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Factors affecting oxidative browning of white wine

by

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Ursachen der oxidativen Bräunung von Weißweinen

Zusammenfassung. — Um Indikatoren der Bräunungsanfälligkeit zu ermitteln, wurden bei 80 Weißweinen aus Handelskellereien die „Gesamtphenole", die optische Dichte bei 280 und 320 nm sowie die Zusammensetzung der UV-absorbierenden Komponenten bestimmt. Die monomeren Katechine und die dimeren Proanthocyanidine erwiesen sich trotz ihrer relativ niedrigen Konzentration als wichtige Merkmale der Bräunungsanfälligkeit.

Introduction

Numerous studies have been made on factors affecting the susceptibility of wines to develop brown colour in the presence of air (see SINGLETON and ESAU 1969, SIMPSON 1980 and references cited therein). In many of these studies, however, few factors were considered and small numbers of wines were analysed. Consequently, the findings have been somewhat inconsistent and insufficiently general to be helpful in winemaking practice.

The colour of white wine is due primarily to intense absorbance in the u. v. region which extends slightly into the visible part of the spectrum. The materials responsible for wine colour can be analysed and separated into specific chemical groups and individual compounds by gel chromatography (SOMERS and ZIEMELIS 1972, CASTINO and DI STEFANO 1976, MYERS and SINGLETON 1979). This technique, therefore, provides a convenient means of examining white wine composition and its relationship with oxidative browning.

It is beneficial to the winemaker to be able to determine which wines are more susceptible to oxidative browning, so that these wines can be given good protection against oxygen contact. Rapid tests to show the overall browning potential of wine can be performed by heating the wine in the presence of air or oxygen (e. g. DU PLESSIS and UYS 1968, SINGLETON and KRAMLING 1976). The usefulness of such tests and the factors influencing browning in a study of 80 commercial wines are considered in this article.

Materials and methods

1. Wines

Dry white table wines made from *Vitis vinifera* grapes and containing no sorbic acid were obtained from 13 commercial wineries in Australia. Some were collected prior to protein stabilisation, in which case bentonite was added at 1.0 g/l, the wines were filtered through sterile grade pads using a laboratory filter and potassium meta-

bisulphite was added to give 20—30 mg free SO_2/l prior to bottling. All analyses and tests on the wines were made during the year of vintage.

2. Analytical methods

Free and total SO_2 contents were determined by the method of RANKINE and POCOCK (1970), iron and copper by atomic absorption spectroscopy (WILSON and RANKINE 1978) and "total phenolics" by the FOLIN-CIOCALTEAU procedure (SINGLETON and ROSSI 1965). U. v. spectra were recorded using a Varian Techtron model 635D u. v.-visible spectrophotometer and 1 mm quartz cells. U. v. elution profiles were obtained using gel chromatographic techniques similar to those described by SOMERS and ZIEMELIS (1972), except that the solvent flow rate was 180 ml/h, 2 ml aliquots of neat wine were applied to the Sephadex G-25 Fine column and the eluent was monitored at 280 nm. The u. v. profiles and peak areas were obtained using a Linear model 252A integrating chart recorder.

3. Isolation and identification of wine components

Wine (1.5 l) was saturated with AR NaCl and extracted with redistilled ethyl acetate, b. p. 77—78 °C (2 × 100 ml). The solvent was removed under vacuum at < 35 °C and the total extract was separated into fractions using the same column and conditions as with the u. v. elution profiles. The materials in each fraction were isolated by extracting into ethyl acetate and were further purified by preparative cellulose TLC. Acetate derivatives of these materials were prepared by treatment with anhydrous pyridine and acetic anhydride at ambient temperature for 16 h. I. r. spectra were recorded using a Perkin Elmer model 237 spectrophotometer and NMR (proton) spectra in $CDCl_3$ were obtained using a Brüker 90 MHz spectrometer with Fourier transform.

4. "Browning" and accelerated "browning" tests

Wines were filtered through a membrane filter with 0.22 µm pore size and 5 ml lots were sealed in the presence of air in 10 ml glass ampoules (actual capacity ca. 12.2 ml). The ampoules and other glassware were sterilised by heating at 150 °C for 4 h prior to use. "Browning" was measured as the increase in optical density at 420 nm after 30 weeks at 15 °C. "Accelerated browning" tests were performed in a similar manner except that readings were made after storage at 50 °C for 3 weeks. Values after storage at 50 °C for 1 week were also recorded.

5. Statistical methods

The relationships between "browning", "accelerated browning" and other analytical values were examined and expressed as correlation coefficients.

