ELSEVIER

Food Chemistry 85 (2004) 259–266

Food Chemistry

www.elsevier.com/locate/foodchem

Improvement on enzymatic hydrolysis of resveratrol glucosides in wine

Giovanna Loredana La Torre, Giuseppina Laganà, Ersilia Bellocco, Fortunato Vilasi, Francesco Salvo, Giacomo Dugo*

Department of Organic and Biological Chemistry, University of Messina, Salita Sperone, 31-98166 Messina, Italy

Received 5 February 2003; accepted 24 June 2003

Abstract

An enzymatic hydrolysis method, useful for the analysis of total wine resveratrol, was optimised. The wine resveratrol O-glycosides were totally hydrolysed in ~9 h after incubation with β-glucosidase at 50 °C; then the *trans*- and *cis*-aglycones were measured by high-performance liquid chromatography after solid phase extraction (SPE). Kinetic study of enzymatic hydrolysis at different temperatures showed that hydrolysis times were reduced with increasing temperature, up to 50 °C, because the enzyme retains its activity even at this temperature.

Keywords: Resveratrol; Enzymatic hydrolysis; Wine; Piceid; Glucosidase

1. Introduction

The chemical composition of a wine is influenced by the climatic and atmospheric conditions, soil type, vine cultivation and the treatment to which it is subjected. Phenolic compounds are secondary metabolites of plants and are responsible for colour and contribute to the bitter flavour of wines (Reyonolds & Wardle, 1996; Singleton & Noble, 1976). They are also bactericidal agents and impart antioxidant properties (Goldberg, 1996), being especially found in the skins and seeds of the grapes.

The phenolic compounds, resveratrol, flavonoids, and furanocoumarins, have many ecologic functions and affect human health. Ecological functions include defence against microbial pathogens and herbivorous animals (Bennet & Wallsgrove, 1994).

In recent years, more interest has been focused on *trans*-resveratrol (Arce, Tena, Rios & Valcàrcel, 1998; Goldberg, Karumanchiri, Yan, Diamandis, Soleas, & Ng, 1996; Goldberg, Ng, Karumanchiri, Yan, Diamandis, & Soleas, 1995; Gu, Chu, O'Dwyer, & Zeece, 2000; Jeandet, Bessis, Adrian, Weston, Peyron, & Trollat, 1995; Rodrìguez-Delgado, Gonzàles, Pérez-Trujillo, & Garcìa-Montelongo, 2002; Romero-Pérez, Lamuela-Raventòs, Andrés-Lacueva, & de la Torre Boronat, 2001; Soleas, Diamandis, & Goldberg, 1997a).

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring phytoalexin produced by some spermatophytes, such as grapevines, in response to injury (Bavaresco, Fregoni, Cantu, & Trevisan, 1999). This compound is reputed to benefit persons afflicted by a wide range of disorders including those affecting the liver, skin, heart, circulation, and lipid metabolism (Soleas, Diamandis, & Goldberg, 1997b).

As a phenolic compound, resveratrol contributes to the antioxidant potential of red wine and thereby may play a role in the prevention of human cardiovascular diseases. Resveratrol has been shown to modulate the metabolism of lipids, and to inhibit the oxidation of low-density lipoproteins and the aggregation of platelets. Moreover, as a phytoestrogen, resveratrol may provide cardiovascular protection. This compound also possesses anti-inflammatory and anticancer properties (Frankel, Waterhouse, & Kinsella, 1993; Fremont, 2000; Teissedre, Frankel, Waterhouse, Peleg, & German, 1996). In grape species, resveratrol reaches concentrations of 50–400 μg/g fresh weight in the leaves.

Resveratrol is also synthesised in the berries and in lignified plant tissues. Concentrations in the skin (pericarp) of the berries are high compared with those in the flesh. During mashing, a part of the

* Corresponding author. Tel./Fax: +39-90-6765180.
E-mail address: dugogia@isengard.unime.it (G. Dugo).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.06.019

resveratrol from the skins is dissolved in the must, in fact, only low levels occur in white wine ($\leqslant 1$ mg/l), whereas red wines normally contain 0.9–8.7 mg/l and even higher levels on occasions (Siemann & Creasy, 1992; Goldberg, Yan et al., 1995).

Resveratrol exists as *cis*- and *trans*-isomers and as *trans*- and *cis*-piceid (β-glucosides of resveratrol) (Vrhovsek, Wendelin, & Eder, 1997). Both isomeric forms were detected in white, rosé, and red wine (Goldberg, Ng et al., 1995; Nevado, Salcedo, & Penavo, 1999). *Cis*-isomers are probably synthesised during the vinification process but do not reach the concentration of *trans*-isomers in the wine (Nevado et al., 1999; Sato, Suzuki, Okuda, & Yokotsuka, 1997). The ability to synthesise resveratrol decreases with ripening. Therefore, increased contents at harvest time cannot originate from an increased synthesis. Because resveratrol increases simultaneously with a decrease of piceid (Nevado et al., 1999), the increase of resveratrol might be related to the hydrolysis of the resveratrol glucosides.

The synthesis of *trans*-resveratrol in the plants can be induced by microbial infections and UV radiation. The content of resveratrol in grape determines natural resistance to the fungus *Botrytis cinerea* (grey mould). After an infection of a single berry, the resveratrol content in the neighbouring berries increases within a short time (Bavaresco et al., 1999; Bennet et al., 1994).

