Vitis **24**, 43—50 (1985)

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Flavonol haze in white wines

by

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Flavonoltrübung in Weißweinen

Z u s a m m e n f a s s u n g : Die Bildung einer gelbfarbenen Trübung in Handels-Weißweinen und gelber Niederschläge sowohl in Mosten wie Weinen ging auf die Ausfällung von Quercetin zurück. Die Rebblätter erwiesen sich als die Hauptquelle löslicher Quercetinglycoside; dies führte nach Hydrolyse während der Lagerung der Moste oder Weine zu Überschußkonzentrationen an freiem Quercetin. Die Flavonole wurden mittels HPLC (reversed phase) analysiert. Der verbreitete Einsatz von Erntemaschinen und die damit verbundene Verunreinigung des Preßgutes durch Blätter wird als die primäre Ursache dieser neuartigen Weintrübung angesehen. Es wird ein enzymatisches Verfahren beschrieben, das die Früherkennung gefährdeter Moste und Weine erlaubt.

K e y w o r d s : wine disorder, analysis, phenol, grape harvest, technique, leaf.

Introduction

An unusual form of instability in white wines has been of sporadic occurrence in the Australian wine industry since the 1977 vintage, when substantial yellow or yellow-green deposits were observed by winemakers during bulk storage of white juices and wines. In a few instances, hazing from finely particulate yellow matter occurred in wines which had previously undergone normal treatments against protein instability and tartrate precipitation, followed by final filtration before bottling.

The deposits were found to consist principally of the flavonol quercetin, a minor phenolic constituent of table wines (RIBÉREAU-GAYON 1964; SINGLETON and ESAU 1969). The emergence of this previously unrecognised phenolic instability suggested that it could be associated with some recent change in viticultural or winemaking practice. In this paper we report our interpretations of the likely origins of flavonol haze and deposition in commercial white juices and wines.

Materials and methods

I d e n t i f i c a t i o n o f f l a v o n o l s

Reference compounds were quercetin, kaempferol and rutin (Fluka). Standard analytical procedures (cellulose and polyamide TLC) were used for examination of hydrolysis products after either acid or enzyme treatment, and for spectral characterisation (MABRY *et al.* 1970; HARBORNE *et al.* 1975). Yellow sediments from juice or wine were washed well with water before extraction of phenolic materials by solution in cold methanol. Wine samples showing haze (50 ml) were membrane filtered (0.45 µm), the residue washed with water and then dissolved from the membrane surface with a few

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ml methanol. Routine confirmation of the yellow matter as being mainly quercetin was by spectrophotometry and HPLC.

HPLC analysis

Phenolic extracts and potentially unstable juices or wines were analysed with a Waters Associates Liquid Chromatograph fitted with Model 6000A Delivery System and Model 440 Absorbance Detector. The column used was reversed-phase μ-Bondapak C_{18} (30 cm × 3.9 mm i. d.). Typical conditions for separation and estimation of flavonols were injection volume 20 or 50 μl (membrane filtered), mobile phase 5 % acetic acid in 35 % aq. methanol, detector 365 nm, full scale deflection 0.1 or 0.05 a. u., flow rate 2.0 ml/min, chart speed 0.5 cm/min. The column was flushed with methanol after each run and re-equilibrated with mobile phase.

Vine leaf extraction and analysis

Fresh leaves (about 40 g), randomly sampled from vines at grape harvest time, were extracted in a blender for 5 min with hot water (400 ml) containing potassium hydrogen tartrate (3 g) and sodium metabisulfite (0.5 g). The extract was chilled and filtered through Celite before making to standard volume (400 ml). Samples for analysis were refined by use of C_{18} Sep Pak Cartridges (Waters Associates). The extract (2.0 ml) was applied to C_{18} Sep Pak, the cartridge washed with water (40 ml) and phenolics then eluted with methanol (20 ml). For measurement of available quercetin content, a concentrate (5.0 ml) of the above was diluted with 4 N aq. HCl (5.0 ml) and the solution refluxed for 5 min on a steam bath before analysis by HPLC.

