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Analytical, Nutritional and Clinical Methods Section

Liquid chromatographic determination of organic acids in txakoli from Bizkaia

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A direct injection high performance liquid chromatographic (HPLC) procedure has been developed for the separation and determination of the major carboxylic acids in wine. The acids are separated on a reversed-phase column C-18, eluted with dilute acetic acid and monitored by UV spectrophotometry at 254 nm. Tartaric acid, malic acid, lactic acid, and citric acid can be determined by this method. The method has been applied to grape must, red wine and white wine without interference from sugars. The samples analysed were txakoli from Bizkaia (wine from the north of Spain). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Many analytical methods are reported in literature for the separation of organic acids. The more usual methods are enzymatics (Joyeux & Lafon-Lafourcade, 1979; Boehringer Mannheim, 1992), electrophoresis (Levi *et al.*, 1993) and chromatography (Symonds, 1978; Auguste & Bertrand, 1980; Rajakylä, 1981; Gonnet & Marich, 1982; Goiffon *et al.*, 1985; Rapp, 1988; Polo *et al.*, 1986). Some of these methods haven been devoted to the determination of the wine acids and the control of malolactic fermentation (Brogley *et al.*, 1993). This fermentation is very important, for wines coming from cool wine regions where grapes have a high acidity due to relatively high concentration of malic acid. This substance and the tartaric acid are the main substances responsible for wine pH.

The aim of this work is to characterize txakoli (Escobal *et al.*, 1993), a kind of wine from the Bizkaia region, located in the north of Spain with an Atlantic climate. The txakoli shows acidic characteristics and it is known that in this kind of wine the exent of malolactic fermentation is very important (Del Campo *et al.*, 1990; Bravo, 1992). Malic acid is of particular interest due to an additional naturally ocurring fermentation step that causes the wine to become less acidic (González-Larraina, 1987).

We have used the traditional methods recommended in the European Community Bulletin to determine the tartaric acid, malic acid and lactic acid (Diario de las Comunidades Europeas, 1990; OIV, 1990). However, these methods involve either tedious separation of organic acids over an anion exchange column; dilution or concentration procedures or quantitation are achieved through the laborious collection of numerous measured fractions (Shaw & Wilson, 1983; Yokosuka *et al.*, 1983; McCord *et al.*, 1984; Polo *et al.*, 1986; Calull *et al.*, 1992). Consequently, these methods have found very limited applications in the industrial analytical laboratory.

The HPLC analysis of organic acids in grape juice and wine generally involves a sample preparation step. To date, in most cases, direct injection chromatographic evaluated systems have evidenced coelution or masking problems of neutral compounds (sugars, glycerol and ethanol) with the organic acid, probably due to the low wavelength (210–220 nm) utilized or the use of refraction index detection or mixtures of methanol and water as mobile phases.

In this paper, a liquid chromatographic procedure which separates the main wine acids is reported. This method is quick; the four main acids are analysed in 6 min and sample preparation procedures are not necessary as the wine is directly injected, thus avoiding the possible error due to losses in recovery in the anion exchange pretreatment. The column is the more usual method used (C-18); the mobile phase is the cheapest and the acetic acid seems to be less corrosive to stainless steel than inorganic acids (HNO_3, H_2SO_4, etc.).

MATERIALS AND METHODS

High performance liquid chromatography

The samples were analysed using a Model HP-1090 Hewlett Packard liquid chromatograph with Diode Array Detector and a C-18 Supelcosil (25 cm×4.6 mm)

5 μm column, with a 5 μm RP-18 guard column. The column was operated at –40°C using 0.25% (v/v) acetic acid as the mobile phase and a flow rate of 0.6 ml/min. The injection volume was 2 μl.

Standard solution and calibration

Tartaric, lactic, malic and citric acids were purchased from Aldrich Chemicals. The experiment was carried out with a stock solution of the main acids found in wine and grape must at concentrations of 10 g/l for tartaric acid, 10 g/l for malic acid, 0.5 g/l for lactic acid and 0.5 g/l for citric acid. These concentrations are the highest values usually found in wine. Calibration solutions were prepared by dilution with distilled water and injected six times.