Because all the chemical forms of resveratrol, including its glucosides, show similar biological activities, it is important to know the true concentrations of these compounds.

Several studies about resveratrol content in Sicilian wines have been done which show that free *cis*- and *trans*-resveratrol concentrations are usually between 0.2 and 13 mg/l in red wines and between 0.1 and 0.8 mg/l in white wines, (Dugo, Bambara, Salvo, Saitta, & Lo Curto, 2000). No proportionality between the levels of free isomers of resveratrol and their glucosides is reported (Waterhouse & Lamuela-Raventòs, 1994), whereas piceid concentrations from 0.3 mg/l to 9 mg/l in red wines and from 0.1 mg/l to 2.2 mg/l in white wines have been detected (Viñas, Lòpez-erroz, Marin-Hernàndez, & Hernàndez-Cordoba, 2000).

Quantitative analysis of resveratrol glucosides is difficult because the reference compounds are not commercially available. Hydrolysis of these glucosides to their corresponding aglycones offers a practical method for the quantification of resveratrol in wines and juices. Several hydrolysis methods can be applied for studies of polyphenol glucosides and alkaline hydrolysis is particularly used to study flavonoids, resveratrol and related compounds in red juices (Rommel & Wrolstad, 1993a, 1993b). Enzymatic hydrolysis with β-glucosidase has been used by Jeandet et al. (1997) to study resveratrol compounds and offers a method for the cleavage of specific monosaccharides from resveratrol O-glucosides.

In the present study, enzymatic hydrolysis of wine resveratrol glucosides with β-glucosidase, to obtain all the glucosides as aglycones, was optimised.

2. Materials and methods

2.1. Chemicals and materials

Trans-Resveratrol was purchased from Sigma Chemical Co. Stock solution of *cis*-resveratrol was produced by UV irradiation (using an 8 watt lamp) of *trans*-resveratrol in methanol for 30-min at 254 nm. For the quantitation of the *cis*-form, a standard curve was obtained with five known amounts of the *trans*-resveratrol irradiated, as described by Romero-Pérez, Lamuela-Raventòs, Waterhouse, and de la Torre-Boronat (1996).

Solid-phase extraction (SPE) cartridges were Supelco Supelclean LC-18 SPE tubes (3 ml). The salicin and 2-hydroxybenzyl alcohol were supplied by Sigma-Aldrich.

α-D-glucosidase from yeast (lyophilised powder), 65U/mg, (EC 3.2.1.20) and β-D-glucosidase from almonds (lyophilised powder), 6U/mg (EC 3.2.1.21) were from Fluka.

Acetonitrile and H_2O were solvent HPLC grade, obtained from Carlo Erba.

2.2. Wines analysed

The samples tested were two red Italian wines (cv. *Nero d'Avola,* and cv. *Sangiovese*) and a white (cv. *Inzolia*) Italian wine, all from Sicily. All wines were stored in the dark at 4 °C, and each was opened immediately prior to analysis.

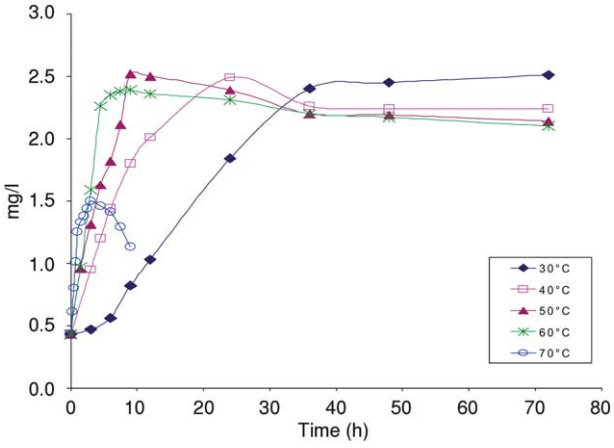

Fig. 1. Influence of incubation temperature on the yield and rate of formation of *trans* and *cis*-resveratrol from resveratrol glucosides hydrolysates using β-glucosidase at pH 6.0. See Section 2 for enzymatic hydrolysis conditions.

Table 1
Resveratrol concentrations (mg/l) at different temperatures, during enzymatic hydrolysis with β-glucosidase

