Vitis 16, 286—294 (1977)

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Oxidative pinking in white wines

by

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Oxydative Rosafärbung in Weißwein

Zusammenfassung. — Es wird eine spektrophotometrische Methode beschrieben, die es ermöglicht, die Intensität der Rosafärbung in Weißwein zu messen. Bei Weinen, die zu Rosafärbung neigen, wird diese durch Zugabe von Wasserstoffperoxid herbeigeführt. Weine, die mit 75 mg Wasserstoffperoxid/l behandelt und 24 Stunden lang bei 25 °C gehalten wurden, entwickelten eine rosa Verfärbung, die Rückschlüsse auf den Präcursorgehalt im Wein erlaubt. Zugabe von 15 mg Wasserstoffperoxid/l führte zu einer weniger starken Verfärbung, die sich in Abhängigkeit vom SO_2-Gehalt des Weines entwickelte; diese Reaktion ermöglicht es nachzuweisen, in welchen Weinen während der Lagerung eine rosa Verfärbung entstehen könnte, so daß gegebenenfalls eine PVPP-Behandlung vorgenommen werden kann.

Der Einfluß des Wein-pH, der Lagerungstemperatur, des SO_2-Gehaltes und der PVPP-Behandlung auf die Entstehung der durch Zusatz von Wasserstoffperoxid ausgelösten rosa Weinverfärbung wurde geprüft. Die durch Zugabe von SO_2 und Änderungen im pH verursachten Reaktionen zeigen, daß die rosa Substanzen keine einfachen (monomeren) Anthocyane sind. Die spektralen und chemischen Eigenschaften dieser rosa Substanzen ähneln jenen, die von Anthocyanen mit höherem Molekulargewicht und Aryl-Substitution in Stellung 4 zu erwarten sind. Solche Anthocyane könnten von Proanthocyanen herstammen, deren Vorkommen in den Trauben bekannt ist und die wahrscheinlich auch im Wein vorhanden sind.

Introduction

With white table wines the term "pinking" is used to describe the troublesome discolouration which sometimes develops during the later stages of production or storage. Pinking is most likely to occur following contact with air and in these instances the colour development is fairly rapid (i.e. over several days). Even where the extent of pinking is slight the colour of the wine is unfavourably affected; where pinking is more intense the wine may be commercially unacceptable.

Wines made from the main white grape varieties (vine sp. *Vitis vinifera)* grown in Australia (Muscat Gordo Blanco, Sultana, Palomino, Riesling and Crouchen) have been found susceptible to pinking with, however, considerable regional variation in such susceptibilities. The reported incidence of pinking has increased in recent vintages and this has coincided with an increase in the use of colder fermentation and inert gas to protect against oxidation. Wines produced under these conditions are often those exhibiting higher susceptibility to pinking; this is in agreement with observations made by Singleton (1972).

Pinking can be removed or prevented by treating the wine with nylon, casein or PVPP (polyvinylpolypyrrolidone). As it is desirable to keep the number of wine-making operations to a minimum and because of increased production costs, treatments to protect against pinking should only be considered where the development

of pinking is likely. A better understanding of the causes and nature of pinking in white table wines would assist in overcoming this problem.

In the present studies, a spectrophotometric method of quantitatively measuring pinking has been developed and an assay formulated to determine the potential of a wine to develop pink colour.

Materials and methods

Spectral measurements: Visible spectra and optical densities were obtained using a Varian Techtron model 635D u.v.-visible spectrophotometer and Linear model 252A chart recorder. The optical densities (E) using 10 mm sample cells were corrected by subtracting the values obtained at the same wavelength (λ) with distilled water. Wine samples were filtered through a membrane filter with 1.2 μm pore size prior to measurement.

Determination of pinking: From an examination of the visible spectra of wines having a range of pink colour it was evident that the wines with more pinking were those with spectra exhibiting higher optical densities at about 500 nm and which deviated by a greater extent from a smooth curve near this wavelength. The visible spectra of two wines, one having no pinking and the other appreciable pinking are shown in Figs. 1 and 2, respectively. In these studies the pinking values were determined using statistical means. A least squares fit to the empirical formula

$$\ln E = a_0 + a_1 \ln(\lambda - 350) + a_2 \ln(\lambda - 350)^2$$

using E_{400}, E_{410}, E_{420}, E_{600}, E_{625} and E_{650} determined the values of the coefficients a_0, a_1 and a_2 for each curve, i.e. spectrum (for example, see Williams 1959, 41—49). For wines showing no pinking a consistently good fit was obtained in the range 420—600 nm using the derived formulae. Estimation of pinking for a wine is based on the difference between E_{500} (observed) and the unpinked (calculated) value at 500 nm. For convenience, the pinking values were multiplied by a factor of 10^3.

