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Degradation of oligomeric procyanidins and anthocyanins in a Tinta Roriz red wine during maturation

by

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S u m m a r y : A young red Tinta Roriz wine was stored for three months at different temperatures (12, 22, 32, 42 °C) under anaerobic conditions and after adjusting the SO_2 to 0, 50, 100 mg/l. Changes in wine phenolic composition, especially the procyanidins and anthocyanins were measured using HPLC reverse phase. Dimeric (B1, B2, B3, B4), galloylated dimeric (B1-3-O-gallate, B2-3-O-gallate, B2-3'-O-gallate) and trimeric procyanidins (C1, T2) were quantified during the maturation of the red wine, and their losses were found to be logarithmic with time. Temperature exerts a marked influence on the progressive degradation of procyanidins, while the presence of SO_2 slows down the degradation. Comparing their activation energies the dimer procyanidins B1, B2 and B3 appear to be more stable to degradation while trimer T2 and B2-3-O-gallate are most reactive. Concerning the anthocyanins, the acylated monoglucosides degrade faster than the other monoglucosides and the p-coumaric acid acylated pigments disappear faster than the acetic acid acylated pigments.

Dégradation des procyanidines oligomères et des anthocyanines d' un vin rouge de Tinta Roriz pendant la phase de maturation

R é s u m é : Un vin rouge préparé à partir du cépage Tinta Roriz (*Vitis vinifera*) a été conservé pendant 3 mois aux températures de 12, 22, 32, 42 °C sous azote et ayant ajusté le SO_2 à 0, 50, 100 mg/l. Les procyanidines, dimères (B1, B2, B3, B4) galloylées (B1-3-O-gallate, B2-3-O-gallate, B2-3'-O-gallate) et trimères (C1, T2) préalablement purifiées sur colonne de polyamide ont été quantifiées par HPLC phase-reverse pendant la conservation des vins. La dégradation des procyanidines suit un modèle cinétique de premier ordre comme cela déjà a été observé pour les 3 anthocyanines (malvidine-3-glucoside, acetylglucoside et p-coumarylglucoside). Les énergies d'activation ont été calculées pour chacque composé; les procyanidines B1, B2 et B3 semblent être les plus stables tandis que les T2 et B2-3-O-gallate sont les plus réactives. La température a une influence très importante sur la dégradation progressive des procyanidines et anthocyanines, tandis que la présence du SO_2 semble ralentir cette dégradation.

K e y w o r d s : procyanidins, anthocyanins, HPLC, degradation rate constant, activation energies, maturation, Tinta Roriz.

Introduction

The phenolic compounds of wines extracted during primary fermentation are of undoubted importance during maturation and aging. Progressive changes are inevitable because of the reactivities of these compounds, but the rate of phenolic interactions and degradations may be subject to many influences.

During the aging of a red wine a decrease in the concentration of anthocyanins and other phenolic compounds and an increase in coloured polymeric pigment (Somers 1971) were observed. It was proposed (Jurd 1965, 1967) that the anthocyanins were condensed directly with other phenolic compounds such as catechin or procyanidins. Haslam (1980) studied the role of the oligomeric procyanidins in the aging of red wine. He observed that at wine pH the familiar acid-catalysed equilibration of procyanidins was occurring. Reaction between anthocyanins, flavan-3-ols, procyanidins and probably additional compounds (hydrolytic degradation products of anthocyanins) were responsible for the precipitation of the oligomeric procyanidins from solution.

In wines the major proanthocyanidins are formed by condensation of units of (+) catechin and (-) epicatechin. The highest compounds cited are hexamers (Lee 1980) but only the structures of dimer, trimer procyanidins and their acylated derivatives (esters of gallic acid) have been elucidated (Weinges and Piretti 1971; Piretti *et al.* 1976; Czochanska *et al.* 1979; Lee 1979; Lunte *et al.* 1988; Lee *et al.* 1990; Oszmianski and Sapis 1989; Oszmianski and Lee 1990; Ricardo-da-Silva *et al.* 1990, 1991 b). Procyanidins contribute to the sensory properties of wine (Timberlake and Bridle 1976), and they have an important role for the oxidation reaction (Simpson 1982; Oszmianski *et al.* 1985; Cheynier *et al.* 1988; Lee and Jaworski 1988; Cheynier and Ricardo-da-Silva 1991). Recently they have received considerable attention owing to their pharmacological effects, in particular on arteriosclerosis and their oxygen free-radical scavenger ability (Laparra *et al.* 1978; Masquellier 1988; Ricardo-da-Silva *et al.* 1991 c).