Time (h)	30 °C			40 °C			50 °C			60 °C			70 °C		
	Trans[a] (mg/l)	*Cis*[b] (mg/l)	Total[c] (mg/l)	*Trans*[a] (mg/l)	*Cis*[b] (mg/l)	Total[c] (mg/l)	*Trans*[a] (mg/l)	*Cis*[b] (mg/l)	Total[c] (mg/l)	*Trans*[a] (mg/l)	*Cis*[b] (mg/l)	Total[c] (mg/l)	*Trans*[a] (mg/l)	*Cis*[b] (mg/l)	Total[c] (mg/l)
0	0.39	0.04	0.43	0.39	0.04	0.43	0.39	0.04	0.43	0.39	0.04	0.43	0.39	0.04	0.43
15′	–[d]	–	–	–	–	–	–	–	–	–	–	–	0.53	0.08	0.61
30′	–	–	–	–	–	–	–	–	–	–	–	–	0.69	0.11	0.80
45′	–	–	–	–	–	–	–	–	–	–	–	–	0.87	0.14	1.01
1.00	–	–	–	–	–	–	–	–	–	–	–	–	1.07	0.18	1.25
1.30	–	–	–	–	–	–	0.79	0.17	0.96	0.79	0.18	0.97	1.13	0.20	1.33
2.00	–	–	–	–	–	–	–	–	–	–	–	–	1.17	0.21	1.38
2.30	–	–	–	–	–	–	–	–	–	–	–	–	1.21	0.23	1.44
3.00	0.40	0.07	0.47	0.79	0.16	0.95	1.07	0.24	1.31	1.31	0.28	1.59	1.26	0.24	1.50
3.30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4.30	–	–	–	0.95	0.25	1.20	1.34	0.29	1.63	1.87	0.39	2.26	1.23	0.23	1.46
5.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5.30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6.00	0.47	0.09	0.56	1.12	0.32	1.44	1.48	0.34	1.82	1.90	0.45	2.35	1.20	0.21	1.41
7.30	–	–	–	–	–	–	1.71	0.40	2.11	1.91	0.47	2.38	1.11	0.18	1.29
9.00	0.71	0.11	0.82	1.40	0.40	1.80	1.99	0.53	2.52	1.91	0.48	2.39	0.97	0.16	1.13
12.00	0.87	0.16	1.03	1.56	0.45	2.01	1.97	0.53	2.50	1.88	0.48	2.36	–	–	–
24.00	1.48	0.36	1.84	1.99	0.50	2.49	1.88	0.51	2.39	1.79	0.42	2.21	–	–	–
36.00	1.92	0.48	2.40	1.79	0.47	2.26	1.78	0.42	2.20	1.79	0.41	2.20	–	–	–
48.00	1.95	0.50	2.45	1.77	0.47	2.24	1.78	0.41	2.19	1.77	0.40	2.17	–	–	–
72.00	1.96	0.55	2.51	1.76	0.48	2.24	1.75	0.39	2.14	1.72	0.38	2.10	–	–	–

[a] *Trans* corresponding to total *trans*-resveratrol.
[b] *Cis* corresponding to total *cis*-resveratrol.
[c] Total corresponding to total (*trans*- + *cis*-) resveratrol.
[d] –, not measured at the given time.

2.3. Instrumentation

The instrument used for analysis of *cis*- and *trans*-resveratrol and salicin was an HPLC-UV/Vis System (Shimadzu) equipped with two pumps (LC-10A), a Controller-System (SCL-10A), a UV/vis detector (10 AV), and an injector (Rheodyne model 7725i with a 20 μl loop).

2.4. Sample preparation and HPLC analysis of cis- and trans-resveratrol

The determination of *cis*- and *trans*-resveratrol in wine samples required an extraction procedure, using SPE cartridges, prior to the HPLC analysis. The SPE cartridges were preconditioned with 3 ml of MeOH, followed by 3 ml of H_2O. 1 ml of the red wine sample was then loaded and allowed to flow through the C-18 bed. After the wine had passed through, the cartridge was washed with 1 ml of H_2O. The bound materials were eluted from the cartridge with 3 × 1 ml of ethyl acetate. Ethyl acetate was evaporated under N_2 flow and the residue filled up to 1 ml with MeOH. The extraction procedure was carried out in subdued light to reduce the light-induced isomerisation of the *trans*-stilbene compounds to the *cis*-isomer during sample handling.

Liquid chromatographic separation was performed using the HPLC system (Shimadzu). A C-18 ODS Hypersil 5 μm (250×4,6 mm i.d.) column was used as the stationary phase and it was preceded by a precolumn of

Table 2
Resveratrol contents (mg/l) in wines with different alcoholic concentrations

Wine	30° / 17h			50° / 9h		
	Trans[a] (mg/l)	*Cis*[b] (mg/l)	Total[c] (mg/l)	*Trans*[a] (mg/l)	*Cis*[b] (mg/l)	Total[c] (mg/l)
Wines with different alcoholic concentration (% v/v)						
3	1.28	0.19	1.47	2.01	0.51	2.52
7	1.36	0.15	1.51	1.91	0.54	2.45
9	1.32	0.16	1.48	1.92	0.56	2.48
10	1.32	0.16	1.48	1.92	0.54	2.46
11	1.33	0.17	1.50	1.99	0.51	2.50
12	1.35	0.17	1.52	1.96	0.56	2.52
13	1.24	0.21	1.45	1.99	0.49	2.48
14	1.35	0.14	1.49	1.94	0.54	2.48
15	1.38	0.12	1.50	1.99	0.50	2.49
16	1.41	0.10	1.51	1.98	0.52	2.50
Wine not treated						
	1.39	0.11	1.50	2.03	0.49	2.52

[a] *Trans* corresponding to total *trans*-resveratrol.
[b] *Cis* corresponding to total *cis*-resveratrol.
[c] Total corresponding to total (*trans*- + *cis*-) resveratrol