Assay of pinking susceptibility: Pink colour could be produced in susceptible wines (i.e. wines of which some lots had pinked during storage) by the addition of hydrogen peroxide. The extent of pinking present after treatment with hydrogen peroxide was determined by the method outlined above. As such the value obtained is the sum of the pinking initially present in the wine (generally a low value) and the pink colour produced by the hydrogen peroxide treatment.

Wine samples (10 ml lots) were placed in screw-capped test tubes (20 ml). Hydrogen peroxide was added and the samples were stored for 24 h at 25 °C in the dark, unless otherwise stated. The samples were mixed thoroughly (i) after the addition of hydrogen peroxide and (ii) before measuring optical densities.

Effect of hydrogen peroxide concentration on pinking produced in wine: 10 ml lots of 5 wines showing a range of ability to pink were treated with 0.05 to 0.40 ml of freshly prepared 0.3 % (w/v) hydrogen peroxide solution (1 ml of 30 % hydrogen peroxide made up to 100 ml with glass-distilled water) to give a concentration of 15 to 120 mg hydrogen peroxide per l. The commercial hydrogen peroxide solution was stored at 4 °C. Analyses by the method of Kolthoff and Sandell (1952) showed a concentration of 29.9 % (w/v).

Effects of reaction time and temperature on pinking produced in wine: The pinking produced in 5 wines with the addition of

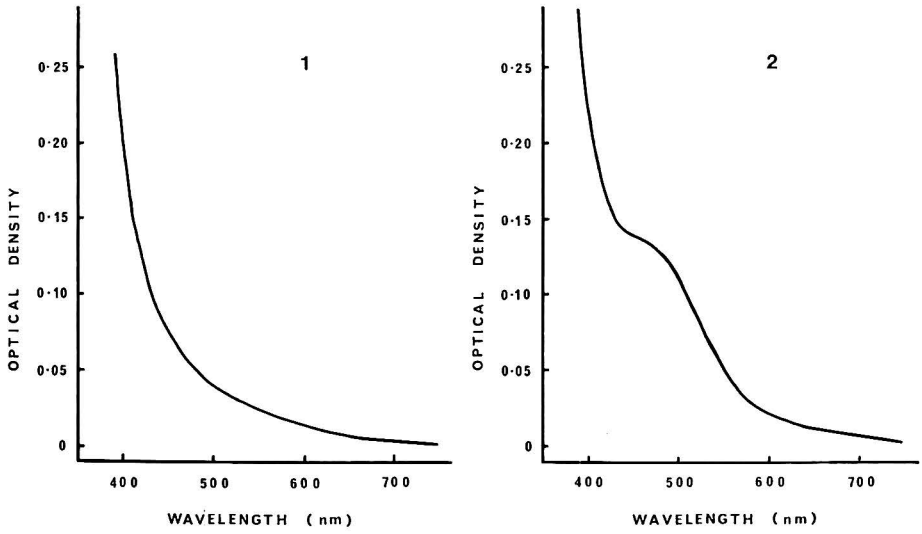

Fig. 1: Spectrum of wine showing no pinking. Pinking value, 0 (absorbance units × 10^3).
Fig. 2: Spectrum of wine showing pinking. Pinking value, 27 (absorbance units × 10^3).

Abb. 1: Spektrum eines Weines ohne Rosafärbung. Farbintensität = 0 (Absorptionseinheiten × 10^3).
Abb. 2: Spektrum eines Weines mit Rosafärbung. Farbintensität = 27 (Absorptionseinheiten × 10^3),

75 mg hydrogen peroxide per l and storage at 25 °C was determined after 3, 6 and 12 h, and over 14 days. The pinking produced in the same wines stored at 3 °C for 1 and 2 days was similarly obtained.

Effect of SO_2 content on pinking present and produced in wine on the addition of hydrogen peroxide: After acidification with 5M HCl (to give a pH <1) wine samples were sparged with nitrogen to remove free SO_2. 5M NaOH was added until the pH of each wine was returned to the original value. Potassium metabisulphite was added to the 5 wines to give a range of samples having up to 60 mg free SO_2 per l. After allowing the wines to equilibrate (2 days at room temperature) free and bound SO_2 levels were determined by the method of RANKINE and POCOCK (1970). Pinking values were obtained after the addition of 15 and 75 mg hydrogen peroxide per l.