Studies concerning the procyanidins composition and the effect of technological winemaking process in red wine have been reported by many authors (Bourzeix *et al.* 1986;

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SALAGOITY-AUGUSTE and BERTRAND 1984; ETIEVANT *et al.* 1988; REVILLA *et al.* 1989; RICARDO-DA-SILVA *et al.* 1991 a, 1992 a, b, 1993). However few studies concerning the evolution of oligomeric procyanidins and the effect of technological treatment during the wine maturation phase have been reported.

The aim of the present work was to study the effect of the temperature and SO_2 content on the degradation of oligomeric procyanidins (dimers, trimers and galloylated dimers) and anthocyanins in a Portuguese red wine during maturation.

Materials and methods

1. Grapes - Wines: Tinta Roriz is a red *Vitis vinifera* grapevine variety very typical in Portugal. The grapes were harvested the last week of September 1992 from the Douro valley (North of Portugal) at commercial maturity.

A lot of 100 kg of grapes were crushed, destemmed and fermented at 22 °C to dryness (<2 g/l residual sugar). The fermenting must was punched down twice daily and after seven days of pomace contact, we pressed it. Both press and free run wines were assembled and after malolactic fermentation, the Tinta Roriz red wine was racked and filtered. Three lots of wines were prepared by adjusting the SO_2 to 0, 50 and 100 mg/l. Each wine was then divided into 4 flasks under nitrogen and hermetically sealed to minimise oxygen contact, and stored for 3 months at 12, 22, 32, 42 °C in darkness.

2. Sample purification: Each sample of wine was purified on a polyamide chromatographic column as described by RICARDO-DA-SILVA (1990). Three succesive elutions with neutral water (pH 7.0), acetonitrile/water (30:70 v/v) and acetone/water (75:25 v/v), allowed us to eliminate HPLC interfering compounds such as phenolic acids, to separate catechins from procyanidins and to obtain a procyanidin fraction. This fraction was evaporated to dryness and redisolved in a solution of methanol/water (50:50 v/v) for subsequent chromatographic analysis.

3. Procyanidin and anthocyanin standards: Procyanidins B1, B2, B3, B4, B1-3-O-gallate, B2-3-O-gallate, B2-3'-O-gallate trimer 2 (epicatechin 4β→8 epicatechin 4β→8 catechin) and trimer C1 (epicatechin 4β→8 epicatechin 4β→8 epicatechin) were isolated and identified following the procedure described by RICARDO-DA-SILVA *et al.* (1991 b). Each standard was injected in duplicate in a HPLC system and their retention time was used to identify the procyanidins in the wine. Procyanidins B2 and B2-3'-O-gallate were used as external standards and the response factors for each compound were calculated.

Malvidin-3-glucoside chloride (Extrasynthese, Lyon, France) was used as an external standard to quantify the monomeric anthocyanins.

4. HPLC analysis

Procyanidins: A Merck model L-6200A pump equipped with a Rheodyne manual injector model 7125-A fitted with a 50 ml loop was used. The column was a reversed phase Superspher 100, C18 (Merck, Germany), 5 mm packing (4.6 mm id x 250 mm) protected with a guard column of the same material. Detection was made at 280 nm with a Konic detector coupled to a Konichrom data treatment system.

The solvents were: A acetic acid/bidistilled water (10:90 v/v) and B, bidistilled water. A linear gradient was run from 10 vol A + 90 vol B to 70 vol A + 30 vol B during 45 min followed by another one from 70 vol A + 30 vol B to 90 vol A + 10 vol B for 82 min and then to pure A during 10 min. The flow rate was 1 ml/min and the injection volume was 30 ml. All the samples were filtered through 0.45 mm membrane filters.

Anthocyanins: The HPLC elution conditions and other details were described in a previous paper (DALLAS and LAUREANO 1994).

Results and discussion

Procyanidins: A HPLC chromatogram shown in Fig. 1 presents the separation of the procyanidin fraction performed by the method described above.