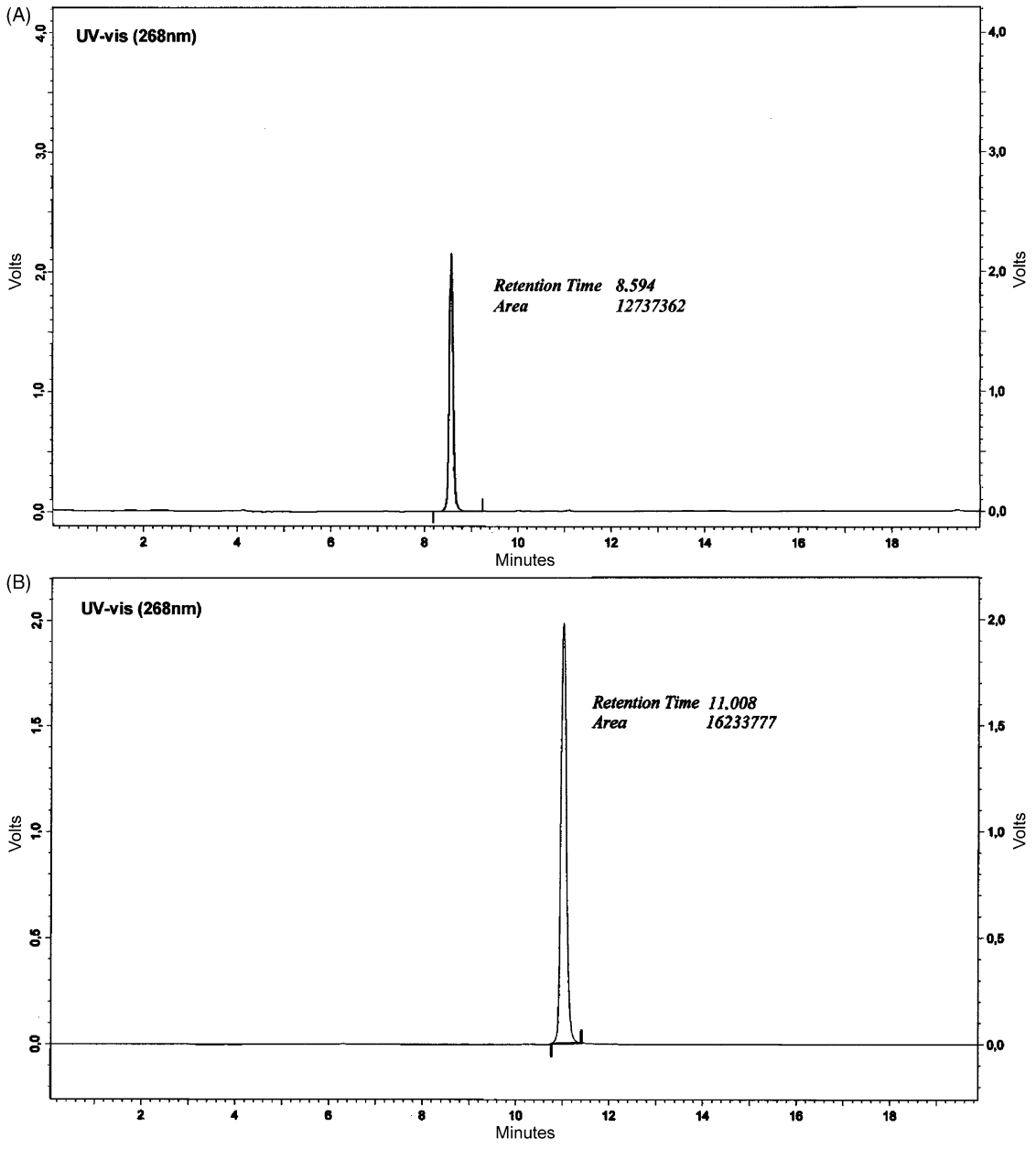

Fig. 2. HPLC chromatogram (at 268 nm) of salicin. (A) Before hydrolysis. (B) After hydrolysis at 30 °C for 17 h. (C) After hydrolysis at 50 °C for 9 h. See Section 2 for chromatographic conditions.

the same material. The mobile phase consisted of phase (A) water/acetic acid (pH = 3) and phase (B) acetonitrile/acetic acid (pH = 3).

cis- And *trans*-resveratrol were eluted with the gradient time programme: 0–0.01 min: 5% B; 0.01–10.00 min: 25% B; 10.01–20.00 min: 40% B; 20.01–35.00 min:100% B.

The eluate was monitored at two different wavelengths, 285 and 307 nm, where *cis*- and *trans*- isomers have absorbance maxima, respectively. All chromatographic experiments were performed at room temperature with a flow rate of 1 ml/min. Samples for HPLC analysis were filtered through a 0.45 μm glass-microfibre GMF Whatman chromatographic filter.

Peaks were identified by comparison of retention time and ultraviolet spectra with standard solutions of *cis*- and *trans*-resveratrol.

2.5. Calibration, recovery, repeatability and sensitivity for resveratrol extraction

Calibration graphs were performed by plotting concentration (μg/ml) against peak area. The equation obtained for the calibration graphs and the regression coefficients is $y = 207120x - 5603.7$ $\left(R^2 = 0.998\right)$.