Enzymatic screening for flavonol instability

Membrane filtered wine (50 ml) was reduced to about 30 ml by rotary evaporator at 30—40 °C. After making to 50 ml with water and addition of Rohapect C (Röhm GmbH, 10 mg in 2 ml), the sample was held at 25 °C for 24 h, then at 5 ° overnight. Developed haze was examined for quercetin content by spectrophotometry and HPLC. Clarified juice was treated directly as above.

Results and discussion

Nature of the instability

The infrequency of reported observations of yellow deposits in commercial juices or wines, and the fact that samples were received only after such haze or deposition had occurred, hampered earlier interpretation of the instability. To our knowledge, the sole previous account of flavonol deposition from wines concerned that during conservation of young red wine in the 1968 Australian vintage when deposits were found to consist of quercetin, kaempferol and myricetin (ZIEMELIS and PICKERING 1969). The cause of that instability was not identified at that time, and it did prove to be an isolated instance as no similar instability in red wines has since been observed.

Where hazing has occurred in previously clarified white wines, the yellow microcrystalline precipitate has been found to consist almost entirely of quercetin, with traces of kaempferol. The same phenolic composition was found in methanolic extracts of yellow lees observed during conservation of bulk juices or wines. Special properties

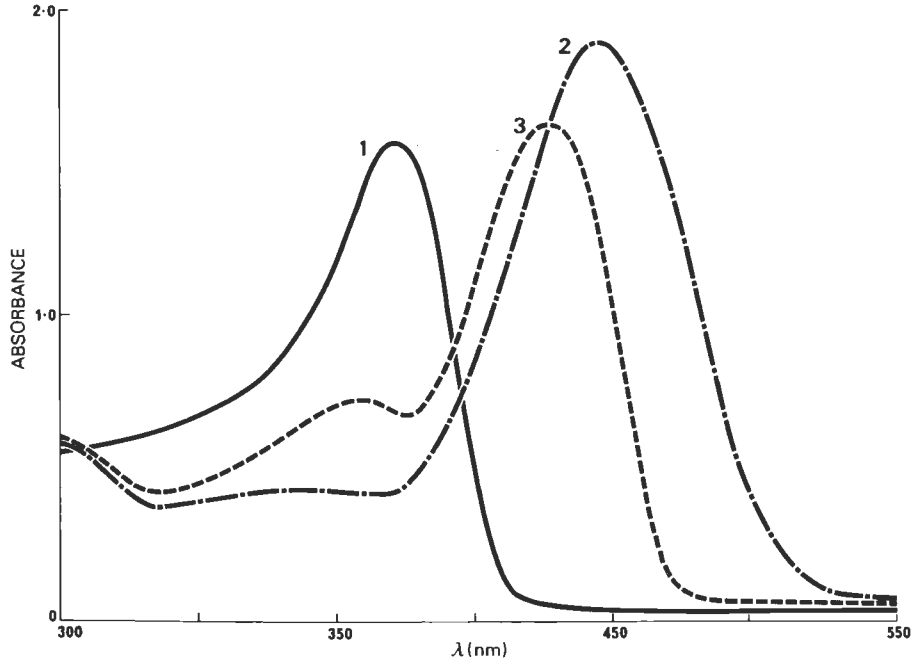

Fig. 1: Ultraviolet spectra of flavonol haze material in methanol solution (1), after addition of $AlCl_3$ (2) and after further addition of HCl (3).

UV-Spektren des Materials von Flavonoltrübungen in methanolischer Lösung (1), nach Zusatz von $AlCl_3$ (2) und nach weiterem Zusatz von HCl (3).

of such extracts were typically λ_{max} 370 nm with bathochromic shift to about 450 nm after $AlCl_3$ addition and further shift to about 430 nm on addition of HCl (Fig. 1); the spectrum and spectral shifts were identical with those for pure quercetin, which was confirmed by TLC with authentic material.

