ELSEVIER

Journal of Chromatography A, 918 (2001) 303–310

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Automated solid-phase extraction for sample preparation followed by high-performance liquid chromatography with diode array and mass spectrometric detection for the analysis of resveratrol derivatives in wine[☆]

C. Domínguez*, D.A. Guillén, C.G. Barroso

Departamento de Química Analítica, Facultad de Ciencias, Universidad de Cádiz, Polígono Río San Pedro s/n, 11510 Puerto Real (Cádiz), Spain

Received 20 November 2000; received in revised form 28 February 2001; accepted 7 March 2001

Abstract

A method has been developed for the simultaneous determination of resveratrol in all its forms (free isomers and glycosylates) in wines by high-performance liquid chromatography with diode array and mass spectrometric detection. Prior to injection into the column, preconcentration of the sample by automated solid-phase extraction is carried out. In the detection by UV absorption, quantitation was carried out at 280 and 305 nm, and in detection by mass spectrometry, quantitation was performed in the selected ion monitoring mode at m/z 228 and at m/z 238. A comparative study between both detection systems was carried out.

Keywords: Sample preparation; Wine; Food analysis; Resveratrol; Glycosides; Phenolic compounds

1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene) and its derivatives are natural phenolic compounds found in many families of plants [1–8]. The grape and related products, such as wine, are probably the food products that contain these compounds in greater proportions.

The presence of these stilbenes in grapes and derived products has been under study for some years now, for two main reasons. First, it has been demonstrated that these compounds act as phytoalexins in the plant [9–15], being synthesized in response to situations of stress, such as attack by pathogens, UV radiation, or lesion. Second, it has also been observed that they are beneficial for human health through their antioxidant properties, since it seems they may be partially responsible for the lower mortality rates from cardiovascular disease observed in populations that customarily consume wine in moderation [15–19]. Further, it has recently been published that it is possible these compounds possess certain chemico-preventive activity against cancer [20–22], as a selective modulator of estrogen re-

[☆]Presented at the 29th Scientific Meeting of the Spanish Group of Chromatography and Related Techniques, Alcalá de Henares (Madrid), 12–14 July 2000.

*Corresponding author. Tel.: +34-956-016-363; fax: +34-956-016-288.

E-mail address: dominico.guillen@uca.es (D.A. Guillén).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00748-8

ceptors [43], as well as having possible application in pathologies of the nervous system [44].

Many different methods have been developed for determining resveratrol in wines. The most common are based on high-performance liquid chromatography (HPLC), using detection by UV absorption [23–26], by electrochemical [27,28] or fluorometric [29,30] methods, or by gas chromatography (GC) [31,32] and GC–mass spectrometry (GC-MS) [33–35] techniques. Recently the introduction of hyphenated techniques, such as LC–MS, have been shown to be powerful tools for the resolution of complex samples, as in this case. As a result, this technique has already been applied for the determination of phenolic compounds in foodstuffs [36].

In respect of the preparation of the sample for its analysis, some authors do not do this, but rather inject the sample directly [23,25,27–30], although the majority use some kind of sample preconcentration and cleaning stage before its injection, such as a liquid–liquid extraction [37–39] or a solid-phase extraction (SPE) [26,40].

The reason for the success of SPE lies in the numerous advantages it offers, such as a high selectivity, fast speed and facility of automation of the procedure [41,42].

This study presents the development of a method for determining simultaneously all the monomers of resveratrol in wine, by the application of HPLC with diode array (DAD) and MS detection. Prior to the injection in HPLC, a stage of preconcentration of the sample by automated SPE is performed, employing a polymeric adsorbent (polystyrene–divinylbenzene) which gives better results than C_{18} since it has a better capacity for retention of compounds with a wide range of polarities. The separation by elution gradient in HPLC and the conditions of MS detection were optimized with the aim of achieving well-resolved peaks and the maximum possible signal in the mass detector.