PVPP treatment of wine and the effect on pinking: 100 ml lots of 5 wines were placed in stoppered measuring cylinders with 12 to 100 mg polyvinylpolypyrrolidone (PVPP) and briefly shaken at 10 min intervals over 1 h. The wines were filtered using a 1.2 μm membrane filter. Pinking values were obtained prior to and after the addition of 75 mg hydrogen peroxide per l.

Effects of acidification and pH change on pinking: Additions of 5M HCl and 5M NaOH to 50 ml lots of 5 wines having appreciable pink colour were made to provide a range in pH (for each wine) from 2.75 to 4.00. Pinking values were obtained (after 4 h) prior to the addition of hydrogen peroxide, after the addition of 75 mg hydrogen peroxide per l and after acidifying the treated samples with 5M HCl (to give a pH <1).

Analysis of errors (in assays using hydrogen peroxide): Triplicate determinations were made and the pinking values shown (see Tables) are means of these determinations. Examination of the data collected for each table showed that the standard error was proportional to the pinking value. Since a similar relationship was found in each instance, the standard error for the pooled data was calculated. This was found to be 3.0 % (of the mean).

Results and discussion

The pinking present in a wine can be assigned a numerical value on the basis of the visible spectral characteristics of the wine. The deviation from a smooth curve between 420 and 600 nm can be estimated by various means (drawing in by hand, comparison with spectra of wines showing no pinking, and statistical analysis as was used in the present studies). Measurement of pinking was based on the optical densities at 500 nm since the greatest differences occurred at about this wavelength. Numerical values obtained and the amount of pinking observable in wines correlated well; the method allows a better comparison to be made with wines having dissimilar colour composition. For most white table wines pinking is recognisable with values >5 (absorbance units $\times 10^3$) and the colour which might otherwise be a desirable light yellow or greenish yellow is unfavourably affected.

As a guide to pinking susceptibility an assay has been described by the manufacturers of Polyclar AT (GAF Corporation 1975) in which the wine is aerated and stored overnight; treatment with PVPP is recommended if pinking develops in the wine. Clearly, the assay is limited because rate determining factors such as temperature and amount of oxidant are not accounted for. In addition, the degree of pinking produced cannot readily be used as an indication of PVPP requirements.

Singleton (1972) had indicated that pinking could be produced in susceptible wines by the addition of a chemical oxidant. Hydrogen peroxide is effective in producing this colour development, and, from the evidence provided by Wildenradt and Singleton (1974) is likely to be present in a wine after exposure to atmospheric oxygen. Pinking which occurs under winery conditions or with storage may result from the *in situ* production of hydrogen peroxide.

In the studies presented, optimum conditions to produce pinking in a range of wines over 24 h by the addition of hydrogen peroxide were determined and the effects of SO_2 content, pH and PVPP treatment were examined. Samples were stored in the dark since it was found that light could affect the rate of pinking. It was also observed that the upper portion of the wine sample occasionally developed a more intense pink colour, therefore, the samples were mixed thoroughly before optical density readings were obtained. This layering of colour was noteworthy because similar observations have been made with pinking occurring in storage tanks.

Factors affecting pinking susceptibility: The pinking produced initially increased with concentration of hydrogen peroxide (Table 1). Even for wines developing considerable pinking 75 mg hydrogen peroxide per l produced maximal or near maximal pinking under the conditions used (i.e. 24 h at 25 °C). Although browning, which can be measured by the increase in absorbance at 420 nm (Singleton and Kramling 1976), also occurs with the addition of hydrogen peroxide, browning and pinking are two distinct phenomena; browning in a wine may occur in the absence of pinking.

Table 1

Effect of hydrogen peroxide concentration on pinking produced in wine (24 h at 25 °C)
Einfluß der Wasserstoffperoxid-Konzentration auf die Rosafärbung von Wein (24 h bei 25 °C)

Wine	Hydrogen peroxide added (mg/l)								
	0	15	30	45	60	75	90	105	120
	Pinking (absorbance units × 10^3)[1]								
A	2	5	26	36	39	41	37	34	41
B	6	8	7	23	50	60	75	66	83
C	0	1	1	2	3	3	3	6	7
D	6	18	37	45	52	49	52	49	51
E	3	5	16	29	39	47	48	51	50

[1]) Mean of 3 determinations.

