of varietal wines at about four months of age are presented in Fig. 5. The low levels of acylated anthocyanins in the two Shiraz wines relative to those in the corresponding grape material (Fig. 3) are again evident, and the shift in position of peak II in these wines is regarded as evidence also of selective extraction within the acylated anthocyanins fraction of Shiraz grapes (see also Fig. 4). A similar shift in peak II was observed in the Malbec wine, in which the tannin pigment fraction is also particularly prominent. However, with these qualifications, the wine pigment profiles at this stage are seen to closely resemble those of the grape varieties (Fig. 1).

The elution volumes of all wine tannin pigment fractions (I) were identical, and were the same as found for these fractions from the grape pigment extracts. Also, there was no significant change in the elution volumes of the anthocyanin fractions (III). Chromatographic examination of fractions II and III revealed no obvious differences from these fractions of grape pigment, but showed the presence in fractions I of a more dense area of uniform streaking of pigments in BAW than found for the corresponding grape tannin pigment fractions.

All of the wine tannin materials isolated were deep red to purple in colour during separation on the gel column, and were fairly uniform in their spectral properties (Table 3). Obvious points of difference from the grape tannin spectra (Table 1) are the shift in the area of minimum absorbance from about 480 nm to 420 nm, and the generally lower mean extinction coefficients of the new wine tannin fractions. These observations indicate significant structural differences between the two types of tannin pigment, and support the theory that wine tannin pigments are derived only in part from the grapes and are mainly formed in the wine after vinification, most probably by interaction between monomeric pigments and reactive tannin precursors, the seeds being a likely source of such materials. Striking evidence of large differences between the tannin pigment fractions of grapes and of wines, and good circumstantial support for this theory are also provided by inspection of a paper chromatogram of total Shiraz grape pigments alongside total wine pigments at various stages of ageing (Fig. 6). The greater mobility of the wine tannins in BAW is quite evident from the chromatogram.

The colour characteristics of the particular varietal wines examined are described in Table 4, together with an assessment of the contents of tannin pigments and of total anthocyanins from the pigment profiles of Fig. 5. The Malbec and Cabernet

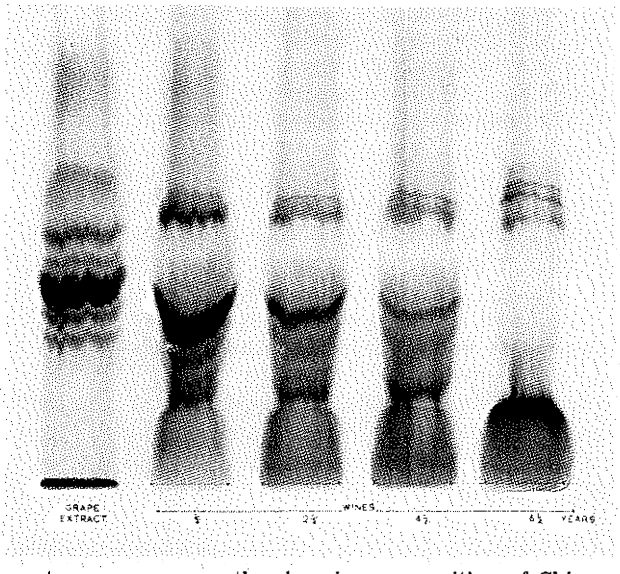

Fig 6 The chromatogram compares the changing composition of Shiraz wine pigments during ageing with total extractible Shiraz grape pigments.

Sauvignon wines contain exceptionally high levels of tannin pigments, and the absence of any correlation between wine colour densities and pigment contents of the Grenache, Mataro and Murray Valley Shiraz wines is also notable. The latter is of course due in part to the differing mean extinction values of the respective tannin pigment fractions (Table 3), but colour density in young wines is obviously not explicable in simple terms of pigment content.

c) Aged Wines

The overall colour density of a red wine remains fairly constant during ageing, but the hue or tint changes continuously with accompanying subtle changes in astringency and flavour of the wine. Spectral measures of these two colour factors were introduced by SUDRAUD (1958) who noted that the tint index, expressed here as $E_{min.}/E_{max.}$ increases steadily during ageing. In aged wines, generally those over about ten years, there is no visible maximum in the red region, the spectra showing dec-

Table 4

Colour characteristics and pigment contents of young varietal wines (4—5 months old)

