ELSEVIER

Food Chemistry **50** (1994) 313–315

0308-8146/94/$7.00
Analytical Methods Section

Separation and identification of phenolic acids in wine vinegars by HPLC

M. C. García-Parrilla,[a] M. León Camacho,[b] F. J. Heredia[a] & A. M. Troncoso[a]*

[a] *Department of Nutrition and Food Science, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain*
[b] *Instituto de la Grasa y sus Derivados, 41013 Sevilla, Spain*

(Received 21 July 1993; revised version received 20 October 1993; accepted 25 October 1993)

The phenolic fraction of wine vinegars was studied by HPLC. The technique proposed gives a good separation of the great majority of the phenolics in current wine vinegar as well as in Jerez vinegars. The identification of the peaks was based on UV-spectra and capacity factors. Some differences in composition were found in the samples analysed.

INTRODUCTION

Phenolic compounds play an important role in wines and have been exhaustively reviewed. Their relation with many phenomena occurring in wines (browning, aging ...) as well as with organoleptic properties, taste and colour and their taxonomical significance, has been clearly demonstrated (Singleton & Esau, 1969; Bourzeix, 1982; Glories, 1984; Condal *et al.*, 1989).

Wine vinegar produced in areas with oenological tradition, such as Jerez, is a product of major economical importance and much appreciated in gastronomy. The use of its volatile compounds as an indicator of quality has been previously reported by some authors (Troncoso & Guzmán, 1987; Blanch *et al.*, 1992). These vinegars are made by the traditional 'solera' system used for 'Jerez' wines and are subsequently aged for variable periods of time up to 25 years to the highest quality. The phenolic composition of these vinegars has not been studied in detail, and may be a useful indicator of origin, aging, elaboration and acceptance. Phenolic acids were formerly isolated in conventional wine vinegar by means of TLC (Díez de Bethencourt *et al.*, 1980). No other studies of phenols in wine vinegar have been reported.

The development of HPLC techniques in recent years has advanced the study of the phenolic composition of wines. The chromatographic behaviour of several phenolic compounds was studied by Wulf and Nagel (1976) and other authors have performed quantitative analysis of phenolic acids (Bertrand & Salagoity-Auguste, 1980). Several applications of HPLC in the oenology field may be found in the bibliography. Some of them deal with taxonomy studies (Archier *et al.*, 1992), while others attempt to explain the evolution of the phenolic compounds throughout the vinification and elaboration processes (Singleton & Trousdale, 1983; García-Barroso *et al.*, 1987).

* To whom correspondence should be addressed.

The aim of this work was to identify some phenolic components in wine vinegars by means of HPLC and to test the method using two different types of wine vinegar, Jerez wine vinegar and wine vinegar produced by quick acetification processes (conventional wine vinegars), in order to note the special characteristics of Jerez vinegars.

MATERIALS AND METHODS

Apparatus

Sample concentration was performed using a Büchi rotavapor. The HPLC system consisted of a 1050 Hewlett Packard Pump, a Multiple Wavelength Detector HP1050 and a Universal Injector HP1050. Chromatograms were recorded on an HP3396 Series II Integrator. Spectra were also recorded on the HPLC Detector.

The column used was a Merck Lichrospher 100 RP-18 (250 × 4 mm i.d.), particle size 5 μm, protected by a guard cartridge.

Chromatographic conditions

- Mobile phase: acetic acid2-propanol/methanol/water (2:2:9:87 (v/v/v/v)).
- Column temperature: 25°C
- Detection UV absorption, 280 nm.
- Time of chromatogram: 40 min.
- Elution program: a linear gradient of flow from 1 ml to 1·5 ml in 40 min.
- Volume injected: 5 μl.

The mobile phase used was essentially according to that proposed by Roston and Kissinger (1981); the introduction of 2-propanol clearly improved resolution.

Table 1. Retention times (T_R), capacity factors (k), selectivity coefficients (α) and λ_{max} of phenolic compounds

Number	Substance	T_R	k'	α	λ_{max}
1	Gallic acid	3·43	0·49	—	272
2	Pyrogallic acid	3·80	0·65	1·33	—
3	Protocatechuic acid	5·98	1·56	2·46	261
4	Catechin	8·08	2·51	1·57	278
5	Gentisic acid	9·62	3·18	1·27	—
6	*p*-Hydroxybenzoic acid	10·52	3·57	1·12	255
7	Vanillic acid	13·25	4·76	1·33	261
8	Caffeic acid	14·47	5·29	1·11	323
9	Syringic acid	15·45	5·71	1·08	—
10	Vanillin	18·59	7·08	1·24	269
11	*p*-Coumaric acid	26·75	10·63	1·50	309
12	Veratric acid	34·16	13·85	1·30	263
13	*m*-Coumaric acid	37·92	15·48	1·12	—

Reagents

All solvents were HPLC-grade and degassed with helium. Samples of phenols used as standards were obtained from Merck, Fluka, Carlo Erba and Sigma, and were used as received.

