Vitis **29**, 71—78 (1990)

Istituto Sperimentale per l'Enologia, Asti, Italia

XAD2 resin in separation of phenolics

by

R. Di Stefano and S. Guidoni

La résine XAD2 dans la séparation des composés phénoliques

Résumé: On a séparé les acides hydroxy cinnamyl tartriques, les acides phénoliques libres, les catéchines, les proanthocyanidines et les pigments libres et polymérisés du vin par adsorption sur la résine XAD2 suivie par l'élution avec des solvents organiques inertes. Les fractions ainsi obtenues sont analysées par CLHP.

Key words: phenol, polyphenol, phenolic acid, catechin, proanthocyanidin, polymeric pigment, anthocyanin, analysis, resin.

Introduction

Separation of phenolics from other compounds of wine is usually obtained by
(1) Solvent extraction (Glorie 1978; Rossi and Pompei 1987);
(2) 'demixtion' (Glorie 1978);
(3) polymer adsorption and subsequent solvent elution.
The last one yields the best results.

A great number of polymers have been used as adsorbents. The most common ones are: Sephadex G25 (Somers 1968), Biogel P4 (Castino and Di Stefano 1976), Sephadex LH20 (Moutounet 1981; Di Stefano and Ciolfi 1983), polyvinylpirrolidone (Glorie 1978), polyamide (Bourzeix *et al.* 1986), XAD2 adsorbent resin (Di Stefano and Guidoni 1989).

In addition, the C_{18} reversed phase seems very useful for sample preparation (Oszmianski *et al.* 1988; Di Stefano and Guidoni 1989).

Nevertheless, the problem of separating different classes of phenolics in order to obtain more efficiency in liquid chromatography (HPLC), in TLC or in other chromatographic techniques has not yet been solved satisfactorily.

In the present work, a new method is proposed for separation of phenolic acids, catechins and proanthocyanidins.

For this purpose, the XAD2 adsorbent resin has been used. Unlike the other adsorbent materials mentioned above, the XAD2 resin allows by means of inert solvents, in short time and at very low cost, separation of phenolics even in great quantities. Consequently, the method is useful for sample preparation in chromatographic analysis as well as for concentration of phenolic compounds contained only in traces.

Materials and methods

Solvents

Methanol, dichloromethane, diethyl ether, ethyl acetate, formic acid, all from Merck. Before use the diethyl ether was distilled on $FeSO_4$.

Resin

XAD2 0.1—0.2 mm, Serva.

Resin treatment and column preparation

5 g of XAD2 resin is suspended in methanol in a 50 ml beaker. After it has been allowed to stand, the liquid phase and the smallest suspended particles are eliminated and a column is packed. To eliminate the impurities, the column is eluted first with 50 ml of dichloromethane and then with 50 ml of diethyl ether, 50 ml of ethyl acetate, 50 ml of methanol and 50 ml of H_2O. Before any separation, it is necessary to activate the column with 25 ml of methanol and 50 ml of H_2O.

Separation of phenolics

10 ml of red wine diluted 1 : 1 with H_2O are eluted through a column of XAD2 resin prepared as above. Then the impurities are eliminated by washing with 40 ml of H_2O. The wine not adsorbed by the resin and the volume of H_2O used for washing are collected. They form the first fraction. The second fraction is eluted with 50 ml of CH_2Cl_2, the third fraction with 50 ml of $(C_2H_5)_2O$, the fourth fraction with 50 ml of $CH_3COOC_2H_5$, and the fifth fraction with 50 ml of CH_3OH.

All the fractions are collected in 100 ml distillation ballons. As the second fraction contains H_2O, it is useful to collect it in a 100 ml separator funnel.

Before HPLC analysis, all the fractions are evaporated by vacuum and the residue is resolved in 2—5 ml of solvent B.

HPLC analysis

Liquid chromatograph Perkin Elmer series 3B
Column LiCrospher 100 RP-18, 5 μm, Merck
Flow: 1 ml/min
λ = 260, 280, 300, 320 nm
Solvent A: HCOOH 0.5 % in H_2O
Solvent B: HCOOH 0.5 %, CH_3OH 50 % in H_2O
Linear gradient from 75 % A to 5 % A in 40 min, then isocratic at 5 % A for 10 min.