Table 2 a

Effect of reaction time and temperature on pinking produced in wine (75 mg hydrogen peroxide per l)
Einfluß von Reaktionszeit und Temperatur auf die Rosafärbung von Wein (75 mg Wasserstoffperoxid/l)

Wine	25 °C										3 °C	
	Reaction time (d)											
	0	0.125	0.25	0.50	1	2	3	5	7	14	1	2
	Pinking (absorbance units × 10^3)[1]											
A	4	9	20	24	24	51	55	42	30	21	10	18
B	3	11	17	14	23	43	44	38	24	21	12	24
C	4	9	10	7	7	8	10	8	5	3	9	13
D	7	13	21	29	50	61	68	57	44	38	12	20
E	4	11	18	22	31	47	51	46	39	31	11	15

[1]) Mean of 3 determinations.

Table 2 b

Browning in wine treated with hydrogen peroxide (75 mg/l, 25 °C)
Bräunung von Wein nach Behandlung mit Wasserstoffperoxid (75 mg/l, 25 °C)

Wine	Optical density at 420 nm (E_{420})		
	0 d	3 d[1])	14 d[1])
A	0.110	0.260	0.258
B	0.110	0.319	0.326
C	0.111	0.197	0.195
D	0.145	0.256	0.296
E	0.129	0.302	0.295

[1]) Mean of 3 determinations.

The extent of pinking initially increased with reaction time and temperature. The rate of pinking at 25 °C after addition of 75 mg hydrogen peroxide per l was linear over the first 24 h; in the wines examined the pinking value reached a maximum after 3 days and then decreased with time (see Table 2 a). It is likely that the

pink materials are reacting further to give products which no longer show strong absorption near 500 nm. Browning as indicated by the increase in E_{420} was found to be similar after 3 and 14 days (Table 2 b). Pinking occurring under winery conditions, which is generally less intense than that developed by the addition of hydrogen peroxide, shows the same sequence of colour changes.

The SO_2 content of a wine did not affect the pinking produced (after 24 h at 25 °C) with 75 mg hydrogen peroxide per l, provided that the free SO_2 level was not greater than ca. 40 mg/l. Such a value is rarely exceeded in Australian white table wines and RANKINE (1975) has indicated that 20—40 mg free SO_2 per l should be adequate. However, with a lower level of hydrogen peroxide (15 mg/l) the pinking produced was proportionally reduced with increased levels of free SO_2 (Table 3).

Table 3

Effect of SO_2 content on pinking present and produced in wine on the addition of hydrogen peroxide

Einfluß des SO_2-Gehaltes auf die bestehende und die durch Zugabe von Wasserstoffperoxid verursachte Rosafärbung von Wein

Wine	SO_2 content (mg/l)		Pinking (absorbance units × 10^3)		
	Free	Bound	Present	Produced by 15 mg H_2O_2/l[1])	Produced by 75 mg H_2O_2/l[1])
A	15	59	2	3	18
	24	65	3	1	17
	27	69	2	1	16
	49	77	2	1	16
	57	84	1	1	13
B	10	133	0	7	19
	13	144	2	4	21
	27	157	0	5	18
	35	163	1	4	16
	49	165	2	4	12
C	6	144	3	7	16
	12	155	1	4	17
	20	165	2	3	15
	29	171	2	1	13
	41	176	2	1	13
D	7	77	5	9	31
	15	84	4	5	32
	24	94	3	6	27
	35	99	4	4	27
	45	101	5	4	22
E	13	116	2	5	17
	21	122	4	5	15
	30	132	4	4	13
	40	138	3	4	12
	52	146	2	3	9

[1]) 24 h at 25 °C; mean of 3 determinations.

Table 4

Effect of PVPP treatment on pinking present and produced in wine on the addition of hydrogen peroxide

Einfluß der Behandlung mit PVPP auf die bestehende und die durch Zugabe von Wasserstoffperoxid verursachte Rosafärbung von Wein

Wine		PVPP added (mg/100 ml) 0	12	25	50	75	100
		Pinking (absorbance units × 10^3)					
A	Before hydrogen peroxide addition	4	3	3	4	4	1
	After hydrogen peroxide addition[1])	40	39	37	32	28	24
B	Before hydrogen peroxide addition	9	6	5	6	5	1
	After hydrogen peroxide addition[1])	30	32	30	25	16	16
C	Before hydrogen peroxide addition	0	0	0	0	0	0
	After hydrogen peroxide addition[1])	10	9	9	6	4	6
D	Before hydrogen peroxide addition	6	4	4	3	2	0
	After hydrogen peroxide addition[1])	46	45	43	37	33	29
E	Before hydrogen peroxide addition	4	7	5	3	4	3
	After hydrogen peroxide addition[1])	41	36	32	30	25	24

[1]) 75 mg hydrogen peroxide per l; 24 h at 25 °C; mean of 3 determinations.

