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Determination of acids and volatile compounds in red Txakoli wine by high-performance liquid chromatography and gas chromatography

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Abstract

The Txakoli is a wine from the north of Spain with acid characteristics and medium ethanol content. We report the comparative results obtained from red Txakoli elaborated with different grape varieties. We have used chromatographic methods for the characterization of this wine. The volatile compounds were analyzed by means of gas chromatography and the organic acid content was determined by a newly validated liquid chromatographic procedure. The aim of this study is to characterize the red Txakoli and to know the major differences between Hondarrabi Beltza grape variety and the other varieties. Hondarrabi Beltza grape variety is the one which was awarded the Generic Label. The major differences observed in the samples obtained from Hondarrabi Beltza variety are their higher contents in ethanol, propanol, ethyl acetate and tartaric and malic acids.

Keywords: Wine; Food analysis; Organic acids; Volatile organic compounds

1. Introduction

The Txakoli from Biscay is a type of wine produced in the Basque Country, a region located in the north of Spain under Atlantic climate, which is undergoing a strong increment in its production due to the aid provided by Autonomous Governmental Institutions and to the fact of having been awarded with the Generic Label [1]. Hondarrabi Beltza grape variety is the one which was awarded the Generic Label. The aim of this study is to characterize the red Txakoli wine and to know the major differences between wine obtained from Hondarrabi Beltza grape variety and the other varieties.

This work is part of a project whose object is the chemical characterization of Txakoli from Biscay [2], as well as the study of several biochemical processes that can take place during the stage of vinification. One of our works studies the influence of malolactic fermentation [3,4] on this kind of wine, and as it can be inferred from the obtained results, the modification of several enological parameters (pH, volatile acidity, total acidity,...) considerably affects its stability.

It is necessary to mention that, up to this moment, Txakoli is a wine meant to be consumed within the year of its production and not to be reserved. The Txakoli wine presents acid characteristics and it is known that the malolactic fermentation in this kind of wine is very important [4,5]. Malic acid is of particular interest due to an additional naturally occurring fermentation stage that causes the wine to become less acid.

In this work we have determined the major

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organic acids and the volatile components of Txakoli by chromatographic methods. The results obtained from Hondarrabi Beltza variety were compared with other varieties.

The European Community Bulletin describes several methods for the determination of tartaric, malic and lactic acid [6]. However, these methods are tedious because they involve either separations of organic acids over an anion-exchange column or different enzymatic methods [7–9]. In our case, chromatography was applied to determine these compounds. The chromatographic methods are considered to be valid alternatives to enzymatic methods for the determination of carboxylic acids in wine. All the carboxylic acids can be chromatographically determined with shorter analysis time and similar accuracy [10].

A newly validated liquid chromatographic procedure has been applied [11] which separates the main wine acids (tartaric acid, malic acid, lactic acid, citric acid and succinic acid). The method is fast, the five main acids are analyzed in a 10-min chromatogram, sample preparation procedures are not necessary and wine is directly injected thus avoiding the possible errors due to recovery losses in the pretreatment. Coelution or masking problems of neutral compounds [12–16] (sugars, glycerol and ethanol) have been avoided as the detection wavelength has been studied and stabilised at 254 nm for all acids except succinic (210 nm).

The column used is the C_{18}, which is the most common one, and the mobile phase is less corrosive for the stainless steel than inorganic acids.

We have used the gas chromatography methods for the determination of some volatile compounds in this fresh wine obtained from different varieties of grapes [10,17]. This wine is a young one and the present compounds are to be found in the undamaged plant cells of the grape or formed during the processing of the grapes, by chemical, enzymatic and thermal reactions in grape must and during the alcoholic fermentation. This wine does not present a maturation bouquet caused by chemical reactions during maturation process.

We have analyzed several samples of red Txakoli wine which were elaborated from the different varieties described in the following section.

2. Experimental

2.1. Samples

Fifty samples, from 1993 and 1994 vintages, obtained from the original elaboration cellars were analyzed. These wines have been bottled when still young and they were six months old when being analyzed. The samples were elaborated from five different varieties of *Vitis vinifera:* Cabernet Sauvignon, Garnacha, Tempranillo, Tintorera and Hondarrabi Beltza.