The presence of quercetin, kaempferol and myricetin at trace levels in wines is due to extraction of the respective glycosides from grape skins and their subsequent hydrolysis to the free flavonols (RIBÉREAU-GAYON 1964), their low aqueous solubilities ensuring that they are minor phenolic constituents of wine. Quercetin has been reported to occur as the 3-glucoside and 3-glucuronide, and it has been noted that the glycosides are easily hydrolysed, accounting for their normal absence *per se* from red wines (RIBÉREAU-GAYON 1964). Since the glycosides are skin extractives, white wines made without skin contact do not contain flavonols (MASQUELIER and POINT 1954; RIBÉREAU-GAYON 1964), and this has also been our observation with experimental white wines made from free-run juices.

Source of the instability

HPLC analysis of an unstable commercial wine from Sultana grapes (Thompson Seedless) showed a major quercetin glycoside fraction (1) at much earlier elution volume than quercetin (Fig. 2 a). Progressive acid hydrolysis of this fraction gave products

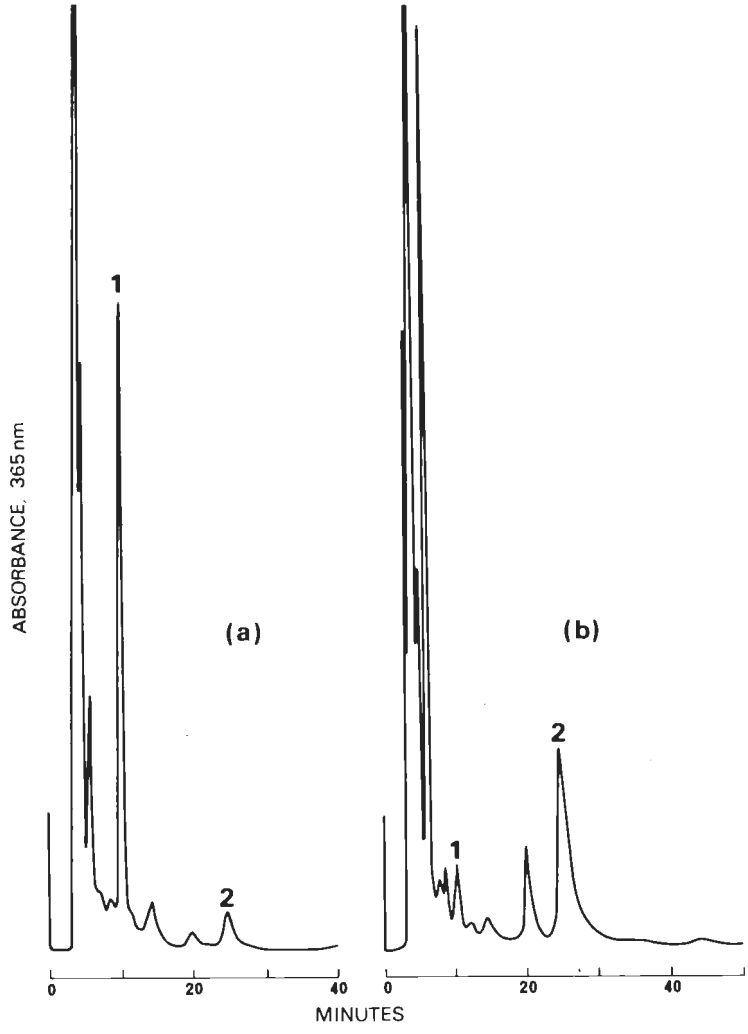

Fig. 2: HPLC analyses of a white wine susceptible to flavonol haze (a), and of the same wine after enzyme treatment (b). Significant components are quercetin glycosides (1) and quercetin (2).

HPLC-Analysen eines zu Flavonoltrübung neigenden Weißweines (a) und desselben Weines nach Enzymbehandlung (b). Quercetinglycoside (1) und Quercetin (2) sind signifikant vorhanden.

identified by HPLC and TLC as quercetin 3-glucoside and rhamnose, then quercetin, glucose and rhamnose. Enzymic treatment of the unstable wine with Rohapect C, and removal of precipitated quercetin by filtration, resulted in virtual disappearance of this major fraction, and an increase in the level of residual quercetin (Fig. 2 b). Similar observations were made from HPLC analyses of Sultana juices susceptible to flavonol haze.