Before injection, the sample and solvents A and B are membrane-filtered, 0.45 μm; freshly distilled H_2O is used.

Results and discussion

For evaluation of the method, the standard compounds (free phenolic acids, catechin, epicatechin) and a series of wines were separated with XAD2 resin and HPLC-analyzed.

The chromatographic peaks of wine having other retention times than the standards were, if possible, tentatively identified by means of their UV spectra. The spectra were registered with the stop flow method. Fig. 1 presents the schedule of XAD2 separation and HPLC analysis. In particular are eluted:

(1) In the first fraction (H_2O): 100 % of gallic acid, 47 % of protocatechuic acid, 49 % of gentisic acid, the hydroxy cinnamoyl tartaric acids;
(2) in the second fraction (CH_2Cl_2): p-hydroxybenzoic, vanillic, syringic acids, 70 % of p-coumaric acid, 95 % of ferulic acid, 14 % of salicylic acid, tyrosol, tryptophol;
(3) in the third fraction ($(C_2H_5)_2O$): 53 % of protocatechuic, 51 % of gentisic, 86 % of

salicylic, 30 % of p-coumaric, 5 % of ferulic and caffeic acids, catechin and epicatechin;

(4) in the fourth fraction ($CH_3COOC_2H_5$): compounds having U.V. spectra similar to that of catechin and compounds with maximum in U.V. spectra at 270 nm, both tentatively attributed to proanthocyanidins;

(5) in the fifth fraction (CH_3OH): monomeric and polymeric anthocyanins, polimeric proanthocyanidins and polimeric oxidated proanthocyanidins.

Every fraction contains a lot of unidentified compounds. The study and the identification of eluted compounds revealed to be very difficult because our chromatographic system permits only a single-wavelength analysis. The diode array U.V.-VIS spectrophotometer would be more useful.

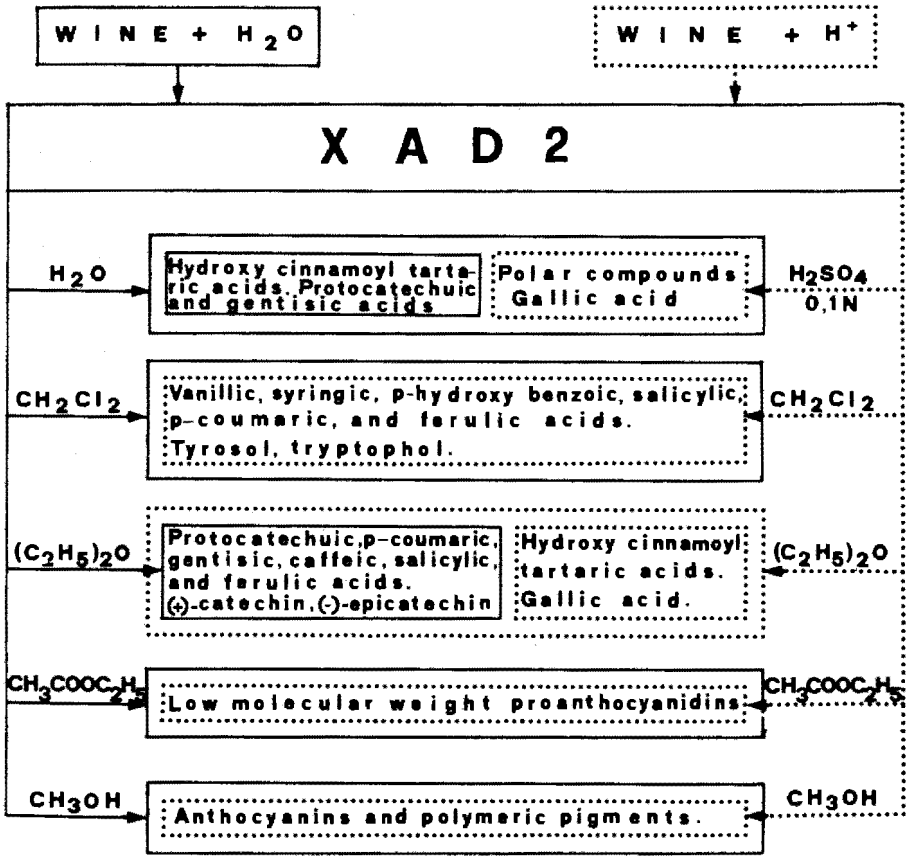

Fig. 1: Neutral and acidic XAD2 separation of phenolic compounds.