Sample preparation

Extracts of polyphenolic substances in vinegars were obtained from the wine vinegar samples essentially in the same way as described by Díez de Bethencourt *et al.* (1980) for wine and vinegar samples. The concentration was performed below 30°C, the concentrate extracted with diethyl ether (4 × 15 ml) and the residue dissolved in methanol water (2:1 (v/v)) (3 ml). The solution obtained was filtered through a 0·45 μm Millipore membrane and kept in the refrigerator if not injected into the chromatograph immediately.

In order to acquire the spectra of small peaks, it was necessary to dissolve the residue in a small volume of methanol (0·5 ml).

Standard preparation

The concentration of the stock solutions of each phenolic compound was of 1 mg ml^{-1}. These solutions were stored in the refrigerator for a period no longer than one month.

Identification

The identification of the chromatographic peaks was carried out according to two criteria. A comparison between the capacity factors (k) and spectra of unknown sample components and those exhibited by the pure phenolic acids (13 in total) in standards solutions (Table 1) was performed. The λ_{max} values for these compounds agree well in samples and standards and with those found in the literature (Cartoni *et al.*, 1991; Archier *et al.*, 1992). Retention times and selectivity coefficients are also shown in Table 1.

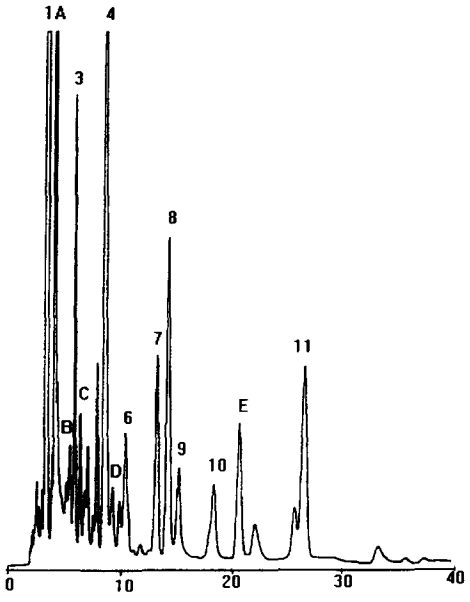

Fig. 1. Chromatogram obtained from a 'Jerez' wine vinegar. Peak numbers are defined in Table 1.

RESULTS AND DISCUSSION

The method was performed with two kinds of vinegar sample belonging to those types of vinegar most consumed in Spain, as described earlier. Figure 1 shows a representative chromatogram corresponding to a Jerez sample whilst Fig. 2 represents that exhibited by a conventional wine vinegar. Components named A–E are still unidentified.

The proposed method allows the analysis of the characteristic phenolic acids and aldehydes of vinegars in 40 min using isocratic elution. Ten phenolic compounds have been identified, some of which have not been previously reported in vinegars. Table 2 shows the individual percentages of the free phenolic compounds found in the samples analysed. Gallic acid is the main component of the fraction, followed by caffeic, *p*-coumaric and catechin. Catechin is an important component of this fraction, except in sample 2 (Jerez wine vinegar aged during 25 years). One reason for this reduction could be the polymerization of catechins

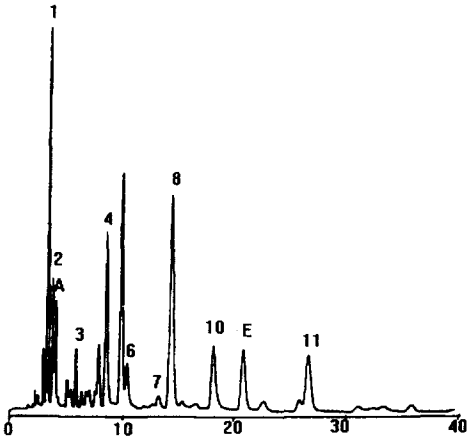

Fig. 2. Chromatogram obtained with a vinegar by quick acetification. Peak numbers are defined in Table 1.

Table 2. Relative percentages of phenolic acids in the vinegars

Number	Substance	Relative percentages			
		Jerez vinegars		Conventional vinegars	
		1	2	3	4
1	Gallic acid	48·0	57·0	21·2	50·9
2	Pyrogallic acid	—	—	3·0	2·1
3	Protocatechuic acid	4·9	4·8	3·4	4·9
4	Catechin	16·9	—	14·7	6·0
5	Gentisic acid	—	3·8	—	2·8
6	*p*-Hydroxybenzoic acid	3·3	4·2	4·9	3·7
7	Vanillic acid	5·0	3·1	1·9	1·4
8	Caffeic acid	8·3	11·8	28·6	14·1
9	Syringic acid	2·9	2·3	—	1·8
10	Vanillin	3·1	2·5	10·8	3·2
11	*p*-Coumaric acid	7·6	10·5	11·5	9·1
12	Veratric acid	—	—	—	—
13	*m*-Coumaric acid	—	—	—	—

during the aging process, which results in the formation of other polyphenolic substances.