2. Experimental

2.1. Reagents and standards

The acetonitrile, methanol and tetrahydrofuran, of HPLC quality, and the other reagents were supplied by Scharlau (Barcelona, Spain). The *trans*-resveratrol was acquired from Sigma (St. Louis, MO, USA) and the 3,4,5-trimethoxycinnamic acid, employed as internal standard, from Fluka (Buchs, Switzerland).

The water employed was previously purified in a Milli-Q system (Millipore, Bedford, MA, USA). The aqueous solutions were filtered using cellulose acetate membranes of 0.45 μm (47 mm) (Micron Separation, Westboro, MA, USA) and were degasified in an ultrasonic bath before being used.

2.2. Samples

A variety of different types of wines were analyzed: red Rioja wines, port and fino sherry wine supplied by Bodegas Osborne (El Puerto Sta. María, Cádiz, Spain), and double-macerated reds and white table wines, produced in Spain and acquired commercially.

2.3. Instrumentation and conditions

The analyses were performed using a Waters Integrity HPLC–DAD–MS system (Waters, Millipore, Milford, MA, USA). The chromatographic system consisted of a Model 616 pump and a Model 600S gradient controller, to which were connected a Model 717 plus autosampler, a Model 996 detector of aligned photodiodes and a Thermabeam MS detector with modified particle beam interface, equipped with a source of ionization by electron impact (70 eV), a quadrupole analyzer and an electron multiplier detector.

The separation was performed using a Symmetry C_{18} column (150×2.1 mm I.D., 5 μm particle size). The control of the equipment, and the acquisition and treatment of data was performed with the Millenium 2010, version 2.21, software.

The chromatographic conditions were the following: 0.2 ml/min flow-rate; 50 μl injection volume; eluent A was distilled water (Milli-Q quality), adjusted to pH 2.5 with sulfuric acid; and eluent B was pure acetonitrile; the elution gradient is shown in Table 1.

Detection by UV absorption was performed by sweeping between 240 and 305 nm, with a resolution of 1.2 nm, and the quantitation was done at 280 and 305 nm. The detection and quantitation by MS was

Table 1
Elution gradient programme

t (min)	A (%)	B (%)	Curve
0	100	0	–
60	50	50	7
90	50	50	7
100	0	100	7
110	100	0	7

performed in the selected ion monitoring (SIM) mode, at *m/z* 228 (for detection of isomers of resveratrol) and at *m/z* 238 (for detection of the internal standard). The conditions imposed in the interface, previously optimized by means of simplex, were the following: source temperature, 220°C; nebulization temperature, 85°C; expansion region temperature, 75°C, helium flow-rate, 250 ml/min.

2.4. Preparation of the samples

The samples were filtered through nylon filters of 0.45 μm (13 mm) (Osmonics, Minnetonka, MN, USA) before subsequently undergoing an SPE, for the purpose of cleaning and preconcentrating them before injection into the HPLC–DAD–MS system.

The SPE stage was performed by a totally completely automated method using a semi-flexible and automatic robotic system: a Benchmate work station (Zymark, Hopkinton, MA, USA). The cartridges used were LiChrolut EN (Merck, Darmstadt, Germany), containing 200 mg of the polymeric adsorbent, polystyrene–divinylbenzene. These were conditioned first with 5 ml of methanol and followed by 3 ml of water. A sample of 5 ml of wine to which 123 μl of 216 mg/l solution of 3,4,5-trimethoxycinnamic acid as internal standard has been added was diluted 1:1 with water and 9.8 ml of diluted sample was loaded in the cartridge previously conditioned. Then the column was rinsed with 0.6 ml of water and later dried with helium for 150 s. Finally the compounds of interest were eluted with 1.2 ml of tetrahydrofuran and followed by 1.2 ml of water.

The flow-rates applied during the automated procedure are the following: condition flow, 0.25 ml/s; load flow, 0.01 ml/s; wash flow, 0.05 ml/s; elution flow, 0.05 ml/s; air flow, 0.10 ml/s; air factor, 0.6.