The resveratrol compounds recovery was measured in five replicated analyses of a hydroalcoholic solution of

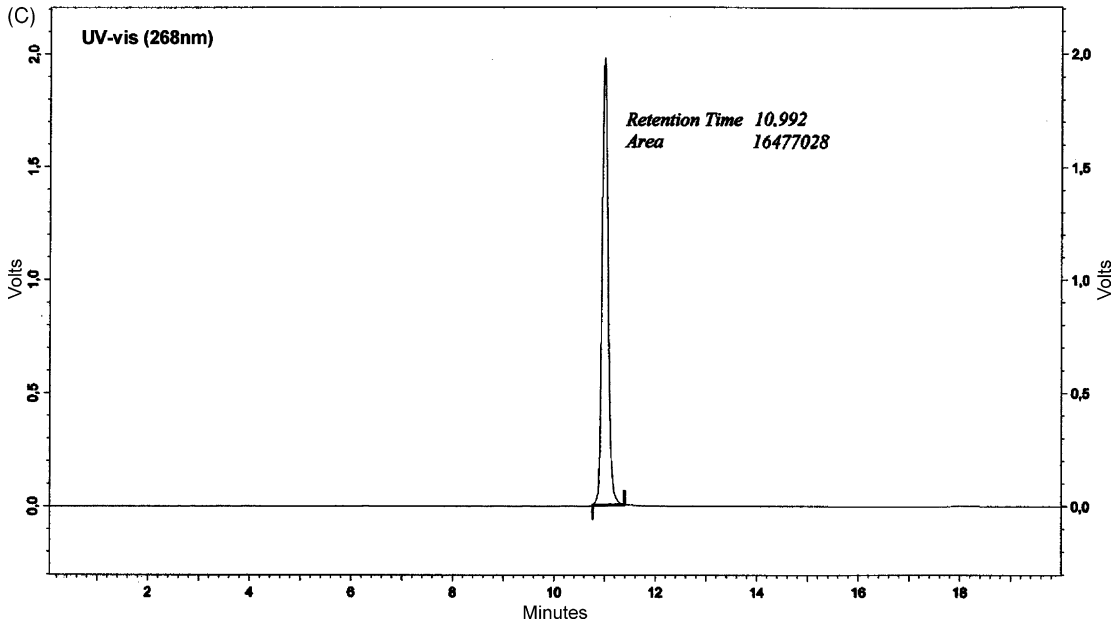

Fig. 2. (*continued.*)

trans-resveratrol standard at the concentration of 10 mg/l. The average recovery was 95.33±0.02%, with an RDS = 2.09%. This was confirmed by adding a standard amount of *trans*-resveratrol to a sample of wine previously tested.

Method sensitivity, which was calculated at a peak height of twice the baseline, was 0.07 mg/l and 0.02 mg/l for *trans*- and *cis*-resveratrol, respectively.

Analyses repeatability, estimated in five replicated analysis of the same sample of wine, was 0.49±0.07 mg/L and 0.47±0.05 mg/L for *trans*- and *cis*-resveratrol, respectively.

2.6. Enzymatic hydrolysis of resveratrol glucosides

The enzymatic hydrolysis was performed by testing a red wine with high resveratrol content. Before the hydrolysis, the pH of red wine was adjusted to 6.0 with 0.1 N NaOH; then β-glucosidase (1 mg/ml) was added. Hydrolysis was carried out, at the beginning, by incubation at 30 °C for 17 h (Jeandet et al., 1997). To prove that the incubation time (17 h), and/or the temperature (30 °C), was able to convert all the resveratrol glucosides into their aglycones, several aliquots (1.5 ml each) of the same wine were incubated at temperatures between 30 and 70 °C. Each aliquot was taken at fixed times and immediately placed in an ice bath to stop the reaction, it was then prepared for the subsequent extraction and HPLC analysis.

The sample of white wine, prepared in the same manner, was submitted to enzymatic hydrolysis at 30 and 50 °C for 17 and 9 h respectively, before the HPLC analysis.

2.7. Enzymatic hydrolysis of standard

The piceid, generally used as standard to value the rate of β-glucosidase activity (salicin), was dissolved in methanolic solution (10% v/v), adjusted to pH 6.0 with 0.01 M HCl to a final concentration of 2.6 mg/ml. Two aliquots of this solution were hydrolysed under the same conditions, as for the white wine, then tested by HPLC.

2.8. Test of influence of alcohol content on enzymatic hydrolysis

An exact quantity of wine was evaporated at 30 °C to remove alcohol. The evaporating solution was protected from light-induced degradation of resveratrol, as described by Romero-Pèrez et al. (1996).

When alcohol was distilled, the obtained sample was divided into two homogeneous portions. One of these was spiked with distilled water to a volume corresponding to half of the original volume; then the alcohol content was measured to verify that the resulting solution was free from alcohol. The second share was divided again into 10 aliquots, which were spiked with an exact volume of hydroalcoholic solutions to obtain samples with different alcoholic concentrations. Then, each of these solutions, together with a sample of wine (not subjected to evaporation), were spiked with β-glucosidase, in order to perform the enzymatic hydrolysis, both at 30 °C/17 h and 50 °C/9 h.