Séparation des composés phénoliques par XAD2 en milieu neutre ou acide.

From Fig. 2 the dramatic differences in elution profiles at two different wavelengths are to be seen. In the ethyl ether fraction, for example, the catechins are detected at 280 nm, the hydroxy cinnamoyl tartaric acids at 300 nm.

Fig. 3 shows HPLC chromatograms of four fractions from XAD2. The above system allows to separate the hydroxy cinnamoyl tartaric acids from free phenolic acids. Thus

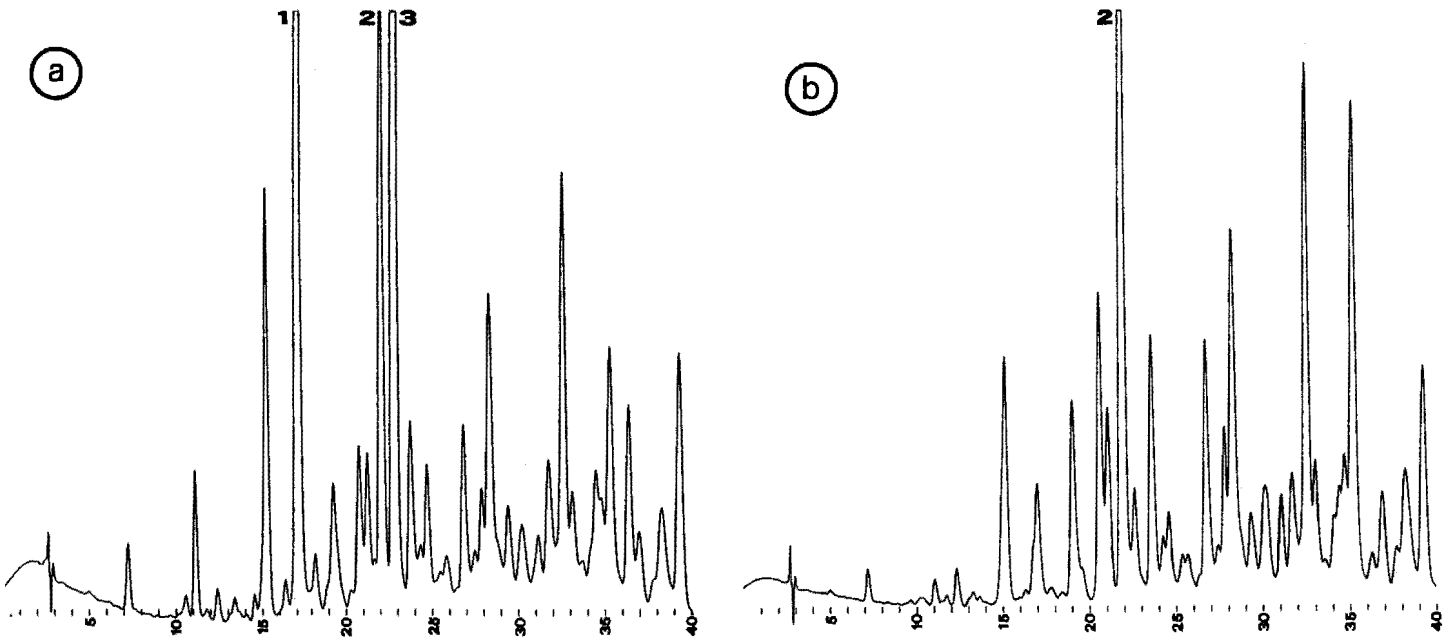

Fig. 2: HPLC analysis of the $(C_2H_5)_2O$ fraction eluted from XAD2 column. a) λ = 280 nm, b) λ = 300 nm. — 1 = (+)–catechin, 2 = caffeic acid, 3 = (–)– epicatechin.