All the steps and flow-rates of the automated SPE procedure were previously optimized by the authors.

2.5. Calibration

For the *trans*-resveratrol, the calibration curves were drawn for the detection both by UV absorption and by MS, from various standard solutions prepared by diluting a stock solution of 100 mg/l of *trans*-resveratrol in aqueous methanol at 60%, such that they cover a range of concentration between 2.5 and 50 mg/l. All the solutions were stored at 4°C and protected from light.

For the calibration of the *cis*-resveratrol, since its commercial standard is not available, a fraction of the stock solution of 100 mg/l *trans*-resveratrol prepared for its own calibration was taken and then irradiated for 15 min in a climatic chamber with control of temperature, humidity and illumination. In this chamber, by a method of accelerated environmental ageing using a solar radiation panel by xenon (1500 W) and at 28°C and 92% humidity, the *trans*-resveratrol is converted into *cis*-resveratrol.

The lower level observed in the peak corresponding to *trans*-resveratrol in the irradiated solution was proportional to the height of the new peak which appeared following the *trans*-resveratrol, corresponding to *cis*-resveratrol, which was identified by its UV absorption and mass spectra. Since no other peaks were detected under these conditions, the concentrations of *cis*-resveratrol were assigned on the basis of the reduction observed for *trans*-resveratrol after its irradiation.

All the samples and calibration standards were quantified by the internal standard method. The compound used as internal standard, 3,4,5-trimethoxycinnamic acid, was selected from the collection of polyphenolic compounds maintained by the research group, bearing in mind that this compound was not present in the samples of wine under study. This compound was added at a fixed and known concentration to all the samples from the commencement of the analysis procedure.

3. Results and discussion

3.1. Chromatographic procedures

First the chromatographic conditions were optimized to ensure that the compounds of interest were

well resolved. For this, the various elution phases and corresponding chromatographic gradient were studied in such a way that, as well as achieving an appropriate resolution, a better signal was obtained in the mass detector; this is because the composition of the phases in which the compounds of interest are eluted considerably affects the yield obtained in the transference of the analyte from the liquid phase to the mass spectrometer. The optimum conditions reached are those described in the preceding section.

Next the stage of preconcentration by automated SPE was studied. Taking as the starting point the conditions previously devised by the authors for the determination of the phenolic compound content of sherry wine [40], the conditions and the instrumental parameters were slightly modified to adapt the method to other types of wine. Fig. 1 shows the chromatograms obtained with UV detection at 305 nm from the direct injection of a red wine (Fig. 1a) and following the stage of automated SPE (Fig. 1b); as can be observed, the increase of the signals

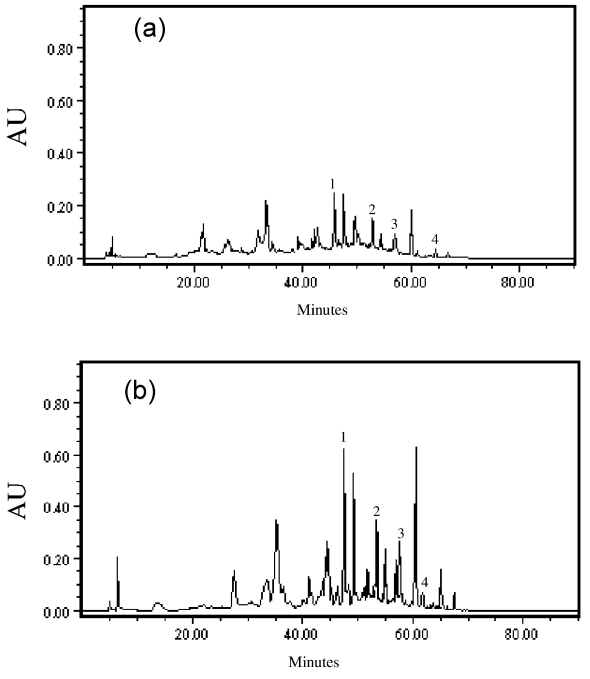

Fig. 1. Chromatograms at 305 nm, corresponding to a red wine. (a) By direct injection. (b) After the stage of automated SPE. Peaks: 1=*trans*-resveratrol glycoside, 2=*cis*-resveratrol glycoside, 3=*trans*-resveratrol, 4=*cis*-resveratrol.