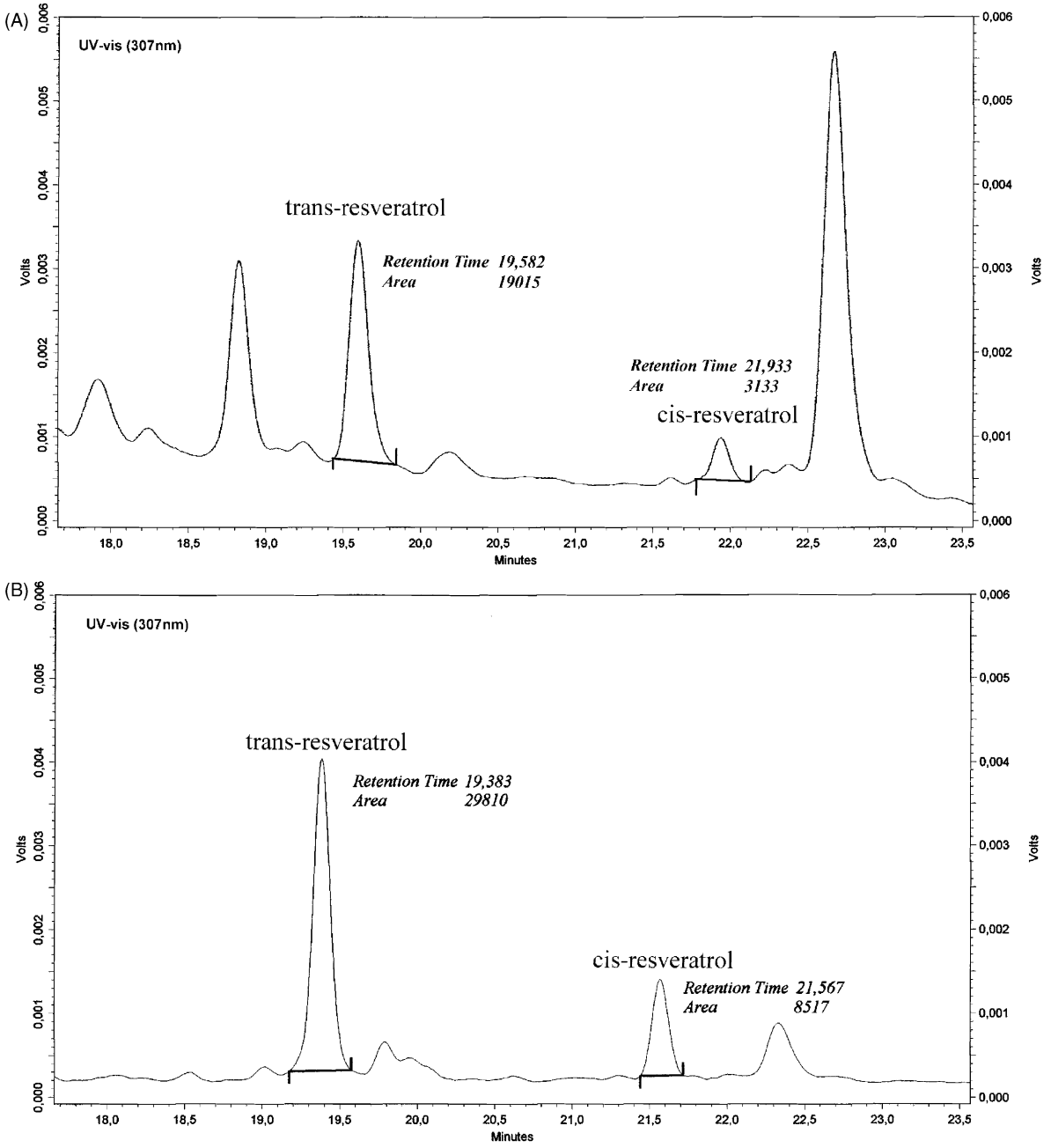

Fig. 3. HPLC chromatogram (at 307 nm) of white wine (cv. *Inzolia*). (A) Before hydrolysis. (B) After hydrolysis at 30 °C for 17 h. (C) After hydrolysis at 50 °C for 9 h. See Section 2 for chromatographic conditions.

3. Results and discussion

Fig. 1 shows the plot of resveratrol content versus time after enzymatic hydrolysis with β-glucosidase at different temperatures.

Examining the kinetics, it is evident that the resveratrol content increases with a rate dependent on the hydrolysis temperature. At 70 °C, hydrolysis is much faster than at the other tested temperatures but, already after 1 h, the rate decreases and the maximum value is equal to 60% of those obtained at 30, 40 and 50 °C. The lower product concentrations in the hydrolysis at 70 °C was caused by the fast deactivation of the enzyme.

In all the kinetics it was observed that the *trans*-resveratrol was the highest represented isomer. During the hydrolysis, its concentration rose five times in value and *cis*-resveratrol content also rose 12.5 times. Moreover, the total resveratrol content decreased, after the maximum value, because of a degradation process caused by the long incubation times. This degradation is quicker at higher temperatures and particularly at 70 °C (see Fig. 1 and Table 1).