This unstable glycoside fraction was not rigorously examined, except as a source of quercetin. It appeared to consist largely of quercetin 3-rutinoside (rutin) which was

found to co-elute with fraction 1 (Fig. 2 a). Our analyses of stable wines and juices have generally shown the latter early-eluting glycosides to be absent or present at very low levels, lower than that of free quercetin, which has a solubility limit of only 3—4 mg/l in wine. In contrast, the diglycoside derivative rutin has much higher aqueous solubility, about 120 mg/l, which is equivalent to about 60 mg/l available quercetin.

To determine the source of the instability, all parts of the vine were examined for the presence of rutin or other soluble quercetin derivatives. Though absent from extracts of grape skins and stalks, such glycosides, corresponding to fraction 1 (Fig. 2 a), were found to occur at surprisingly high levels in vine leaves. Assays of available quercetin content, made by HPLC analyses of the hydrolysed extracts, showed that vine leaves can contribute several mg quercetin per g fresh weight (Table).

Amounts of available quercetin present as glycosides in leaves of several grape varieties
Gehalte des verfügbaren Quercetins (Glycosidform) in den Blättern verschiedener Rebsorten

	mg/leaf	mg/g leaf
Sultana	14.5	5.1
Muscat Gordo Blanco	18.5	6.4
Riesling	10.4	3.6
Shiraz	13.1	4.3

Though rutin, the 3-rutinoside derivative of quercetin, has been found in the leaves of many plant species (HARBORNE *et al.* 1975), the principal flavonol glycosides occurring in leaves of *Vitis vinifera* have been reported to be the 3-glucuronides (EGGER *et al.* 1976), along with 3-rutinosides and 3-glucosides of quercetin, kaempferol and myricetin. The predominance of this troublesome fraction in the flavonol composition of Sultana leaf extract, and the effect of partial acid hydrolysis of the whole extract are shown in Fig. 3. Kaempferol was noted in the hydrolysate in addition to quercetin, and acid hydrolysis of fraction 1 also showed traces of kaempferol. It has been reported that gradient elution systems, not used in this investigation, are necessary for adequate resolution of flavonol diglucosides (MCMURROUGH 1981). On the other hand, it has been advantageous here to use an isocratic HPLC system in which the major flavonol leaf extractives are confined to one fraction (1, Figs. 2, 3).

Thus the analytical system is considered to give indications of prior leaf contamination of the grapes and of consequent susceptibility to flavonol haze. However, such instability in juices or wines can be more readily detected in practical terms by use of the enzyme Rohapect C, which induces flavonol haze within 24 h by hydrolysis of the more soluble precursor glycosides. The effectiveness of this procedure was verified in laboratory trials of samples to which vine leaf extracts had been added. Though Rohapect C is a 'pectinase' which has been promoted for use in the citrus industry, the product apparently has the required glycosidase activity for release of free quercetin from vine leaf extracts, activity not found in many other commercial enzyme preparations tested.

Finally, it seems to be worth noting that in studies of any actual sensory effects arising from leaf contamination of the crush, the volatile extractives, particularly cer-