corresponding to the compounds of interest is significant. This result is important particularly bearing in mind that the system of MS detection used in this present study (LC–MS interface; particle beam; ionization by electron impact) has a fairly limited sensitivity. These assays were performed with real samples, with their peaks being identified by both their UV spectra absorption and their mass spectra.

3.2. Calibration

Considering that only the *trans* isomer of resveratrol is commercially available as a standard, the possibility of obtaining a calibration curve for each of the species under study is rather limited. There are two alternative solutions to this difficulty described in the bibliography: one is the calculation of the concentrations of the different compounds using exclusively the calibration curve of *trans*-resveratrol; the other is the generation of the *cis* isomer by means of irradiation of a standard solution of *trans*-resveratrol and then quantifying the amount of *cis*-resveratrol thus formed in function of the quantity of *trans*-resveratrol seen to have ''disappeared''.

The first of these options is arguably inadequate, especially considering the spectral characteristics of the two compounds. Fig. 2 shows the UV absorption and mass spectra for the two isomers of resveratrol. The mass spectra is the same for both isomers, but the UV absorption spectra are clearly fairly different: the *trans* isomer presents an absorption maximum at 305.7 nm (ϵ=26 000) while that presented by the *cis* isomer is 287.8 nm (ϵ=12 000); consequently there would be a considerable error in quantifying *cis*-resveratrol by means of the curve of the *trans* isomer.

This is not the case with the glycosides since the spectra of the combined forms are similar to those corresponding to the free forms. Consequently, it would be reasonable, in order to perform a quantitation with the least possibility of error, to have available two calibration curves: one to quantify *trans*-resveratrol and its glycoside, the other for the *cis* isomer and its glycoside.

This is the second alternative described in the bibliography, in which the *cis* isomer is generated by solar light irradiation, from a solution of *trans*-resveratrol of known concentration; the degree of

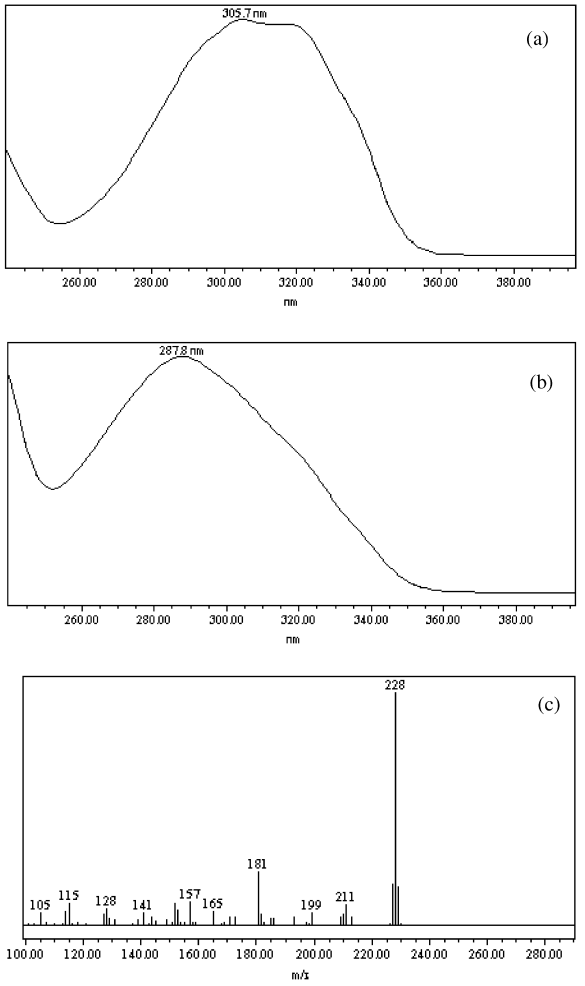

Fig. 2. (a) UV absorption spectra (normalized at absorbance maxima) of *trans*-resveratrol. (b) UV absorption spectra (normalized at absorbance maxima) of *cis*-resveratrol. (c) Mass spectra (normalized at maximum intensity) of both isomers of resveratrol.