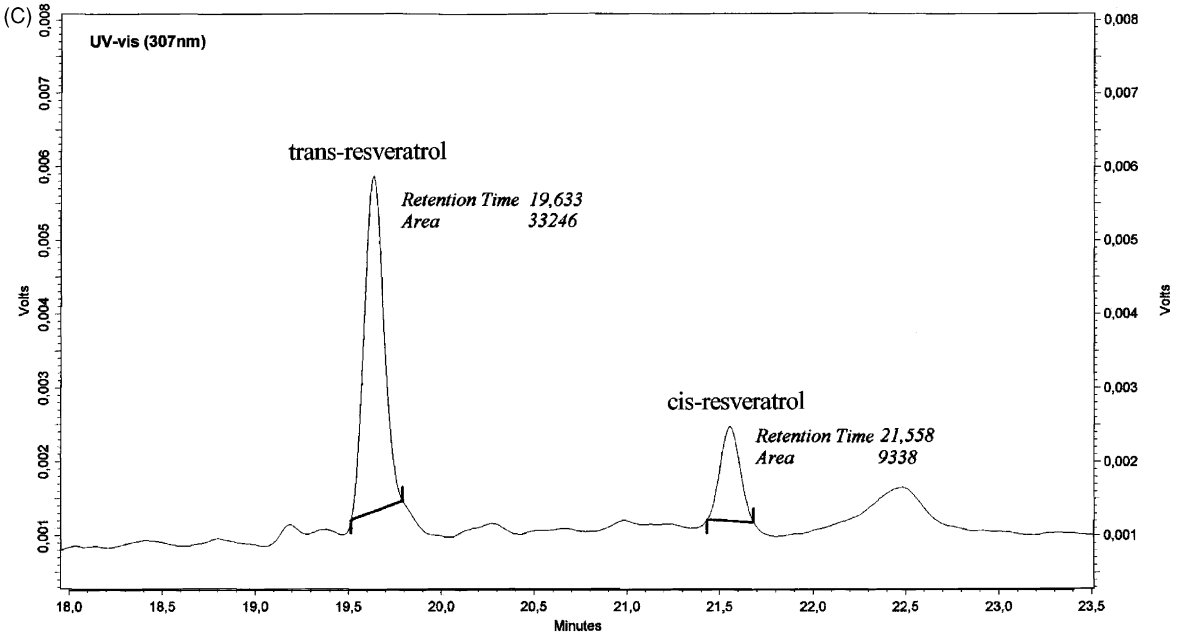

Fig. 3. (*continued.*)

By analysing the values obtained from the kinetic study of hydrolysis with β-glucosidase at different temperatures, it can be seen that the enzyme retains its activity, even at high temperature, for quite long times, and this property makes it possible to reduce the enzymatic hydrolysis times (36–72 h at 30 °C, 24 h at 40 °C, 9 h at 50 and 60 °C), as well as to complete the hydrolytic process.

In fact, the same sample, subjected to hydrolysis for 17 h at 30 °C, gave a total resveratrol content equal to 1.25 mg/l, slightly lower than the value obtained at 50 °C for 3 h and reduced by half if compared with the value determined at the end of hydrolysis (9 h at 50 °C).

The α + β-glucosidase hydrolysis, performed in parallel to that reported above, gave results comparable with those obtained from samples treated only with the β-glucosidase; this is in agreement with our results on other wines and with those one of other authors on the minimum amount of α-bonds in resveratrol glucosides (Goldberg, Ng et al., 1995).

The enzymatic hydrolysis of salicin (Fig. 2) and of a white wine (Fig. 3), containing a low amount of resveratrol, performed at the same time according to the Jeandet method and at 50 °C for 9 h, gave almost the same results, with slightly greater values for the second. In this case, incubation at 50 °C for 9 h has to be preferred because of the shorter hydrolysis time.

Moreover, to leave no doubt about the results and to verify that *cis*-resveratrol is obtained by hydrolysis and not by isomeric production, a test was performed with *trans*-resveratrol standard at 50 °C for 9 h. The chromatograms obtained before and after the thermic treatment showed only the *trans*-resveratrol peak.

To verify that the enzymatic method would be applicable, with best results, to wines with different alcoholic concentrations, we performed a test on several samples obtained from the same wine but with different alcoholic concentrations.

Table 2 shows that different alcoholic concentrations do not influence the enzymatic dosage of total resveratrol, either at 30 °C or 50 °C; the small variation in resveratrol contents of individual samples could be attributed to experimental error. Furthermore, the different values confirm that, at 30 °C, the resveratrol concentration was reduced to about 60%. Moreover, the contents of resveratrol isomers, obtained after the analysis of the wine samples with different alcoholic contents, are in agreement with the amounts of the same isomers in the wine that was not subjected to evaporation, confirming that the concentration step, carried out by rotary evaporation, did not cause degradation or loss of the analytes (Romero-Pérez et al., 1996).

In conclusion, a rapid and sensitive method has been developed for the determination of *trans*- and *cis*-piceid (β-glucosides of resveratrol) in wine. This method shows good precision and can be employed as a routine method for quantification of resveratrol glucosides present in wine samples.

References

Arce, L., Tena, M. T., Rios, A., & Valcàrcel, M. (1998). Determination of trans-resveratrol and other polyphenols by a continuous flow sample clean-up system followed by capillary electrophoresis separation. *Analytica Chimica Acta*, *359*, 27–38.

Bavaresco, L., Fregoni, C., Cantu, E., & Trevisan, M. (1999). Stilbene

compounds: from the grape wine to wine. *Drugs under Experimental and Clinical Research*, *25*, 57–63.

Bennet, R. C., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *Tansley Review NO. 72. New Phytol.*, *127*, 617–633.

Dugo, G., Bambara, G., Salvo, F., Saitta, M., & Lo Curto, S. (2000). Contenuto di resveratrolo in vini rossi da uve alloctone e autoctone prodotte in Sicilia. *L'enologo*, *12*, 79–84.

Frankel, E. N., Waterhouse, A. L., & Kinsella, J. E. (1993). Inhibition of human LDL oxidation by resveratol. *Lancet*, *341*, 1103–1104.

Fremont, L. (2000). Biological effects of resveratrol. *Life Sciences*, *66*, 663–674.

Goldberg, D. M. (1996). More on antioxidant activity of resveratrol in red wine. *Clinical Chemistry*, *42*, 113–114.

Goldberg, D. M., Karumanchiri, A., Yan, J., Diamandis, E. P., Soleas, G., & Ng, E. (1996). Method to assay the concentrations of phenolic constituents of biological interest in wines. *Analytical Chemistry*, *68*, 1688–1694.