2.2. Organic acids analysis by high-performance liquid chromatography

2.2.1. Instrumentation

The liquid chromatographic analyses were performed on a Hewlett-Packard Model HP-1090 liquid chromatograph equipped with a diode array detection (DAD) system. Separations were carried out on a C_{18} Supelcosil (25 cm×4.6 mm) 5-μm column, with a 5 μm RP-18 guard column. A loop of 2 μl was used.

2.2.2. Chromatographic conditions and detection

The chromatographic separation was carried out using an isocratic elution with 0.25% (v/v) acetic acid in water. The flow-rate of the eluent was 0.6 ml/min and the column temperature was 40°C. For single-wavelength monitoring, the detector was set at 254 nm for all acids except succinic acid (210 nm).

2.2.3. Standards

Tartaric, lactic, malic, citric and succinic acids were purchased from Aldrich. The experiment was carried out with a stock solution of the main acids found in wine and grape must at concentrations 5 g/l for tartaric acid, 5 g/l for malic acid, 2 g/l for lactic acid, 0.5 g/l for citric acid and 0.5 g/l for the succinic acid. These concentrations are the highest usually found in wine [18]. Calibration solutions

were prepared by dilution with distilled water and injected six times.

2.3. Volatile compounds analysis by gas chromatography

2.3.1. Instrumentation

The Txakoli wine was analyzed using a Model HP 5890 Hewlett-Packard gas chromatograph with a flame ionization detector and a 25 m×0.2 mm I.D. HP-FFAP column (0.3 μm). The sample was directly injected and preparation procedures were not necessary.

2.3.2. Chromatographic conditions and detection

The chromatographic conditions were as follows: initial temperature, 60°C (15 min); 3°C/min ramp to 150°C; and 15 min hold at 150°C. The injector and flame ionization detector temperature were both 200°C and nitrogen was used as the carrier gas, at a flow-rate of 0.6 ml/min. The volume injected was 1 μl.

Fig. 1 shows the chromatogram obtained when the wine sample is passed through a C_{18} Supelcosil column using the reversed-phase (HPLC) method. Fig. 2 shows the chromatogram obtained for a red wine by the gas-chromatographic method.

2.4. Potassium analysis by atomic absorption spectroscopy

2.4.1. Instrumentation

For the determination of this metal, several methods have been described in the literature. In this work we have followed the procedure recommended by the D.O.C.E., 1990 for the determination of potassium, which has been directly analysed by flame atomic absorption spectroscopy (FAAS).

The lamp (HCL) current was 30 mA with the wavelength at 589.0 nm. The slit width was 0.2 nm (N). The gases were air-acetylene and the flame was oxidant, leant and blue.

2.4.2. Standards

The calibration curve was made with a blank and four solutions of the following concentrations: 500, 1000, 1500 and 2000 mg/l. No matrix modifier was used in order to avoid an extra contamination and higher final costs and complexities of the determinations.

2.5. Total and the volatile acidity determination

The two acidities were measured by titration methods. These procedures were recommended by the D.O.C.E. Volatile acidity is determined in the solution obtained from distillation of wine and for

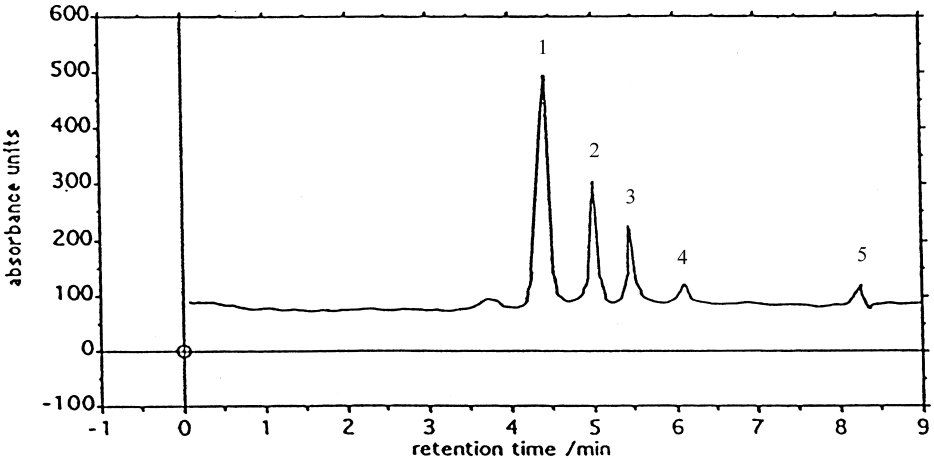

Fig. 1. Chromatogram obtained from a Red Txakoli wine sample by HPLC. (**1**) Tartaric acid, (**2**) malic acid, (**3**) lactic acid, (**4**) citric acid, (**5**) succinic acid.