Analyse CLHP de la fraction rincée par l'éther éthylique de la colonne XAD2. a) λ = 280 nm, b) λ = 300 nm. — 1 – (+)– catéchine, 2 = acide caféique, 3 = (–)– epicatéchine.

superposition of peaks due to the great concentration of these compounds is avoided. In these conditions the fraction of free phenolic acids can be concentrated to make possible detection of compounds even in traces.

The elution in the H_2O fraction of gallic acid and some of protocatechuic and gentisic acids is the weak spot of the method. In order to quantitatively adsorb these two latter acids in XAD2 resin it is necessary to acidify the wine. In this case, 5 ml of wine and 5 ml of H_2SO_4 1 N are adsorbed on XAD2. After washing with 10 ml of H_2SO_4 0.1 N, the phenolic compounds are eluted with the above organic solvents. In the first fraction there is yet some gallic acid but protocatechuic acid, gentisic acid and hydroxy cinnamoyl tartaric acids are retained. All these compounds are recovered in the ethyl ether fraction.

The remarkable presence of hydroxy cinnamoyl tartaric acids can give problems in HPLC separation of free phenolic acids. So the XAD2 separation of not acidified wine is more useful and, for quantitative analysis, preventively testing the column with a pure solution of the above free acids.

The inconvenience of p-coumaric acid and ferulic acid, both in CH_2Cl_2 and in $(C_2H_5)_2O$ fractions, can be avoided eluting the XAD2 column with $(C_2H_5)_2O$ after H_2O washing and making the HPLC analysis with a column more efficient or varying the analytical wavelength.

The method allows the analysis of catechins in the ethyl ether fraction by detection at 280 nm. Studies are carried out to identify the proanthocyanidins in the ethyl acetate fraction. Also the separation of free and polymeric anthocyanins present in the CH_3OH fraction is a matter of subsequent studies (see DI STEFANO and CRAVERO 1990).

Conclusion

The use of the XAD2 resin separation method seems to give a good solution of separation problems with monomeric phenolics as well as low molecular weight proanthocyanidins.

The inert solvents utilized for this separation assure a good stability of these compounds.

The method allows the separation of hydroxy cinnamoyl tartaric acids of the phenolic acids containing $-OCH_3$ groups, of the polyhydroxylated phenolic acids, of the catechins, of the proanthocyanidins and of the compounds responsible for colour of red wine.

After separation, the above fractions can be utilized for characteristic phenolic reactions as well as for HPLC quantitative analysis.

In addition, characteristic profiles of different wines can be obtained by means of XAD2 separation and HPLC analysis. These profiles allow conclusions on both cultivar and technology used for preparation of a wine.

The diode array detector in HPLC analysis can increase the speed and efficiency of the method.

Summary

Hydroxy cinnamoyl tartaric acids, free phenolic acids, catechins, proanthocyanidins and both free and polymeric pigments of wines were separated by adsorption on XAD2 resin and by subsequent elution with organic inert solvents. The fractions obtained in this way were analyzed by HPLC.