Goldberg, D. M., Ng, E., Karumanchiri, A., Yan, J., Diamandis, E. P., & Soleas, G. (1995). Assay of resveratrol glucosides and isomers in wine by direct-injection high-performance liquid chromatography. *Journal of Chromatography A*, *708*, 89–98.

Goldberg, D. M., Yan, J., Ng, E., Diamandis, E. P., Karumanchiri, A., Soleas, G., & Waterhouse, A. L. (1995). A global survey of trans-resveratrol concentrations in commercial wines. *American Journal of Enology and Viticulture*, *46*, 159–165.

Gu, X., Chu, Q., O'Dwyer, M., & Zeece, M. (2000). Analysis of resveratrol in wine by capillary electrophoresis. *Journal of Chromatography A*, *881*, 471–481.

Jeandet, P., Bessis, A. C., Adrian, M., Weston, P., Peyron, D., & Trollat, P. (1995). Effect of enological practices on the resveratrol isomer content of wine. *Journal of Agricultural and Food Chemistry*, *43*, 316–319.

Jeandet, P., Breuil, A. C., Adrian, M., Weston, L. A., Debord, S., Meunier, P., Maume, G., & Bessis, R. (1997). HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorometry. *Analytical Chemistry*, *69*, 5172–5177.

Nevado, J. J. B., Salcedo, A. M. C., & Penavo, G. C. (1999). Simultaneous determination of cis and trans-resveratrol in wines by capillary zone electrophoresis. *Analyst*, *124*, 61–66.

Reyonolds, A. G., & Wardle, D. A. (1996). Impact of viticultural practices on grape monoterpenes and their relationship to wine sensory response. In T. Henick-Kling, T. E. Wolf, & E. M. Harckness (Eds.), *Proceeding of the Fourth International Symposium on Cool Climate Viticulture and Enology*. New York: Comm. Services.

Rodrìguez-Delgado, M. A., Gonzàles, G., Pérez-Trujillo, J. P., & Garcìa-Montelongo, F. J. (2002). Trans-resveratrol in wines from the Canary Islands (Spain). Analysis by high performance liquid chromatography. *Food Chemistry*, *76*, 371–375.

Romero-Pérez, A. I., Lamuela-Raventòs, R. M., Waterhouse, A. L., & de la Torre-Boronat, M. C. (1996). Levels of cis- and trans-resveratrol and their glucosides in white and rosé vitis vinifera wines from Spain. *Journal of Agricultural and Food Chemistry*, *44*, 2124–2128.

Romero-Pérez, A. I., Lamuela-Raventòs, R. M., Andrés-Lacueva, C., & de la Torre-Boronat, M. C. (2001). Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *Journal of Agricultural and Food Chemistry*, *49*, 210–215.

Rommel, A., & Wrolstad, R. E. (1993a). Influence of acid and base hydrolysis on the phenolic composition of red raspberry juice. *Journal of Agricultural and Food Chemistry*, *41*, 1237–1241.

Rommel, A., & Wrolstad, R. E. (1993b). Composition of flavonols in red raspberry juice as influenced by cultivar, processing, and environmental factors. *Journal of Agricultural and Food Chemistry*, *41*, 1941–1950.

Sato, M., Suzuki, Y., Okuda, T., & Yokotsuka, K. (1997). Contents of resveratrol, piceid, and their isomers in commercially available wines made from grapes cultivated in Japan. *Bioscience Biotechnology and Biochemistry*, *61*, 1800–1805.

Siemann, H. E., & Creasy, L. L. (1992). Concentration of the phytoalexin resveratrol in wine. *American Journal of Enology and Viticulture*, *43*, 49–52.

Singleton, V. L., & Noble, A. C. (1976). Wine flavour and phenolic substances. In G. Charalambous, & I. Katz (Eds.), *Phenolic, sulphur and nitrogen compounds in food flavours*, *ACS Symposium Series 26* (pp. 47). Washington: American Chemical Society.

Soleas, G. J., Diamandis, E. P., & Goldberg, D. M. (1997a). Resveratrol: a molecule whose time has come? And Gone? *Clinical Biochemistry*, *30*, 91–113.

Soleas, G. J., Diamandis, E. P., & Goldberg, D. M. (1997b). Wine as a biological fluid: History, production and role in disease prevention. *Journal of Clinical Laboratory Analysis*, *11*, 287–317.

Teissedre, P. L., Frankel, E. N., Waterhouse, A. L., Peleg, H., & German, J. B. (1996). Inibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wine. *Journal of the Science of Food and Agriculture*, *70*, 55–61.

Viñas, P., Lòpez-erroz, C., Marin-Hernàndez, J. J., & Hernàndez-Cordoba, M. (2000). Determination of phenols in wines by liquid chromatography with photodiode array and fluorescence detection. *Journal of Chromatography A*, *871*, 85–93.

Vrhovsek, U., Wendelin, S., & Eder, R. (1997). Effects of various vinification techniques on the concentration of cis- and trans-resveratrol and resveratrol glucoside isomers in wine. *American Journal of Enology and Viticulture*, *48*, 214–219.

Waterhouse, A. L., & Lamuela-Raventòs, R. M. (1994). The occurrence of piceid, a stilbene glucoside, in grape berries. *Phytochemistry*, *12*, 571–573.