conversion is then quantified and the corresponding calibration curve for this isomer is obtained by dilution of the initial solution. However, the procedure followed to achieve these standards by irradiation involves certain errors, which could be avoided if *cis*-resveratrol were available commercially as a standard.

The calibration of *cis*-resveratrol and its glycoside obtained from the irradiated standards of *trans*-resveratrol provides good analytical characteristics, as can be observed from Table 2. However, taking into account the similarity between the mass spectra of the two isomers of resveratrol and their corresponding glycosides, the possibility is still open of using a single calibration curve, in the case of performing the quantification by MS. But for this alternative to be considered valid, the results for the concentration of *cis*-resveratrol calculated using the curve of *trans*-resveratrol should not differ significantly from those obtained by applying the curve for *cis*-resveratrol constructed with the irradiated standards of *trans*-resveratrol. To assess whether or not there is such a difference, the *t*-test for means of two paired samples, for 95% confidence, was applied to an identical series of samples, making a comparison of the concentrations of *cis*-resveratrol calculated from its own curve and those calculated from the curve of *trans*-resveratrol. The result was that there are no significant differences between the results obtained.

Thus by constructing a single calibration curve, from the synthetic compound, it is possible to quantify the four monomers when the detection system used is mass spectrometry. The analytical parameters, obtained from the calibration curves of

Table 2
Analytical parameters of the method

	trans-Resveratrol		*cis*-Resveratrol	
	MS (228 *m*/*z*)	DAD 305 nm	MS (228 *m*/*z*)	DAD 280 nm
Concentration range(mg/l)	2.5–50	2.5–50	1.23–40.06	1.23–40.06
r^2	0.9980	0.9997	0.9972	0.9997
Standard error	0.2324	0.3934	1.2434	0.2614
Detection limit	1.202	0.948	2.558	0.834
Quantification limit	4.005	3.16	8.525	2.781
Analytical sensitivity	0.4625	0.3533	0.9844	0.3211

trans- and *cis*-resveratrol by means of the two detection systems used, is given in the Table 2. It can be seen that both detection systems give similar results.

3.3. Recovery and repeatability

The recovery and repeatability of this analytical method has been studied, by means of the processing of samples of the same wine six times; a known quantity of *trans*-resveratrol had previously been added to this wine. In this way, a series of six results were obtained for each detection system; these are shown in Table 3.

It can be observed that the method provides an acceptable relative standard deviation (RSD) for the four compounds with both systems of detection, since the dispersion of the data is not very wide. Although the highest value of RSD is obtained for *trans*-resveratrol with MS detection, the mean value of concentration obtained by this system is closer to the known figure than that resulting from the detection by UV absorption, in spite of quantifying it at the wavelength of maximum absorption of this compound. It is also observed that for the spiked species, the recovery of the method of analysis is closer to reality when measured by the MS detection system than when measured by the UV absorption system. This could be an indication that for the latter system there exists a systematic error in the method. In contrast, the quantification by UV absorption is more accurate than by MS.

In addition it is notable that in the MS detection system, the lower RSD corresponds to *cis*-resveratrol, the compound present in the lowest concentration in the sample studied. This is explained by the high selectivity of this system of detection, which provides simpler chromatograms that are easier to integrate since no other compounds appear co-eluted with those of interest, thus avoiding a quantitation error.