Wine variety	$\lambda_{max.}$ (nm)	$\lambda_{min.}$ (nm)	Colour density (1 mm cell)	Colour tint	Pigment content g/l	
					Tannin pigments	Total anthocyanins
Shiraz (Barossa Valley)	535	430—460	0.73	0.66	1.9	0.91
Shiraz (Murray Valley)	535	430—460	0.54	0.70	0.9	0.40
Cabernet Sauvignon	538	430—450	0.89	0.62	2.4	0.98
Malbec	535	420—440	1.28	0.49	3.2	0.64
Mataro	540	440—460	0.60	0.67	1.5	0.45
Grenache	528	430—460	0.29	0.78	1.2	0.36

reasing light absorption from 400 to 700 nm. The sum of optical densities at 420 and 520 nm is then used to express colour density, and the ratio of these two values as a measure of colour tint (SUDRAUD, 1958).

Recent investigations of Bordeaux wines aged from two to forty three years included such data, together with separate measures of total phenolics (as permanganate indices) and also of anthocyanins (RIBÉREAU-GAYON 1964, RIBÉREAU-GAYON and STONESTREET 1966). It is evident from these reports that while no correlation exists between colour density and anthocyanin levels in red wines, there is a good proportional relationship between colour density and total phenolics for the wines older than three years. The same has been found to apply in each of two series of experimental Shiraz wines from the Barossa Valley, aged from two to nine years, the Folin Denis assay being used as a measure of total phenols. Younger wines, with high anthocyanin content, do not fit the straight line relationship which exists between the two factors in older wines.

Such general observations as have been made on red wine colour can now be understood in terms of wine pigment profiles and knowledge of the properties and relative amounts of pigment fractions. A real measure of the changing pattern of pigment distribution during red wine ageing is readily provided by gel column analysis, and the dynamic qualities of hue and tint, together with the relative stability of colour density, are then explicable in terms of the spectral properties of the pigment fractions which are isolated.

Whereas the colour of a new wine is largely due to its high anthocyanin content (Fig. 5), the subsequent ageing process involves continuous shift of pigments from the monomeric to the polymeric state, with consequent colour changes. The increasing contribution by tannin pigments to total wine colour is responsible for the higher absorbance of aged wines in the 400—450 nm region, causing continuous increase in wine colour tint and also compensating, in the visual or other assessment of colour density, for the progressive loss of anthocyanins.

The inadequacy of paper chromatography as a technique for examination of aged wine pigments is well demonstrated by the chromatogram (Fig. 6). However, pigment profiles provide an objective measure of the relative quantities of materials present, and effectively represent the 'chemical age' of the wine. Thus the further ageing changes in pigment distribution of Shiraz wines are illustrated in Fig. 7 by profiles of Barossa Valley wines aged from one to sixteen years.

The increasing predominance of tannin pigments during wine ageing, with progressive decrease in monomeric pigments, is evident from the profiles. The presence of residual anthocyanins was invariably indicated by a peak, however slight, at the elution volume of fraction III (70 ml, Fig. 7). This fraction of the 13 year old wine was examined after one further gel filtration, and the presence of the four anthocyanins was verified by co-chromatography with Shiraz grape pigments. However, anthocyanins were absent from the sixteen year old wine, the profile recording only a slight tannin trail.

In contrast to the finding with young wine pigments, gel column analysis did not provide a clean separation of polymeric from monomeric pigments in aged wines — chromatography of fractions always showed evidence of a tannin trail. This was partly due to the presence of intermediate tannins, overlapping with fraction II, and also to other chromatographically indiscrete brown-red pigments, of higher R_f in BAW than the anthocyanins, which are eluted with fraction III. Thus the accurate determination of fractions is not possible by this means in aged wines, although a good measure of pigment distribution is provided. It is noteworthy that the ap-