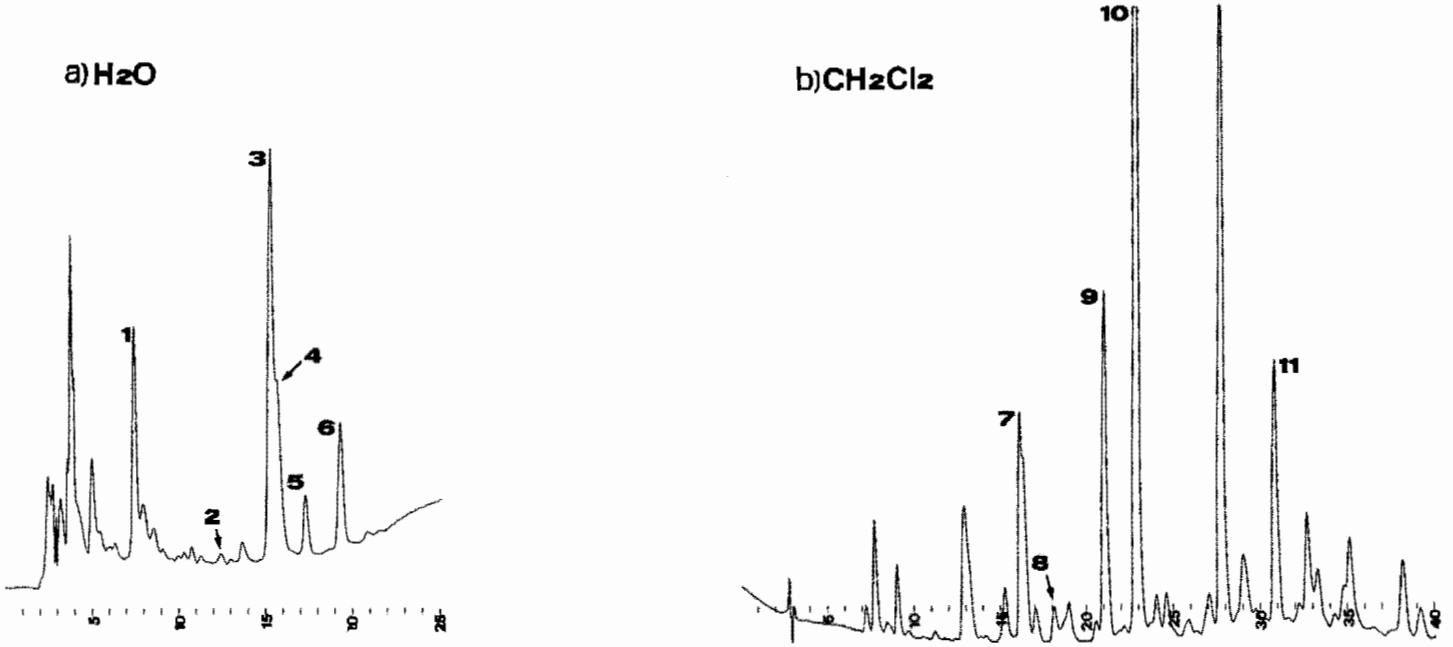

Fig. 3: XAD2 separation of phenolic compounds of a red wine. HPLC analysis. λ = 280 nm. — 1 = gallic acid, 2 = protocatechuic acid, 3 = caffeoyl tartaric acid, 4 = 2-S-glutathionyl caffeoyl tartaric acid, 5 = p-coumaryl tartaric acid glucoside, 6 = p-coumaryl tartaric acid, 7 = tyrosol, 8 = p-hydroxy-benzoic acid, 9 = vanillic acid, 10 = syringic acid, 11 = tryptophol, 12 = (+)–catechin, 13 = caffeic acid, 14 = (–)–epicatechin, 15 = proanthocyanidins.