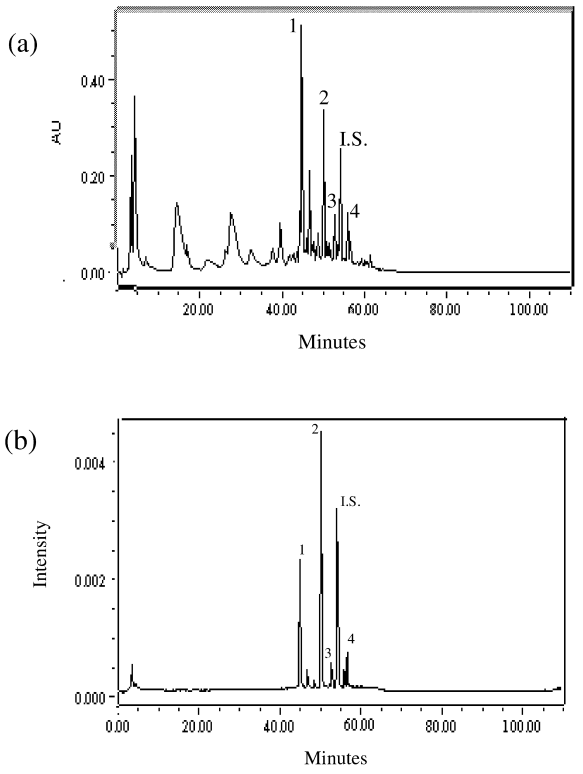

Fig. 3. Chromatograms of an extract of red wine. (a) Detection by UV absorption at 305 nm, (b) detection by MS in the SIM mode, at m/z 228.

In Fig. 3, the chromatograms obtained by UV detection (Fig. 3a) and by MS (Fig. 3b) of the same sample are presented. The simplicity and cleanness of the chromatogram provided by MS, in comparison

Table 3
Repeatability study of a red wine spiked with 15 mg/l of *trans*-resveratrol (six repetitions)

	Glycoside				Glycoside			
	trans-Resveratrol		*cis*-Resveratrol		*trans*-Resveratrol		*cis*-Resveratrol	
	MS	DAD	MS	DAD	MS	DAD	MS	DAD
Mean (mg/l)	1.302	1.598	1.945	1.909	15.606	11.278	0.838	0.720
SD	0.141	0.044	0.160	0.047	2.024	0.744	0.034	0.063
RSD (%)	10.846	2.753	8.219	2.456	12.970	6.594	4.018	8.775
Recovery (%)	–	–	–	–	98.83	71.42	–	–

with that obtained by UV absorption at 305 nm, is very evident. This difference in selectivity between the two systems of detection is due to the fact that, in a sample of wine, there is a greater chance of finding other compounds, apart from those of interest, which absorb at 305 nm than other compounds with molecular fragments of m/z 228.

3.4. Comparative study of the two systems of detection

To compare the two systems of detection and to check whether one of them shows any systematic error, the values obtained by both systems for a series of samples composed of different types of wine fortified with *trans*-resveratrol were analyzed by means of regression. A regression line was constructed in which on the *x*-axis were represented the results of detection by UV absorption and on the *y*-axis the results of detection by MS; an *r* value of 0.9912 was obtained for this line. The slope (*b*) and intercept (*a*), together with the confidence limits for 95%, were: $b=1.7355\pm0.042$ and $a=-2.0048\pm2.301$, which indicates, with respect to the ideal values ($b=1$, $a=1$), that no significant differences exist in the case of the intercept, whereas they do exist in the case of the slope, since a value greater than 1 would indicate the existence of a systematic error in one of the methods.

Lastly, the method of analysis was applied to the four derivatives of resveratrol in various types of wine, and the results obtained are shown in Table 4.

4. Conclusions

The development of hybrid instrumental techniques widens the alternatives for analyzing compounds present in complex samples and at low concentrations. This is the case of the analysis of resveratrol in wines.