Referring to the procyanidin composition of Tinta Roriz wine (Fig. 2) procyanidin B1 was the major component (60 mg/l) found in our study followed by B2 (30 mg/l) while procyanidins B3 and B4 were present in lower concentration (13, 11 mg/l respectively). Besides the previously reported dimeric procyanidins some trimeric and galloylated dimeric procyanidins were found in significant concentrations. In fact trimeric procyanidins, especially trimer 2 was present in important amounts (30 mg/l) compared to the dimeric procyanidins. The galloylated dimeric

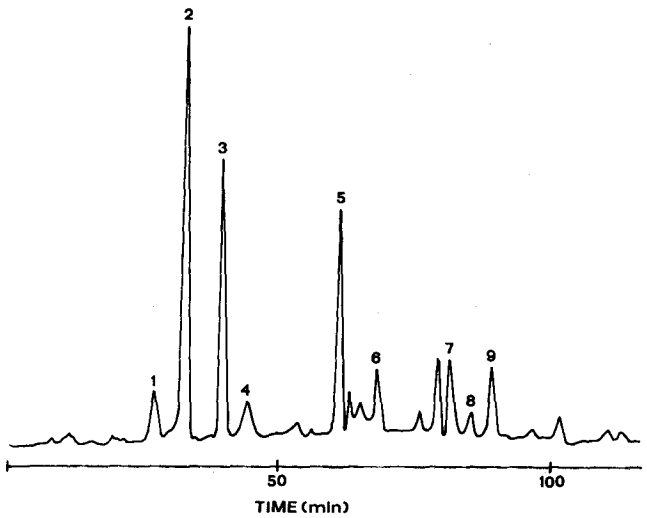

Fig. 1: HPLC chromatogram recorded at 280 nm of a procyanidin fraction from a Tinta Roriz red wine. 1) Procyanidin B3; 2) Procyanidin B1; 3) Procyanidin trimer T2; 4) Procyanidin B4; 5) Procyanidin B 2; 6) Procyanidin B1-3-O-gallate; 9) Procyanidin trimer C1.

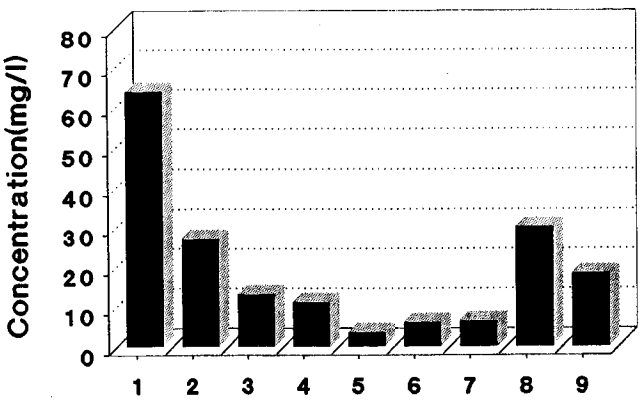

Fig. 2: Procyanidin composition of Tinta Roriz red wine at 22 °C after 4 d: 1) B1; 2) B2; 3) B3; 4) B4; 5) B1-3-O-gallate; 6) B2-3'-O-gallate; 8) T2; 9) C1.

procyanidins B1-3-O-gallate, B2-3-O-gallate and B2-3'-O-gallate were present in lower concentration than the non-galloylated ones. Morever B1-3-O-gallate and B2-3'-O-gallate were degradated faster than the B2-3-O-gallate and it was not possible to study their evolution during maturation.

To determine the rate of the degradative reaction of the procyanidins during maturation a regression analysis has been carried out. A plot of the log. nat. of concentration of the remaining procyanidins against time, produced regression lines with good linearity. Thus, the degradation of the oligomers procyanidins at each treatment temperature appears to be by first order kinetic reaction. The slopes of the above mentioned regression lines (k-values, s^{-1}) the coefficients of determination (R^2) and the probability of the proposed model are listed in Tabs. 1 and 2 for all the procyanidins.

The effect of SO_2 on the rate constants was tested at each temperature. The results obtained show that at both concentration (50 and 100 mg/l of SO_2) the procyanidins degradative rates exhibited for these wines were lower than those calculated for the control wine.

Comparing the results of the degradative rate in order to determine the influence of the storage temperature, we observed that procyanidins decreased 5 or 10 times faster with the higher temperature (32 and 42 °C). The activation energies were calculated by plotting the log. nat. of the first order kinetic constants versus temperature. The results are presented in Tab. 3 and a multiple range test using LSD method at 5 % was applied. Significant differences were observed and different homogeneous groups were formed in each wine. The results for the control wine showed that, the activation energies of the procyanidins decreased in the following order: B1»B3>B4>C1»B2>T2>B2-3-O-gl, while for the wine made with 50 mg/l of SO_2 the order was: B1>B2>B3>C1>B4>T2>B2-3-O-gl. Finally, for the wine stored with the higher level of SO_2 (100 mg/l) the activation energies decreased in the following order: B1>B2>B3>C1»B4>T2>B2-3-O-gl.