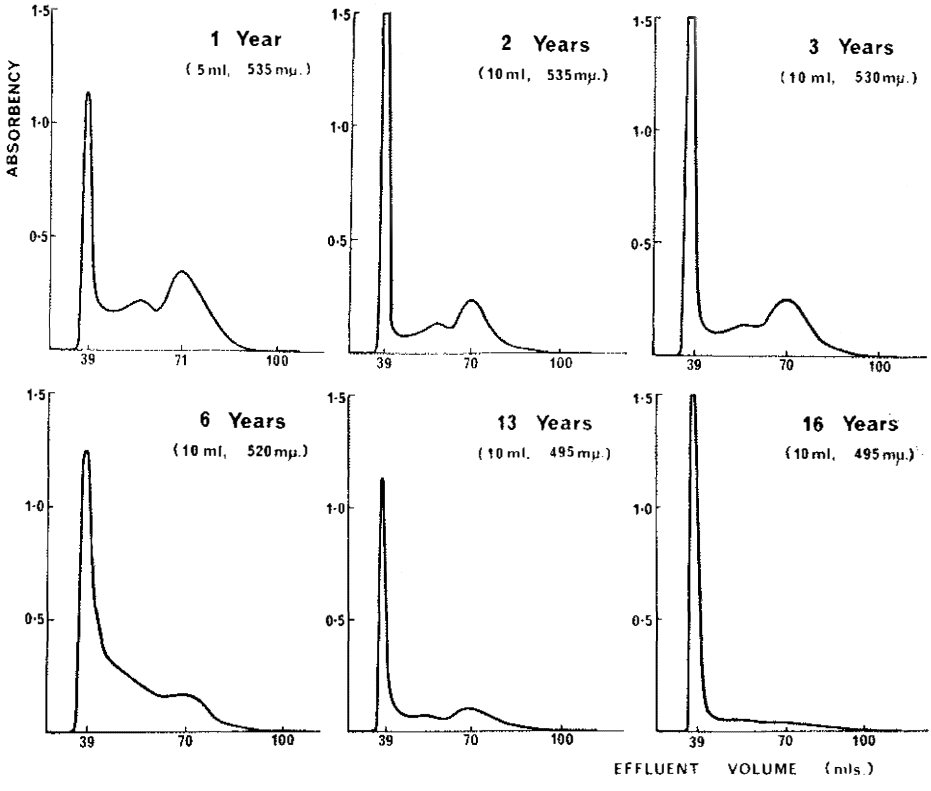

Fig. 7: Pigment profiles of aged Shiraz wines from the Barossa Valley.
The aliquots used and the wavelengths at which the elution curves were recorded are shown in brackets.

parent levels of around 20 mg/l residual anthocyanins in Bordeaux wines up to forty three years old (P. Ribéreau-Gayon 1964) can be attributed to the small anthocyanin-like response shown by high concentrations of tannin pigments in the wine analyses, which were based upon pH effects and the bisulphite reaction.

An important feature of the ageing process in Shiraz wines, which most probably applies generally to dry red wines, is that progressive alteration in the constitution of the tannin pigment fraction was indicated by the steady decrease in $\lambda_{max.}$ (in the eluting solvent) from 540 nm in a new wine to about 490 nm in the oldest wines examined. This of course relates to general observations of wine spectral characteristics during ageing (Sudraud 1958), and it is considered that the progressive changes evident in tannin pigment structures probably relate also to the early observations by J. Ribéreau-Gayon (1933) of reduced methoxyl levels in the colouring matter of older wines. A gross measure of these ageing effects is given by the visible spectral properties of aged Shiraz wine tannin fractions (Table 5). Despite large spectral changes during ageing, the mean extinction values of these fractions remain at a fairly uniform level, somewhat higher than found in the young wine.

With regard to the amounts of tannin pigments present in aged wines, although it is not yet possible to report on the same wine over a number of years, the indications are that, despite large changes in the pigment profile, there is little alteration

Table 5

Visible spectral characteristics of wine tannin fractions from aged Shiraz wines, in 50% aqueous acetone (HCl)

Age of wine (years)	$\lambda_{max.}$ (nm)	$\lambda_{min.}$ (nm)	$E_{min.}/E_{max.}$	Mean $E^{1\%}_{1\,cm}$ at $\lambda_{max.}$
1	537	420	0.56	27
2	535	420	0.58	28
3	530	410	0.55	28
6	520	410	0.64	26
13	492	415	0.81	24
16	494	412	0.76	25

in the total content of tannin pigments in the wine (about 2 g/l in the Shiraz experimental wine series examined, vintages 1959–1966). The decreased astringency and more mellow flavour, which are the important characteristics of ageing in red wines, are most probably due to progressive changes in molecular size distribution within this fraction of total wine phenolics.

d) Regional effects in aged Shiraz wines

Observations of regional variation in the pigment compositions of Shiraz grapes, and of the corresponding young wines from the Barossa and Murray Valley viticultural areas have already been reported (c. f. Figs. 3 and 5, Table 4). Of more practical importance is the related finding of large differences between the pigment profiles of aged wines from these areas, apparently the consequence of greatly differing rates of change of pigment distribution during ageing – there are also notable qualitative differences between the tannin pigment fractions of similarly aged wines from the two districts. The existance and significance of these regional factors, so long indicated by subjective experience, is demonstrated here by an example.