The method of analysis of resveratrol in wines proposed in this study offers many advantages. First, the SPE stage provides good repeatability and selectivity, speed, minimum manipulation of samples and, furthermore, is a procedure that can be fully automated. Second, the quantitation of the four monomers of resveratrol by means of mass spectrometry, employing a single calibration curve constructed from internal standards prepared from a commercially-available standard, simplifies the procedure and offers more reliability. Third, detection by mass spectrometry, being more selective, permits simpler, clean chromatograms to be obtained which facilitate the quantitation.

Table 4
Concentrations (mg/l) found in different types of wine

	trans-Resveratrol glycoside		*cis*-Resveratrol glycoside		*trans*-Resveratrol		*cis*-Resveratrol	
	MS	DAD	MS	DAD	MS	DAD	MS	DAD
Red wine – 1	0.965	1.893	1.191	1.656	n.d.	n.d.	n.d.	n.d.
Red wine – 3	0.727	0.418	0.920	1.524	n.d.	n.d.	n.d.	n.d.
Red wine – 4	0.840	1.447	0.918	0.909	n.d.	n.d.	n.d.	n.d.
Red wine – 5	1.543	2.300	1.040	1.548	n.d.	n.d.	n.d.	n.d.
Red wine – 6	1.223	1.882	1.194	1.505	n.d.	n.d.	n.d.	n.d.
Red wine – 9	1.300	1.912	1.315	1.626	1.073	1.274	1.174	1.024
Red wine – 10	0.727	0.418	0.765	1.015	0.836	0.787	1.295	1.065
Red port wine	1.281	1.812	2.484	2.222	n.d.	n.d.	n.d.	n.d.
Double macerated red wine – 1	6.968	9.442	7.816	5.301	2.009	2.157	2.758	1.660
Double macerated red wine – 2	7.354	8.504	10.553	7.091	2.000	1.844	2.159	1.482
Double macerated red wine – 3	4.078	5.076	6.944	5.002	1.387	1.439	1.917	1.315
White table wine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sherry wine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pedro Ximenez wine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Muscatel wine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Acknowledgements

This study was supported by the CICYT of the Spanish Ministry of Education and Science and FEDER of U.E. (project 1FD97-0683.01). The authors are indebted to Bodegas Osborne & Cia. for supplying samples.