As can be seen, the dimer procyanidins B1, B2, B3 present the highest values of activation energies (except B2 on controled wine). This indicates that the three procyanidins were the most stable compounds compared to the other procyanidins during maturation. This could be not only to their small reactivity, but it could also occur that the other procyanidins, especially C1, T2, B1-3-O-gl, B2-3-O-gl and B2-3'-O-gl were slowly hydrolysed to B1 and B2 at the pH of wine. TIMBERLAKE and BRIDLE (1976) observed that trimer C1 had been transformed into epi-

Table 1

Reaction rates of the disappearance of procyanidins in a Tinta Roriz wine at 12 °C and 22 °C expressed as k-values = $\Delta \ln C/\Delta T$

Procyanidins	SO_2 (mg/l)	Temperature (°C)	k (s^{-1})	R^2	Probability of the model	Temperature (°C)	k (s^{-1})	R^2	Probability of the model
	0		$5.4.10^{-8}$	90	P<0.04		$6.6.10^{-8}$	80	P<0.07
B1	50	12	$0.7.10^{-8}$	86	0.06	22	$3.7.10^{-8}$	95	0.02
	100		$0.5.10^{-8}$	90	0.006		$4.9.10^{-8}$	92	0.01
	0		11.10^{-8}	95	0.02		15.10^{-8}	95	0.03
B2	50	12	$1.8.10^{-8}$	90	0.004	22	$9.0.10^{-8}$	97	0.01
	100		$1.7.10^{-8}$	87	0.06		$9,1.10^{-8}$	94	0.006
	0		$8.4.10^{-8}$	97	0.01		$11,4.10^{-8}$	79	0.06
B3	50	12	$4.0.10^{-8}$	92	0.05	22	$6.6.10^{-8}$	92	0.04
	100		$1.8.10^{-8}$	90	0.01		$9.7.10^{-8}$	94	0.03
	0		27.10^{-8}	86	0.05		$23,3.10^{-8}$	78	0.05
B4	50	12	$14.2.10^{-8}$	81	0.06	22	$13.6.10^{-8}$	98	0.005
	100		$8.3.10^{-8}$	92	0.04		$15,3.10^{-8}$	94	0.05
	0		17.10^{-8}	80	0.01		$21,4.10^{-8}$	86	0.06
B2-3-0-gallate	50	12	$5,9.10^{-8}$	89	0.04	22	$17.0.10^{-8}$	94	0.03
	100		$4.3.10^{-8}$	95	0.02		$11,2.10^{-8}$	75	0.05
	0		$6.2.10^{-8}$	90	0.001		$8.9.10^{-8}$	82	0.06
T2	50	12	$3.2.10^{-8}$	87	0.05	22	$3,3.10^{-8}$	98	0.05
	100		$3.2.10^{-8}$	89	0.05		$4,2.10^{-8}$	91	0.01
	0		$8.2.10^{-8}$	92	0.04		$8.4.10^{-8}$	92	0.04
C1	50	12	$2.9.10^{-8}$	92	0.04	22	$7.4.10^{-8}$	93	0.04
	100		$3.9.10^{-8}$	88	0.05		$6.8.10^{-8}$	78	0.05

T2=Procyanidin trimer 2; C1=Procyanidin trimer 1

Table 2

Reaction rates of the disappearance of procyanidins in a Tinta Roriz wine at 32 °C and 42 °C expressed as k-values = $\Delta \ln C/\Delta T$