Results and discussion

The wines chosen for this study did not contain sorbic acid since this compound would have interfered with some of the assays. Sorbic acid, which is used as a preservative, especially with those wines containing residual sugar, absorbs strongly at 280 nm (ZIEMELIS and SOMERS 1978).

On the basis of similarities observed in the u. v. elution profiles of the 80 wines examined, 10 major peaks were assigned. The elution volumes corresponding with peak maxima showed some variability, especially with peaks 2—7. But this is consist-

ent with the complex nature of these peaks. Table 1 gives the peak areas, elution volumes and identities of the major components as shown by the present study and earlier investigations (SOMERS and ZIEMELIS 1972, MYERS and SINGLETON 1979, NAGEL *et al.* 1979, NICKENIG and PFEILSTICKER 1980).

Table 1

Composition of the u. v. absorbing materials in wine based on gel chromatography

Die Zusammensetzung der UV-absorbierenden Substanz nach gelchromatographischen Analysen

Peak	Approx. elution volume (ml)	Peak area expressed as integration counts		Major components
		Mean	Range	
1	45	11	1—40	Proteins, protein-tannin complexes
2	95	819	448—1 360	Nucleotides, tyrosol
3	130	588	131—1 040	Nucleotides
4	155	51	0—423	Nucleotides
5	180	400	0—1 270	Hydroxycinnamic acid-tartaric acid esters including caffeoyltartaric acid
6	205	72	0—532	Hydroxycinnamic acids, ethyl caffeate
7	235	213	0—745	Hydroxycinnamic acids including caffeic acid
8	280	20	0—75	(−)-Epicatechin
9	340	38	2—199	(+)-Catechin
10	415	37	0—165	Dimeric procyanidins B1—B4

Most of the materials with elution volumes greater than 200 ml and virtually all the materials corresponding with peaks 7—10 were extracted into ethyl acetate. Tyrosol, ethyl caffeate, caffeic acid, epicatechin and catechin were isolated from the extracts of 4 wines considered representative of the 80 wines on the basis of the u. v. profiles. The identities of these materials were determined from their i. r. spectra and the NMR spectra of their acetate derivatives. Identical spectra were obtained under similar conditions from authentic materials. The major components of peak 10 were the dimeric procyanidins B1—B4 as indicated by their u. v. spectral properties and by 2-dimensional cellulose TLC using conditions similar to those described by LEA *et al.* (1979). Minor quantities of flavonol glycosides were also present in peak 10 as indicated by monitoring the gel chromatography at 365 nm. The major flavonol glycoside isolated by cellulose TLC, also found to be abundant in *V. vinifera* leaves (ZIEMELIS personal communication), gave quercetin on acid hydrolysis (HARBORNE 1973). WEINGES and PIRETTI (1972) and PIRETTI *et al.* (1976) reported that quercetin 3-glucoside was the major flavonol glycoside present in Trebbiano and Albana grapes (*V. vinifera* cvs.). The presence of ethyl caffeate in wine has only been reported in recent times (NICKENIG and PFEILSTICKER 1980), but this compound and larger quantities of the free acid were major phenolic components in many of the Australian wines examined. Monocaffeoyltartaric acid, expected to be the major hydroxycinnamic acid-tartaric acid ester present in

Table 2

Correlations between browning and analytical parameters

Die Korrelation zwischen der Bräunung und den analytischen Parametern

	Correlation coefficient (r)			
	"Browning"		"Accelerated browning"	
Increase in optical density at 420 nm over 1 week at 50 °C	0.361	**[1]	0.692	***[1]
"Browning"	—		0.686	***
"Accelerated browning"	0.686	***	—	
Wine pH	−0.127	NS	0.074	NS
Free SO_2	−0.027	NS	−0.109	NS
Iron content	0.272	*	0.209	NS
Copper content	0.024	NS	0.062	NS
"Total phenolics"	0.438	***	0.255	*
Optical density at 280 nm	0.452	***	0.230	NS
Optical density at 320 nm	0.331	**	0.108	NS
Peak 1	−0.065	NS	0.010	NS
Peak 2	0.220	NS	0.104	NS
Peak 3	0.080	NS	0.135	NS
Peak 4	−0.052	NS	−0.154	NS
Peak 5	0.346	**	0.254	*
Peak 6	−0.044	NS	−0.141	NS
Peak 7	0.093	NS	−0.058	NS
Peak 8	0.513	***	0.383	***
Peak 9	0.366	**	0.336	**
Peak 10	0.418	***	0.438	***

[1] NS = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

these wines (see NAGEL *et al.* 1979), was found to have an elution volume of 176 ml and, therefore, is considered to be a major component of peak 5.