Amongst the many Shiraz wines examined, there was a 2.7 fold difference in colour density between two particular wines of identical age (four years) and winery treatment. The more deeply coloured wine contained a lower level of anthocyanins (170 mg/l, by bisulphite assay) than did the other (220 mg/l). The two wines were from grapes grown in the Barossa and Murray Valleys respectively. Wines from the Barossa Valley are invariably more deeply coloured, with much higher initial anthocyanin levels than those from the irrigated Murray Valley district, but the features noted generally typify wines from these areas at all stages of wine ageing after about one year, i. e. comparable anthocyanin levels are then found in wines of the same age but having up to three-fold differences in colour density.

Pigment profiles from equal volumes of the two wines (Fig. 8) illustrate and explain the above observations. There are obviously major quantitative differences between the tannin pigment fractions of each wine, which account for the marked disparity in colour densities. Also, the higher anthocyanin content of the Murray Valley wine is evident from the profiles, even though accurate assessment of fractions is not possible here because of the complexity of total pigments.

However, the important point is that there is little evidence, from the profile of this four year old Murray Valley wine of any maturation changes having occurred during ageing to this stage (compare Fig. 8 with Fig. 5). In contrast, Shiraz wines

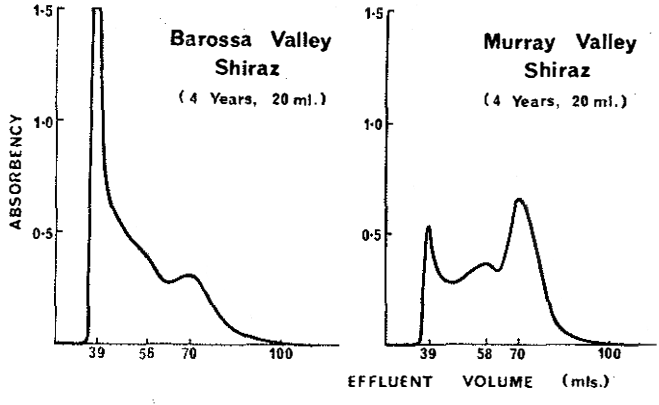

Fig. 8: Comparison of the pigment compositions of aged Shiraz wines from the Barossa and Murray Valley regions.

from the Barossa Valley (and similar viticultural areas also) show quite dramatic changes in pigment composition during the first two years (Figs. 5 and 7).

Qualitative differences between the tannin pigments of these regional wines were indicated by the fact that $\lambda_{max.}$ of the Murray Valley wine tannin fraction was 536 nm and that of the Barossa Valley fraction 525 nm – the mean extinction values at these maxima were 27 and 29 respectively. The spectral properties, which probably relate to different molecular size distributions, suggest that ageing rates within each tannin pigment fraction are also quite dissimilar, being much slower in the Murray Valley wine – the corresponding $\lambda_{max.}$ in new wines from each region is 542 nm.

These particular observations have been supported by gel column analyses of many other wines from these areas, aged from one to eight years. Some variation in pigment profiles of different wines and equal age does occur within each region, and this was particularly evident in the Murray Valley wines; but comparisons of such profiles and of the tannin pigment fractions have confirmed the general conclusion that the rates of change of total pigment distribution, and also of the tannin pigment fraction, as judged by positions of $\lambda_{max.}$, are much slower in wines from the Murray Valley region. The latter feature has been consistent enough to suggest that $\lambda_{max.}$ of the tannin fraction in Murray Valley wines decreases by 1–2 nm per year of ageing, compared with a decrease of 3–4 nm/year in wines from the Barossa Valley, over the eight year period of wine age examined. ($\lambda_{max.}$ of this fraction in the oldest wine analysed here, a Barossa Valley Shiraz, was 490 nm.)

These large regional differences between the ageing characteristics of Shiraz wines are possibly not directly related to the differing pigment compositions of grapes from the two areas, as illustrated in Fig. 3. They are considered to be more likely due to a relative deficiency of reactive tannin-precursor materials in the Murray Valley grapes, but closer study of total phenolic constituents is obviously required to decide the question. In any case, it is significant that such wines, from irrigated viticultural areas, are generally considered to be inferior in quality to those from areas such as the Barossa Valley.