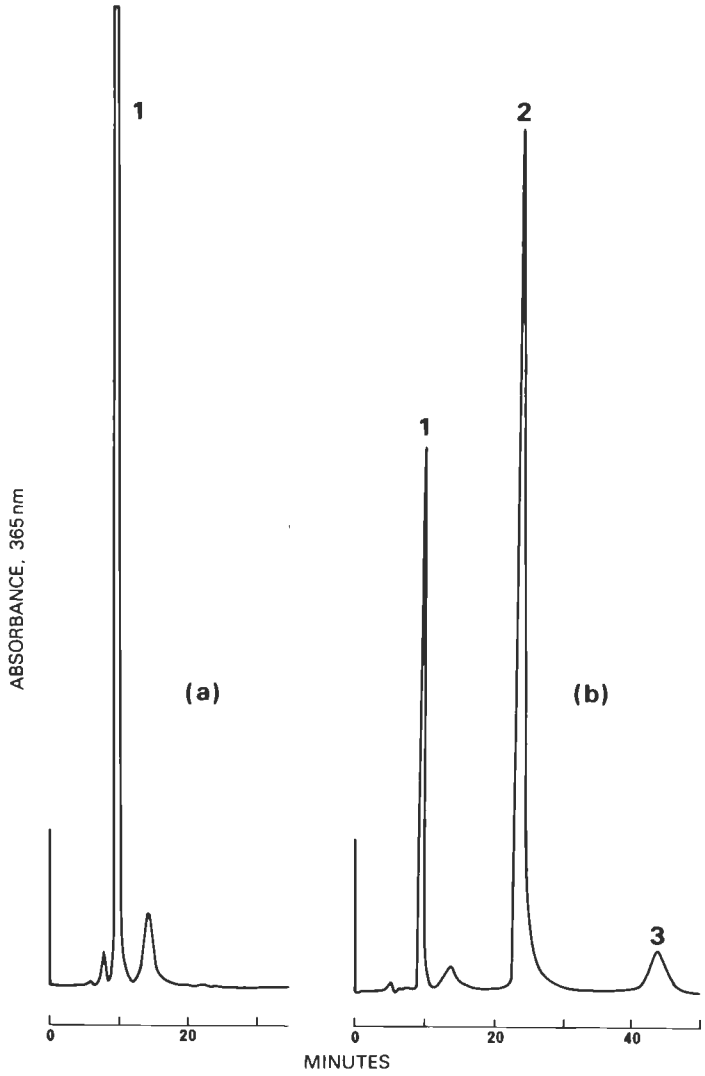

Fig. 3: HPLC analyses of aqueous vine leaf extract (a) and of the same extract after partial acid hydrolysis (b). Significant components are quercetin glycosides (1), quercetin (2) and kaempferol (3).

HPLC-Analysen eines wäßrigen Extraktes aus Rebblättern (a) und desselben Extraktes nach partieller saurer Hydrolyse (b). Quercetinglycoside (1), Quercetin (2) und Kaempferol (3) sind signifikant vorhanden.

tain C_6 components, have been given attention (JOSLIN and OUGH 1978, WILDENRADT *et al.* 1975). Though machine harvesting has been reported not to incorporate a sufficient level of leaf extractives to influence wine quality (JOSLIN and OUGH 1978; NOBLE *et al.* 1975), these C_6-volatiles are apparently responsible for the 'grassy' flavour from exces-

sive leaf contact in fermentations from Chenin Blanc (WILDENRADT *et al.* 1975). As flavonol extraction is also a feature of leaf contact, it seems possible that abnormally high residual levels of quercetin or its glycosides could be responsible for the bitter characteristics sometimes evident in such wines.

Conclusions

The evidence suggests that flavonol haze in white wines is a direct result of excessive leaf content in the grape crush. The appearance of this phenomenon in reeent years can be attributed both to the widespread use of machine harvesting of grapes in Australia and to an accompanying trend towards earlier finishing of white wines. Thus some leaf contamination is inevitable with machine harvesting, allowing the possibility of relatively high levels of unstable quercetin glycosides in the new wine. It has been evident that hydrolysis to free quercetin would then eventually occur during vinification and storage, but insufficient time allowed for its precipitation can lead to the occurrence of quercetin haze in the finished wine. Though Sultana was the variety usually involved in such flavonol instability, the occurrence of similar flavonol content in leaves of other varieties suggests the absence of any varietal factor affecting incidence of the problem.

Summary

The formation of a yellow haze in commercial white wines, and of yellow sediments from both juices and wines, was due to precipitation of quercetin. The vine leaves were found to be the major source of soluble quercetin glycosides, leading to excessive levels of free quercetin after hydrolysis during juice or wine storage. Flavonols were analysed by reversed-phase HPLC. Widespread use of machine harvesting, with higher leaf contamination of the grape crush, is considered to be the primary cause of this new form of wine instability. An enzymatic procedure for detecting susceptibility to such deposition in juices and wines is described.

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Eingegangen am 10. 9. 1984

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