Under winery conditions, moderate to high levels of free SO_2 should inhibit or limit the extent of pinking, as it is probable that the pinking precursors are competing with free SO_2 for the available oxidant.

Increasing levels of PVPP removed greater amounts of pink materials and their precursors from wine (Table 4). The significance of high concentrations of the precursors in some wines even after addition of comparatively large amounts of PVPP has yet to be determined.

The influence of pH on the absorbance of pinking materials in the range 2.75—4.00 was slight (Table 5 a). Also, acidification of the same wines after treatment with hydrogen peroxide (75 mg/l, 24 h at 25 °C) did not enhance the pink colour (Table 5 b). These findings show that the pink materials are not simple flavylium salts or their glycosides, such as are present in red grapes (SINGLETON and ESAU 1969, p. 31) and young red wines, since the colour of these materials is strongly pH dependent (SOMERS 1971). However, with alkyl or aryl substitution at the 4-position the flavylium nucleus is relatively insensitive to pH change (SOMERS 1971) or bleaching by SO_2 (TIMBERLAKE and BRIDLE 1968). Flavylium salts substituted at the 4-position are derivable from procyanidins indicated as being present in white wines (WEINGES and PIRETTI 1972) by oxidation of intermediate flav-2-enes. The formation of cyanidin from leucoanthocyanidins by this reaction mechanism as suggested by SINGLETON (1972) does not account for pinking in white table wines.

Conditions to produce pink colour in susceptible white table wines have been determined. Addition of 75 mg hydrogen peroxide per l and storage at 25 °C for 24 h produces pinking, the intensity of which indicates the content of precursors in the wine. Addition of 15 mg hydrogen peroxide per l under similar conditions produces less pink colour and the colour development is influenced by SO_2 content of the wine. Provided that SO_2 levels in the wine are maintained, this method measures pinking susceptibility of the wine. An assay incorporating addition of 15 mg hydrogen peroxide per l is being used routinely by one of the major wineries in Australia to determine which wines require PVPP treatment (private communication, B. Tyson).

Table 5 a

Effect of pH on pinking in wine
Einfluß des pH auf die Rosafärbung von Wein

Wine	pH 2.75	3.00	3.25	3.50	3.75	4.00
	Pinking (absorbance units × 10^3)					
F	7	6	3	5	4	4
G	6	9	7	9	6	6
H	11	14	10	13	11	9
I	10	11	10	10	10	9
J	7	6	10	7	6	6

Table 5 b

Effect of acidification on pinking after the addition of hydrogen peroxide (75 mg/l; 24 h at 25 °C)
Einfluß des Ansäuerns auf die Rosafärbung nach der Zugabe von Wasserstoffperoxid (75 mg/l; 24 h bei 25 °C)

Wine	Pinking at wine pH[1] (absorb. units × 10^3)	Pinking at pH <[1])[2] (absorb. units × 10^3)
F	48	43
G	74	67
H	76	62
I	49	52
J	94	70

[1]) Mean of 3 determinations.
[2]) 2 h after acidification.

Summary

A spectrophotometric method is described which is able to measure the amount of pink colour in white wine.

Addition of hydrogen peroxide produced pinking in susceptible wines. Wines treated with 75 mg hydrogen peroxide per l and held at 25 °C for 24 h produces pink colour indicative of the quantity of precursor in the wine. Addition of 15 mg hydrogen peroxide per l gave less intense pinking and its development was influenced by SO_2 content of the wine; this reaction provides a means of showing which wines are liable to develop pink colour during storage and consequently, whether PVPP treatment is advisable.

The influences of wine pH, storage temperature, SO_2 content and PVPP treatment on the production of pink colour in wine after hydrogen peroxide addition were examined.

Effects of SO_2 addition and pH change showed that the pink materials were not simple (monomeric) anthocyanins. The spectral and chemical characteristics of the pink materials are similar to those expected of higher molecular weight anthocyanins having aryl substituents at the 4-position, and which could be formed from proanthocyanins. These materials (proanthocyanins) are known to occur in grapes and are likely to be present in wine.

Acknowledgements

The author thanks Mr. G. C. MILLER for technical assistance, Mr. L. VEITCH of the Division of Mathematical Statistics, C.S.I.R.O., Glen Osmond, South Australia for advice and statistical analysis, Drs. B. C. RANKINE and T. C. SOMERS for helpful suggestions, and the Australian Wine Board for financial support. Thanks are also due to the numerous wine companies for the supply of 1976 vintage dry white table wines (*Vitis vinifera*) used in these studies.

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Eingegangen am 19. 7. 1977

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