Conclusion

The unique value of gel column analysis and of the pigment profile concept in the study of grape and wine pigments has been demonstrated by partial solution

of several long standing problems in areas of viticulture and oenology where little comprehensive data was previously available. The nature of grape and wine tannins as polymeric pigments related also to accompanying anthocyanins had escaped earlier attention because of the absence of suitable means for their routine separation from the anthocyanins, but it is now evident that these tannin pigments are of prime importance in fruit maturation and wine ageing reaction processes.

Before their recent isolations from red wines and from wine grapes, no such materials had been specifically reported in the literature of natural product chemistry. It is therefore relevant to report here that similar tannin pigments having anthocyanin like properties have also been isolated in this laboratory from the colouring matters of anthocyanin containing fruits and flowers — excellent gel column separations from anthocyanins were obtained with extracts of plum, cherry, rose and hibiscus pigments. It has become clear that polymeric pigments related to anthocyanins are actually quite widespread in nature, and that they may be, as in wine grapes, a major weight fraction of plant pigments.

At this stage, no information is available on the nature of reactions leading to their formation, but it seems evident in this regard that what occurs in the grape or other fruit to a limited extent under biochemical control is a continuous process during ageing of a red wine. All of the tannin pigment fractions isolated are quite obviously molecular mixtures, and are probably very diverse both in structural type and molecular dimensions. These features are generally considered to be responsible for the varying sensory properties of tannins (GOLDSTEIN and SWAIN 1963). Because of the prohibitive structural problems presented here, it is indeed fortunate that the molecular size of tannins seems likely to be the more important feature in objective considerations of astringency and flavour in wines.

The gel system chosen for pigment analysis, using Sephadex G-25, provides no more than a separate measure of gross fractions. It cannot distinguish between tannin fractions of different grape materials or of wines, all of which are excluded from the gel. However, qualitative differences between these fractions, especially those from ageing wines, have been indicated by spectral characteristics, and the use of more loosely crosslinked gels, with larger exclusion limits, does apparently allow fractionation of tannins according to molecular size (SOMERS, 1966 a). Thus the measurement of differences in molecular size distribution of tannin fractions seems possible by this means. Further work, involving the use of such gel systems and determinations of mean molecular weights, should lead to useful knowledge of their special physical, chemical and physiological properties.

However, the present availability of means for routine determination of total colour components has many important applications. Areas of research and development in which the pigment profile concept can be usefully applied include the following:

a) The assessment of wine grape materials in terms of available phenolic pigments. Varietal characteristics and regional differences within varieties are important to those concerned with vine breeding, clonal selection and wine making.

b) Processes for colour extraction before fermentation can now be properly evaluated, as also can the nature of extraction occurring during the traditional fermentation on skins. Colour has too often been regarded in the industry as a single entity.

c) Quality control of tannins and other pigments is perhaps now possible in blending operations, as the stage of maturation, or 'chemical age' of a red wine, with respect to pigment composition, can be routinely assessed in an objective fashion.

d) The effects of the various fining materials on pigment content can be accurately determined and related to organoleptic evaluations.

e) Proposed treatments for acceleration of the natural ageing processes in wine may be examined objectively.

Summary

Gel column analysis of pigment extracts has been automated, and the distribution of total coloured constituents in terms of tannin pigments, acylated anthocyanins and anthocyanins can now be measured routinely, enabling rapid evaluation of materials. Quantitative determinations of polymeric and monomeric pigments in grape extracts and in young wines have been made from the pigment elution curves.

Wine grape varieties from the same viticultural area were found to have characteristically different elution curves. Evidence is presented for one variety that some degree of selective extraction of pigments occurs during normal fermentation on skins, the anthocyanins being more efficently extracted than other fractions — thus the composition of pigments in the new wine differs from that in the grape. Other young varietal wines are also qualitatively different from one another in pigment compositions, which generally resemble those of the grape varieties.

Regional differences between grapes and wines of one variety have been demonstrated, which relate to colour extraction and to the ageing characteristics of the wines from these regions.

The gross changes in pigment distribution which occur during ageing of red wines, with progressive loss of monomeric pigments and increase in the relative proportions of tannin pigments are described. The latter fraction is seen to be of primary importance to red wine technology.

The elution curves obtained are regarded as pigment profiles of the grapes or wines, and the utility of this concept in viticultural and oenological research is demonstrated.

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