References

[1] J.L. Ingham, Phytochemistry 15 (1976) 1791.
[2] R.G. Powell, M.R. Tepaske, R.D. Plattner, J.F. White, S.L. Clement, Phytochemistry 35 (1994) 335.
[3] R.J. Kumar, D. Jystostna, G.L.D. Krupadanam, G. Srimannarayana, Phytochemistry 27 (1988) 3625.
[4] J. Gorham, S.J. Coughlan, Phytochemistry 19 (1980) 2059.
[5] V.S. Sobolev, R.J. Cole, J.W. Dorner, J. AOAC Int. 78 (1995) 1177.
[6] R. Hain, B. Bieseler, H. Kindl, G. Schröder, R. Stöcker, Plant Mol. Biol. 15 (1990) 325.
[7] P. Langcake, R.J. Pryce, Phytochemistry 16 (1977) 1193.
[8] P. Langcake, A. Cornford, R.J. Pryce, Phytochemistry 18 (1979) 1025.
[9] R. Bessis, P. Jeandet, M. Adrian, A.C. Breuil, S. Debord, Rev. Oenologues 85 (1997) 5.
[10] R.J. Pryce, P. Langcake, Phytochemistry 16 (1977) 1452.
[11] M. Barlass, R.M. Miller, T.J. Douglas, Am. J. Enol. Vitic. 38 (1987) 65.
[12] A.A. Calderón, J.M. Zapata, R. Muñoz, M.A. Pedreño, A.R. Barceló, News Phytol. 124 (1993) 455.
[13] J.P. Roggero, M.C. García-Parrilla, Sci. Aliments 15 (1995) 411.
[14] J.P. Blond, M.P. Denis, J. Bezard, Sci. Aliments 15 (1995) 347.
[15] F. Bravo, Alimentaria, En-Febr (1996) 71.
[16] I. Hurtado, P. Caldú, A. Gonzalo, J.M. Ramón, S. Mínguez, C. Fiol, J. Agric. Food Chem. 45 (1997) 1283.
[17] C.S. Stockley, in: B. Rankine (Ed.), Information Report, Austr. Soc. Wine Educ. (1995) 1.
[18] P. Fürst, Proc. Nutr. Soc. 55 (1996) 945.
[19] E.N. Frankel, A.L. Waterhouse, P.L. Teissedre, J. Agric. Food Chem. 43 (1995) 890.
[20] S. Brun, Cah. Nutr. Diét. 30 (1995) 224.
[21] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W.W. Beecher, H.H.S. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Science 275 (1997) 218.
[22] M.C. Cravero, V. Dell'Oro, Sevi No. 2557 (1995) 2759.
[23] R. Lamuela-Raventós, A.I. Romero-Pérez, A.L. Waterhouse, M.C. de la Torre-Boronat, J. Agric. Food Chem. 43 (1995) 281.
[24] M. Fregoni, L. Bavaresco, D. Petegolli, M. Trevisan, C. Ghebbioni, Vignevini 6 (1994) 33.
[25] D.M. Goldberg, E. Ng, A. Karumanchiri, J. Yan, E.P. Diamandis, G.J. Soleas, J. Chromatogr. A 708 (1995) 89.
[26] A. Gonzalo, P. Vidal, Alimentación, Equipos Tecnología October (1995) 67.
[27] K.D. McMurtrey, J. Minn, K. Pobanz, T.P. Schultz, J. Agric. Food Chem. 42 (1994) 2077.
[28] K.D. McMurtrey, in: T.R. Watkins (Ed.), Wine Nutritional and Therapeutic Benefits, ACS Symposium Series 661 (1997) 45–55.
[29] R. Pezet, V. Pont, P. Cuenat, J. Chromatogr. A 663 (1994) 191.
[30] T. Okuda, K. Yokotsuka, Am. J. Enol. Vitic. 47 (1996) 93.
[31] G. Revel, T. Hogg, C. Santos, J. Int. Sci. Vigne Vin 30 (1996) 31.
[32] A. Antonelli, C. Fabri, G. Lercker, Chromatographia 42 (1996) 469.
[33] D.M. Goldberg, J. Yan, E. Ng, E.P. Diamandis, A. Karumanchiri, G.J. Soleas, A.L. Waterhouse, Anal. Chem. 66 (1994) 3959.
[34] G.J. Soleas, D.M. Goldberg, E.P. Diamandis, A. Karumanchiri, J. Yan, E. Ng, Am. J. Enol. Vitic. 46 (1995) 346.
[35] D.M. Goldberg, J. Yan, E. Ng, E.P. Diamandis, A. Karumanchiri, G.J. Soleas, A.L. Waterhouse, Am. J. Enol. Vitic. 46 (1995) 159.
[36] A. Gioacchini, A. Roda, G.C. Galleti, J. Chromatogr. A 730 (1996) 31.
[37] E. Celotti, R. Ferrarini, R. Zironi, S.L. Conte, J. Chromatogr. A 730 (1996) 47.
[38] V. Vacca, G. Madau, M.A. Franco, P. Fenu, Riv. Sci. Alimentazione 4 (1995) 565.
[39] F. Mattivi, Z. Lebensm. Unters.-Forsch. 196 (1993) 522.
[40] C. Chilla, D.A. Guillén, C.G. Barroso, J.A. Pérez-Bustamante, J. Chromatogr. A 750 (1996) 209.
[41] D.A. Guillén, C.G. Barroso, J.A. Pérez-Bustamante, J. Chromatogr. A 730 (1996) 39.
[42] D.A. Guillén, Doctoral Thesis, University of Cadiz, Cadiz, 1994.
[43] J. Pezzuto, K.P.L. Bhat, Sevi No. 2782/83 (1999) 4169.
[44] A. Bertelli, Sevi No. 2782/83 (1999) 4189.