Grape must and wines

Grape must from txakoli, red wine (red txakoli) and white wine (white txakoli) from Bizkaia, Spain, were used. The grape must samples were stored at –20°C and the red and white txakoli at –4°C. The samples were filtered with a 0.45 μm nylon membrane and injected directly into the chromatographic system.

RESULTS AND DISCUSION

As is shown in Fig.1 and Table 1, a good resolution between the different peaks was obtained for the tartaric, malic, lactic and citric acids.

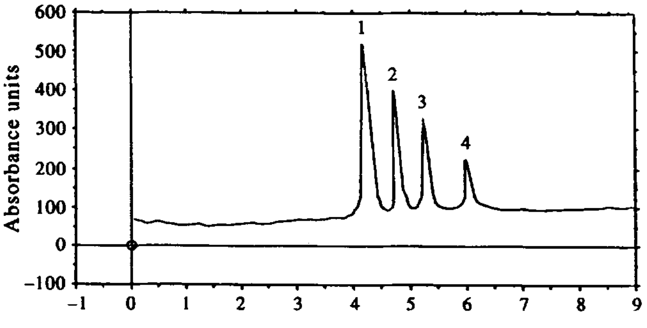

Fig. 1. Separation of standard mixture. Chromatographic conditions: Column (C-18) Supelcosil 30 cm×4.6 mm, I.D. 5 μm. Temperature 40°C, UV monitoring at 254 nm, eluent 0.25% acetic acid at flow rate 0.6 ml/min. Peaks: 1 = tartaric, 2 = malic, 3 = lactic, 4 = citric.

Table 1. Values of retention time (t_R), capacity factor (k') and resolution (Rs) between peaks

Acids	t_R (min)	k'	Rs[a]
Tartaric	4.29	1.86	3.30
Malic	5.03	2.35	1.64
Lactic	5.54	2.69	2.08
Citric	6.25	3.16	—

[a] The resolution has been calculated between the peak and the next

Figure 1 shows the chromatogram obtained for a standard mixture: tartaric acid 5 g/l, malic acid 4 g/l, lactic acid 0.1 g/l and citric acid 0.3 g/l. Values of retention time, capacity factor and resolution between different peaks can be found in Table 1.

Table 2. Values of within day and between day reproducibilities in a stock solution (tartaric acid 5 g/l, malic acid 4 g/l, lactic acid 0.1 g/l and citric acid 0.3 g/l)

Compound	Within day (n = 10) RSD (%)	Between day (n = 10) RSD (%)
Tartaric acid	3.65	3.94
Malic acid	0.98	1.08
Lactic acid	1.29	1.48
Citric acid	4.36	4.62