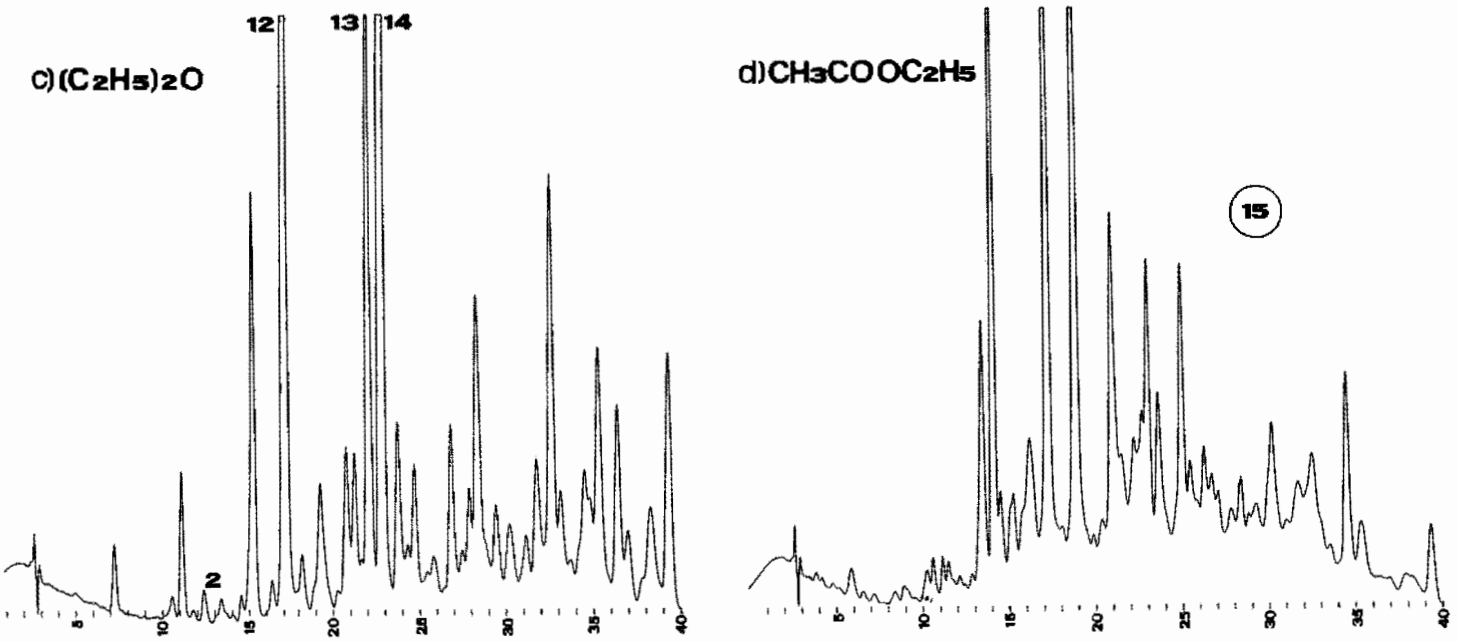

Fig. 3: Séparation des composés phénoliques d'un vin rouge par XAD2. Analyse CLHP. λ = 280 nm. — 1 = acide gallique, 2 = acide protocatéchique, 3 = acide caféyl tartique, 4 = acide 2-S-glutathionyl caféyl tartrique, 5 = acide p-coumaryl tartrique glucoside, 6 = acide p-coumaryl tartrique, 7 = tyrosol, 8 = acide p-hydroxy-benzoïque, 9 = acide vanillique, 10 = acide syringique, 11 = tryptophol, 12 = (+)–catéchine, 13 = acide caféique, 14 = –(–)epi-catéchine, 15 = proanthocyanidines.

Literature

BOURZEIX, M.; WEYLAND, D.; HEREDIA, N.; 1986: Etude des catéchines et des procyanidols de la grappe de raisin, du vin et d'autres dérivés de la vigne. Bull. O.I.V. **59**, 1171—1254.

CASTINO, M.; DI STEFANO, R.; 1976: Frazionamento degli acidi fenolici dei vini bianchi per gel filtrazione. Riv. Viticolt. Enol. **29**, 290—305.

DI STEFANO, R.; CIOLFI, G.; 1983: Formazione di antociani polimeri in presenza di flavani ed evoluzione degli antociani monomeri durante la fermentazione. Riv. Viticolt. Enol. **31**, 325—338.

— — ; GUIDONI, S.; 1989: La determinazione dei polifenoli totali nei mosti e nei vini. Vignevini **16** (1—2), 47—52.

— — ; CRAVERO, M. C.; 1990: Frazionamento dei composti polifenolici dei vini rossi. L'Enotecnico **26** (3), 99—106.

GLORIE, Y.; 1978: Recherches sur la Matière Colorante des Vins Rouges. Thèse, Univ. de Bordeaux II.

MOUTOUNET, M.; 1981: Dosages des polyphenols des moûts de raisin. Connaiss. Vigne Vin **15**, 287—301.

OSZMIANSKI, J.; RAMOS, T.; BOURZEIX, M.; 1988: Fractionation of phenolic compounds in red wine. Amer. J. Enol. Viticult. **39**, 259—262.

ROSSI, M.; POMPEI, C.; 1987: Ottimizzazione della separazione per HPLC di acidi fenolici e catechine applicazione ai mosti d'uva. Riv. Viticolt. Enol. **40**, 24—38.

SOMERS, T. C.; 1968: Pigment profiles of grapes and wine. Vitis **7**, 303—320.

Eingegangen am 13. 6. 1989

R. DI STEFANO
Istituto Sperimentale
per l'Enologia
Via Pietro Micca 35
14100 Asti
Italy

S. GUIDONI
Istituto Coltivazioni Arboree
Università di Torino
Via Giuria 15
10126 Torino
Italy