Procyanidins	SO_2 (mg/l)	Temperature (°C)	k (s^{-1})	R^2	Probabilty of the model	Temperature (°C)	k (s^{-1})	R^2	Probability of the model
	0		$23.2.10^{-8}$	97	P<0.002		$33.5.10^{-8}$	91	P<0.003
B1	50	32	$21.2.10^{-8}$	94	0.001	42	$31.9.10^{-8}$	97	0.002
	100		$15.9.10^{-8}$	97	0.001		$38.9.10^{-8}$	96	0.001
	0		$29.3\ 10^{-8}$	97	0.002		$41.8.10^{-8}$	96	0.001
B2	50	32	$28.8.10^{-8}$	93	0.001	42	$37.0.10^{-8}$	98	0.001
	100		$17.6.10^{-8}$	96	0.001		$40.5.10^{-8}$	94	0.001
	0		$42.1.10^{-8}$	93	0.001		$54.6.10^{-8}$	96	0.02
B3	50	32	$31.3.10^{-8}$	98	0.001	42	$47.9.10^{-8}$	98	0.001
	100		$26.9.10^{-8}$	97	0.001		$50.8.10^{-8}$	98	0.001
	0		$94.1.10^{-8}$	78	0.05		$93.1.10^{-8}$	90	0.02
B4	50	32	$93.2.10^{-8}$	80	0.05	42	$90.5.10^{-8}$	95	0.02
	100		$39.4.10^{-8}$	97	0.001		$59.9.10^{-8}$	96	0.03
	0		$31.9.10^{-8}$	80	0.04		$33.5.10^{-8}$	94	0.01
B2-3-0-gallate	50	32	$15.1.10^{-8}$	83	0.01	42	$31.0.10^{-8}$	98	0.001
	100		$12.8.10^{-8}$	80	0.02		$20.9.10^{-8}$	78	0.03
	0		$11.5.10^{-8}$	96	0.004		$20.2.10^{-8}$	85	0.01
T2	50	32	$6.5.10^{-8}$	95	0.001	42	$17.0.10^{-8}$	89	0.005
	100		$4.6.10^{-8}$	96	0.001		$15.8.10^{-8}$	84	0.01
	0		$55.9.10^{-8}$	78	0.05		$37.9.10^{-8}$	81	0.02
C1	50	32	$24.8.10^{-8}$	88	0.05	42	$33.7.10^{-8}$	93	0.007
	100		$14.2.10^{-8}$	95	0.01		$31.1.10^{-8}$	97	0.002

T2 = Procyanidin trimer 2; C1 = Procyanidin trimer 1

Table 3

Activation energies (kcal/mol) for disappearance of procyanidins and anthocyanins in a Tinta Roriz red wine

SO_2 (mg/l)	Temperature (°C)	Procyanidins							Anthocyanins		
		B1	B2	B3	B4	B2-3-0-g	T2	C1	Mv-glc	Mv-ac	Mv-coum
	12										
	22										
0	32	12,0	7.7	12.4	8.9	4.3	4.4	7.8	18.3	16.3	15.7
	42										
	12										
	22										
50	32	23.9	18.5	16.0	14.2	8.6	10.0	15.4	18.2	17.3	16.5
	42										
	12										
	22										
100	32	25.2	20.4	19.8	12.3	8.7	8.6	12.5	21.2	18.4	16.7
	42										

B2-3-0-g = Procyanidin B2-3-0-gallate; T2 = procyanidin trimer 2; C1 = Procyanidin trimer C1
Mv-glc = Malvidin-3-glucoside; Mv-ac = Malvidin-3-acetylglucoside; Mv-coum = Malvidin-3-p-coumarylglucoside

catechin, B2 and other procyanidins after seven months of storage, in a control phenol solution and also in a mixture with anthocyanins.

However, the trimer C1 seems to be more stable than the trimer T2 in all the wines, while this one with the B2-3-O-gl were the most reactive procyanidins.

As can be seen, the different oligomeric procyanidins can be classified into various groups according to their activation energies. The high level of SO_2 added before storage, may contribute to the best stability of these compounds. To investigate more deeply the activity of the SO_2 on the thermal degradation of the different oligomeric procyanidins, model solutions with or without the presence of anthocyanins were carried out.

Anthocyanins: A typical HPLC chromatogram of the Tinta Roriz red wine recorded at 520 nm is shown in Fig. 3. Malvidin-3-glucoside, malvidin-3-acetylglucoside and malvidin-3-coumarylglucoside were the more important anthocyanins present in the wines. However small concentrations of unidentified anthocyanins which become more important as the wine ages are present in all the chromatograms. Data obtained by HPLC analysis for each experimental wine during the 3 months of storage showed a decrease in the concentration of individual anthocyanins. The losses of anthocyanins in all the wines were logarithmic with time as was observed by MARKAKIS (1982) and BAKKER *et al.* (1986). Tab. 4 summarizes the rate constants on a per second basis, obtained by plotting log. nat. of concentration of remaining anthocyanins against time.