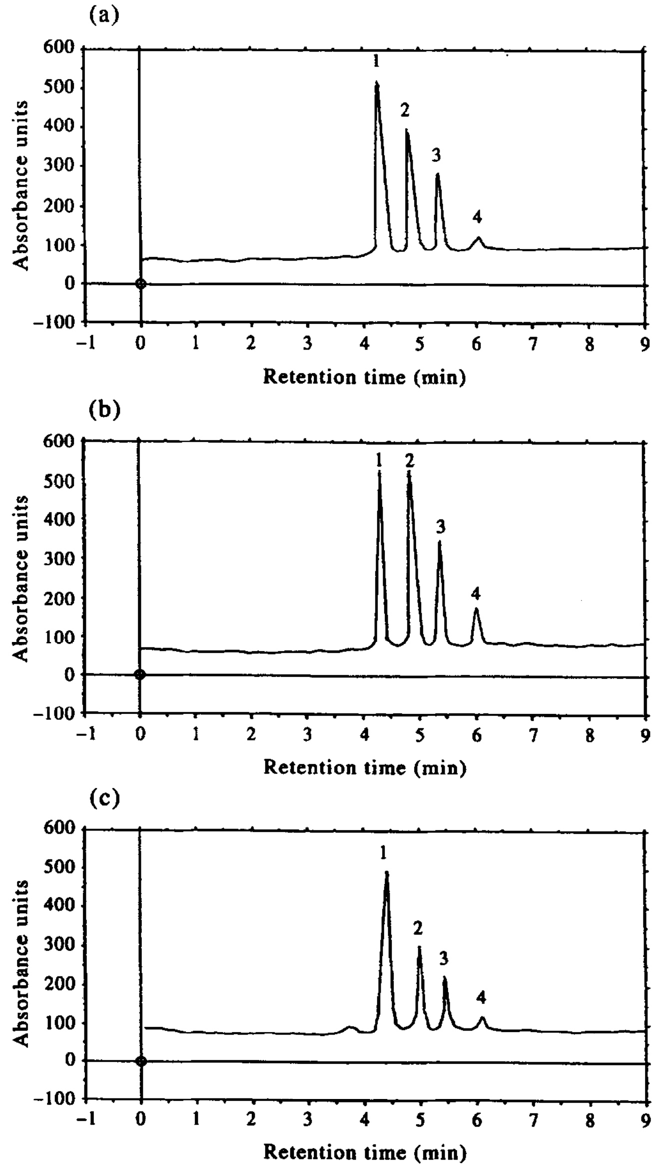

Fig. 2. Chromatograms corresponding to a white txakoli (a), a red txakoli (b) and a grape must of txakoli (c). Conditions as in Fig. 1.

Table 3. Determination of tartaric, malic, lactic and citric acid in grape must of txakoli from Bizkaiala; application of method proposed

Variety	Tartaric (g/l)	Malic (g/l)	Lactic (g/l)	Citric (g/l)
Albariño	5.15	5.90	0.106	N.D.
Chardonay	3.97	8.99	0.124	N.D.
Folle blanche	7.15	7.84	0.077	N.D.
Pinot	5.09	3.43	0.077	N.D.
Sauvignon	4.63	3.55	0.118	N.D.
Riesling	8.93	3.83	0.391	N.D.

N.D., not detected.

The four acids were resolved in only 6 min, without any interference peak. The sugars presented coelution with the organic acid ($t_R = 5.17$ min) but at 254 nm they were not detected. Calibration lines were constructed in the range between the value in the stock solution (maximum level found in the literature for txakoli) and a ten-fold dilution. Good linear responses (correlation coefficients > 0.9999) were obtained for the four acids in the examined range: tartaric acid 0.1–10 g/l; malic acid 0.1–10 g/l; lactic acid 0.05–0.5 g/l; and citric acid 0.05–0.5 g/l.

Table 2 shows the results obtained for the within and between reproducibilities for a standard sample of tartaric acid 5 g/l, malic acid 4 g/l, lactic acid 0.1 g/l and citric acid 0.3 g/l.

Figure 2 shows the chromatogram for a white txakoli (a), a red txakoli (b) and a grape must from txakoli (c). These chromatograms show that the application of this method is possible for all the steps of wine elaboration: grape must and bottled wine and that the application is not only possible for white wine but also for red wine.

Table 3 collects the results obtained for the analysis of different txakoli grape must. These samples correspond to six varieties of grape selected for their importance in the txakoli elaboration processes and they are from the 1992 vintage. The variety election in cool climates is more critical for the quality of final wine than in warm climates. In general, varieties with short maduration times are better for cool regions, high latitudes or elevated altitudes and usually when temperature and light are limited.

The proposed method has been used to monitor the exent of malolactic fermentation in txakoli samples by determining the levels of malic and lactic acids.

CONCLUSIONS

Reversed-phase chromatography with a UV detector is a quick and useful method to determine the organic acids in wine. No pretreatment is needed and the compounds are separated and quantified in 6 min. The coelution with sugars is avoided by detection at 254 nm. This method has been applied for the first time to characterize and determine the organic acids in txakoli.

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