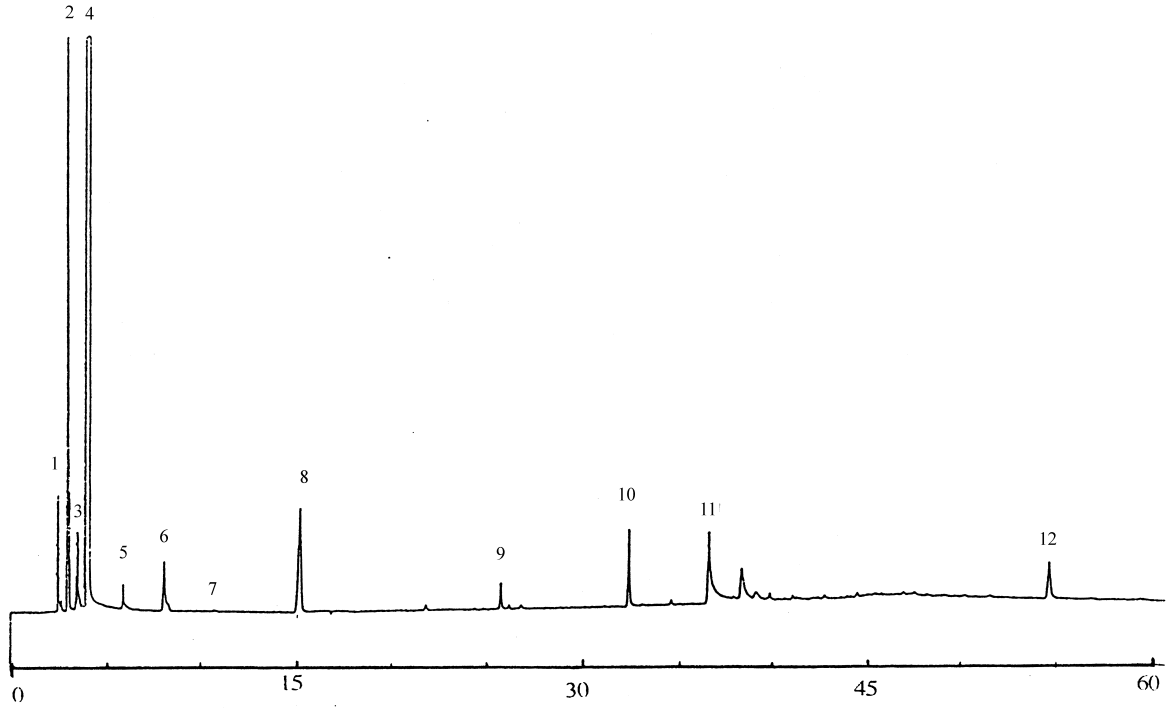

Fig. 2. Chromatogram obtained from a Red Txakoli wine sample by GC. (**1**) Acetaldehyde, (**2**) ethyl acetate, (**3**) methanol, (**4**) ethanol, (**5**) propanol, (**6**) isobutanol, (**7**) butanol, (**8**) isoamyl alcohol, (**9**) ethyl lactate, (**10**) acetic acid, (**11**) 2,3-butanediol, (**12**) 2-phenyl ethanol.

the total acidity, pretreatment of the sample is not necessary.

3. Results and discussion

Table 1 shows the content in volatile compounds found in samples of 1993 and 1994 vintages. The major differences were observed in samples obtained from Hondarrabi Beltza variety which presented the highest content in ethanol, propanol and ethyl acetate. This fact can mean a better adaptation of this variety to the growing zone.