Relationships found between "browning", "accelerated browning" and the increase in optical density at 420 nm over 1 week at 50 °C, wine pH, free SO_2, iron and copper content, "total phenolics", optical densities at 280 and 320 nm and peak areas of the u. v. absorbing components separated by gel chromatography are shown in Table 2. Close correlation was found between "browning" and "accelerated browning". Moderate correlation was found between "browning" and "total phenolics", optical densities at 280 and 320 nm, peak 5 and peaks 8—10. "Accelerated browning" showed lower correlation than "browning" with the analytical parameters examined, which may have been due to the effects of heating and the alteration of reaction rates at higher temperature as observed by BERG and AKIYOSHI (1956).

The "total phenolics" assay has been widely used to determine phenolic content of wine and, in particular, to indicate browning potential of white wines. ROSSI and SINGLETON (1966) did not find a strong correlation between "total phenolics" and browning potential and, recently, SOMERS and ZIEMELIS (1980) reported serious limitations of the assay due to interference by sulphur dioxide. The results from the present study indicate that there are better guides to browning susceptibility although the "total phenolics" assay gives moderate indication of this susceptibility.

The extent of correlation between "browning" and the catechin phenolics (peaks 8—10) is significant because of the relatively low concentrations of these compounds in the wines. On the bases of peak area and calibrations made with model wine solution, the amounts present in the 80 wines were: epicatechin (peak 8), mean value 7.8 mg/l (range 0.0—29.2 mg/l) and catechin (peak 9), mean value 14.8 mg/l (range 0.8—77.6 mg/l). The total quantities of the dimeric procyanidins B1—B4 (peak 10), if all the absorbance at 280 nm is attributed to these materials, would be similar to the quantities of catechin found. The quantities of the catechin phenolics in young wines are influenced by several factors including the extent of extraction of solids by the must and the subsequent losses during fermentation and processing (SIMPSON 1980). In many wines, these materials are important contributors to the colour changes following air contact. Even in the absence of air, these materials presumably polymerise during storage, contributing to the development of additional colour in the wines.

Certain relationships, which might have been expected but were not found, are those between browning and copper content, wine pH, and free SO_2. Copper ions catalyse autoxidative reactions and additions of copper to individual wines increase the initial browning rates (BERG and AKIYOSHI 1956, SIMPSON unpublished results). With extensive oxidation, however, the overall colour production is not necessarily higher in the presence of added copper ions. With both the "browning" and "accelerated browning" tests used, the extent of oxidation of the wines was considerable.

In preliminary trials, a range of samples with pH 3—4 were obtained by adding mineral acid and alkali to the same wines: more colour was consistently produced in the higher pH samples when subjected to the "accelerated browning" tests. The lack of significant correlation between browning and wine pH for the 80 wines examined may be due, in part, to the increased hydrolysis during storage and prior to analysis of complexes containing phenolic residues and the release of more readily oxidised phenolics in the lower pH wines. The strong negative correlation between wine pH and quantities of materials corresponding with peak 5 ($r = -0.477$, $P < 0.001$) suggests that these materials are undergoing acid hydrolysis; this is likely since hydroxycinnamic acid-tartaric acid esters are present in this fraction.

The poor correlation found between "browning" or "accelerated browning" and free SO_2 content of the wines is probably attributable to the extent of browning that occurred. The initial suppression of colour development by the free SO_2 (BERG and AKIYOSHI 1956) apparently did not exert a strong influence on the browning that was measured. Presumably for the same reason, there was little correlation observed between the free SO_2 content and the increase in optical density at 420 nm after 1 week at 50 °C. Free SO_2 in wine gives protection against oxidation and additional quantities increase the extent of protection (BERG and AKIYOSHI 1956, AMERINE *et al.* 1972). With the conditions used in the "accelerated browning" tests, free SO_2 was rapidly lost and the quantity of bound SO_2 was also reduced.

Since browning of white wines during processing and storage occurs at ambient temperatures and is due to some oxygen uptake, the test where conditions are most similar is the "browning" test; this, therefore, must be expected to provide the best indication of browning potential under normal conditions of processing and storage of white wines. Tests of browning of wine in which the conditions are severe, i. e. storage at elevated temperature in the presence of considerable or excess oxygen, are indicators of browning capacity or maximal colour production. They relate more closely with actual phenolic content and the oxidisability of wine, but tend to be less applicable in gauging the effects of slight oxygen contact in the normal course of processing and storage of wine.

Summary

Indications of browning susceptibility of 80 commercial white wines were given by measures of "total phenolics", optical densities at 280 and 320 nm and compositions of the u. v. absorbing components. The monomeric catechins and dimeric procyanidins, despite their relatively low concentrations, were found to be important indicators of browning susceptibility.

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