In general, there appear to be some differences in the qualitative composition of wine vinegars and Jerez wine vinegars. Pyrogallic acid has importance only in the case of rapid-fermentation vinegars. As expected, syringic acid, a product of bacterial fermentation (Díez de Bethencourt *et al.*, 1980) is a usual component of these vinegars, but veratric and *m*-coumaric acids, characteristic products of Jerez wines (García Barroso *et al.*, 1983), were not detected in any of the vinegars.

Díez de Bethencourt *et al.* (1980), separated some phenolic compounds in vinegars by TLC and reported that the presence of protocatechuic, vanillic and *p*-hydroxybenzoic acids in wine vinegars should be proof of their wine origin. The source of phenolic compounds in Jerez vinegar samples can be either the must or the oak barrels in which they are aged. However, vinegars produced by a quick acetification process are not aged, thus the only possible source of phenolic substances is the must. During acetification these compounds will give rise to changes in vinegar composition, due to the oxidation process subsequent to the airation to which they are submitted.

HPLC is a suitable technique not only for the comparison of vinegars of different types but also to follow the acetification process. Therefore the proposed method could be of interest for further research concerning the influence of factors such as the substrate used, elaboration procedure and aging process in the vinegar obtained.

REFERENCES

Archier, P., Coen, S. & Roggero, J. P. (1992). Composition phénolique de vins issus de monocépages. *Sci. Aliment.*, **12**, 453–66.

Bertrand, A. & Salagoïty-Auguste, M. H. (1980). Dosage des acides-phenols dans les vins par chromatographie liquide a haute-pression. *Ann. Fals. Exp. Chim.*, **74**, 17–28.

Blanch, G. P., Tabera, J., Sanz, J., Herraiz, M. & Reglero, G. (1992). Volatile composition of vinegars. Simultaneous distillation-extraction and gas chromatographic–mass spectrometric analysis. *J. Agric. Food Chem.*, **40**(6), 1046–9.

Bourzeix, M. (1982). Compuestos fenólicos de uva y vino. In *Enología, Temas actuales*, ed. C. Llaguno. Fondo Editorial Anque, Madrid, Spain, pp. 177–217.

Cartoni, G. P., Coccioli, F. & Pontelli, L. (1991). Separation and identification of free phenolic acids in wines by high performance liquid chromatography. *J. Chromatogr.*, **537**, 93–9.

Condal, M., Gómez, J. M., De la Torre, M. C. & Villanueva, J. (1989). Influencia del licor de tiraje sobre el contenido polifenólico del cava. *Anal. Bromatol.*, **41**(1), 73–80.

Díez de Bethencourt, C., Gómez-Cordovés, C. & de la Calera, J. G. (1980). Compuestos fenólicos de pequeño peso molecular. Su influencia en la calidad de los vinagres. *Rev. Agroq. Tecnol. Alim.*, **20**(2), 247–55.

García-Barroso, C., Cela Torrijos, R. & Pérez-Bustamante, J. A. (1983). HPLC separation of benzoic and hydroxycinnamic acids in wines. *Chromatographia*, **17**(5), 249–52.

García-Barroso, C., Cela Torrijos, R. & Pérez-Bustamante, J. A. (1987). A study of behavioural groupings of polyphenolic species in "Fino" sherry wine. *Z. Lebensm. Unters. Forsch.*, **185**, 307–14.

Glories, Y. (1984). La coleur des vins Rouges. I. Les equilibres des anthocyanes et des tanins. *Connaiss. Vigne Vin*, **18**(3), 195–217.

Roston, D. A. & Kissinger, P. T (1981). Identification of phenolic constituents in commercial beverages by liquid chromatography with electrochemical detection. *Anal. Chem.*, **53**(11), 1695–9.

Singleton, V. L. & Esau, P. (1969). *Phenolic Substances in Grapes and Wines and Their Significance*, Adv. Food Res. Suppl. 1. Academic Press, New York, USA.

Singleton, V. L. & Trousdale, E. (1983). White wine phenolics: Varietal and processing differences as shown by HPLC. *Am. J. Enol. Vitic.*, **34**(1), 27–34.

Troncoso, A. M. & Guzmán, M. (1987). Volatile components in Andalusian vinegars. *Z. Lebensm. Unters-Forsch*, **185**, 130–3.

Wulf, L. W. & Nagel, C. W. (1976). Analysis of phenolic acids and flavonoids by high-pressure liquid chromatography. *J. Chromatogr.*, **116**, 271–9.