Table 2 shows the contents in organic acids. Tartaric and malic acids found in wines made from Hondarrabi Beltza variety are similar to those from

Table 1
Results obtained from 1993 and 1994 vintages

Volatile compounds	Amount		
	Medium	Maximum	Minimum
Acetaldehyde (mg/l)	39.55	76.13	5.40
Ethyl acetate (mg/l)	109.98	174.70	10.14
Propanol (mg/l)	61.18	100.88	40.34
Isobutyl alcohol (mg/l)	84.36	101.87	72.97
Isoamyl alcohol (mg/l)	142.13	180.34	109.12
2,3-Butanediol alcohol (mg/l)	453.46	689.20	289.31
Ethyl lactate (mg/l)	65.05	100.21	–
Glycerol (g/l)	6.96	9.78	3.90
Methanol (mg/l)	31.22	50.82	10.67
Ethanol (%, v/v)	10.61	12.43	9.12

Table 2
Results obtained from 1993 and 1994 vintages

Organic acid	Medium	Standard deviation	Maximum	Minimum
Tartaric acid	2.65	0.396	3.28	2.30
Malic acid	2.01	1.120	3.32	1.12
Lactic acid	0.91	0.160	2.55	0.30
Citric acid	0.56	0.042	0.60	0.50
Succinic acid	0.54	0.213	0.68	0.21

the samples made from Cabernet Sauvignon. The samples obtained from these varieties have a lower pH because the tartaric and malic contents are higher than the value media.

The relation between glycerol and ethanol content has been established and can give us information about the development of the glyceropiruvic fermentation respective to the alcoholic fermentation. The relation found in the samples from Hondarrabi Beltza variety is similar to the value from the sample from Cabernet Sauvignon.

Table 3 shows the major correlations obtained for organic acids, acidities, pH and potassium. The correlation matrix obtained from volatile compounds is shown in Table 4. The correlation matrix shows a good degree of correlation between the ethanol, the ethyl acetate and the propanol. These compounds are the major difference found in the Hodarrabi Beltza variety. The existent correlation between the acetaldehyde, the 2,3-butanediol and the glycerol compounds wich are interrelated during the glycolysis, should be pointed out.

The processing of the data (obtained from 50 samples) by means of the regression analysis and analysis of variance (ANOVA) has allowed us to stabilise a mathematical relation between the pH, the potassium content and the total amount of acidity for the red Txakoli wine:

$$pH = 3.673328 + 0.000311\,[K] - 0.079288\,[A.T.]$$

We would like to mention that the method we have developed for the evaluation of the organic acids which are present in wine allow the quantification of

Table 3
Correlation matrix for organic acids, acidities, pH and potassium (correlation matrix for variables $X_1 ... X_9$)

	pH	Potassium	Tartaric acid	Malic acid	Lactic acid	Citric acid	Succinic acid	Total acid
pH	1							
Potassium	0.86	1						
Tartaric acid	−0.649	−0.558	1					
Malic acid	−0.339	−0.313	−0.422	1				
Lactic acid	−0.083	0.014	0.288	0.149	1			
Citric acid	0.465	0.306	−0.96	0.595	−0.301	1		
Succinic acid	−0.629	−0.898	0.547	−0.03	−0.276	−0.332	1	
Total acid	−0.831	−0.563	0.438	0.569	0.498	−0.309	0.161	1
Volatile acid	0.986	0.901	−0.559	−0.461	−0.106	0.345	−0.652	−0.838

Table 4
Correlation matrix for volatile compounds (correlation matrix for variables: $X_1 ... X_8$)

	Acetaldehyde	Ethyl acetate	Ethanol	Propanol	Isobutanol	Isoamyl alcohol	2,3-Butanediol	Glycerol
Acetaldehyde	1							
Ethyl acetate	0.23	1						
Ethanol	−0.253	0.57	1					
Propanol	−0.39	0.803	0.952	1				
Isobutanol	−0.73	0.232	0.569	0.699	1			
Isoamyl alcohol	−0.358	−0.157	−0.063	0.144	0.702	1		
2,3-Butanediol	0.641	0.055	−0.178	−0.398	−0.887	−0.931	1	
Glycerol	0.797	0.654	0.18	0.158	−0.291	−50.066	0.247	1

the acids that coelute with the neutral compounds, thanks to the selectivity which the detection at 254 nm presents. At that wavelength, sugars are scarcely absorbed whereas the acids present an important absorption, as it can be observed in the UV–visible spectrum.

By using the proposed method [7], the greatest interferences due to the coelution between the organic acids and the neutral compounds take place in the malic acid and the fructose. The detection at 254 nm makes possible the quantification of this acid because the fructose presents no absorption at this wavelength. Nevertheless, the succinic acid can be detected at 210 nm as no neutral compounds interfere under the above described conditions. A greater sensibility is achieved for this acid at this wavelength than at 254 nm.

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