Some differences were observed when the degradative rates for the control wine (0 mg/l of SO_2) were compared with the corresponding rates of the wines made with 50

Table 4

Reaction rates of the disappearance of anthocyanidins in a Tinta Roriz wine expressed as k-values = Δ ln C/ΔT

SO_2 (mg/l)	Temperature (° C)	Malv-3-gluc k (s^{-1})	Malv-3-gluc R^2	Malv-3-acet k (s^{-1})	Malv-3-acet R^2	Malv-3-coum k (s^{-1})	Malv-3-coum R^2
0	12	$4.3\ 10^{-8}$	96	$6.1\ 10^{-8}$	80	$5.0\ 10^{-8}$	84
	22	$1.8\ 10^{-7}$	97	$2.0\ 10^{-7}$	91	$1.9\ 10^{-7}$	96
	32	$5.1\ 10^{-7}$	98	$5.1\ 10^{-7}$	98	$5.9\ 10^{-7}$	99
	42	$9.0\ 10^{-7}$	97	$9.3\ 10^{-7}$	91	$1.1\ 10^{-7}$	96
50	12	$3.2\ 10^{-8}$	94	$4.5\ 10^{-8}$	80	$3.3\ 10^{-8}$	90
	22	$1.5\ 10^{-7}$	98	$1.6\ 10^{-7}$	90	$1.5\ 10^{-7}$	95
	32	$3.2\ 10^{-7}$	95	$3.4\ 10^{-7}$	94	$4.7\ 10^{-7}$	94
	42	$7.0\ 10^{-7}$	97	$8.6\ 10^{-7}$	81	$3.7\ 10^{-7}$	96
100	12	$1.7\ 10^{-8}$	81	$3.3\ 10^{-8}$	80	$1.9\ 10^{-8}$	82
	22	$8.2\ 10^{-8}$	85	$1.5\ 10^{-7}$	94	$1.5\ 10^{-7}$	88
	32	$3.1\ 10^{-7}$	98	$3.3\ 10^{-7}$	98	$3.7\ 10^{-7}$	94
	42	$6.3\ 10^{-7}$	97	$8.6\ 10^{-7}$	94	$8.1\ 10^{-7}$	95

Malv-3-gluc =Malvidin-3-glucoside; Malv-3-acet= Malvidine-3-acetylglucoside, Malv-3-coum= Malvidine-3-p-coumarylglucoside

and 100 mg/l of SO_2. As we can judge by the k-values the presence of higher concentration of SO_2 on the wines decreases the anthoyanins degradative rate at the temperature studied.

Comparing the losses of the three anthocyanins, both malvidin-3-acetylglucoside and malvidin-3-coumarylglucoside have higher k-values than malvidin-3-glucoside. This could be due to their greater reactivity but it could also be to a hydrolytic degradation of the acylated anthocyanins to malvidin-3-glucoside. McCloskey and Yengoyan (1981) reported that the acylated monoglucoside disappears faster than the other monoglucosides in Cabernet-Sauvignon and Zinfandel wines.

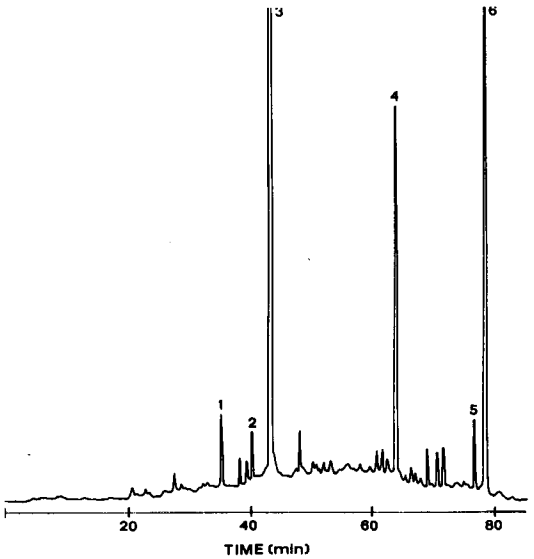

Fig. 3: A typical HPLC chromatogram of the Tinta Roriz wine recorded at 520 nm. 1) Petunidin-3-glucioside; 2) Peonidin-3-glucoside; 3) Malvidin-3-glucoside, 4) Malvidin-3-acetylglucoside; 5) Peonidin-3-p-coumarylglucoside; 6) Malvidin-3-p-coumarylglucoside.

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