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Identification and Quantification of some Wine Phenolic Acids by High-Performance Liquid Chromatography Equipped with Electrochemical Detector

Elektrokimyasal Dedektörlü HPLC Sisteminde Şarapta Bulunun Bazı Fenolik Asitlerin Belirlenmesi ve Tayini

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Abstract

Phenolic acids (gallic acid, p-hydroxybenzoic acid, syringic acid, 2,3-dihydroxybenzoic acid, ferulic acid, p-coumaric acid and vanillic acid) in red (Cabernet Sauvignon, Cabernet Franc, Merlot, Syrah, Carignan, Grenache,Bogazkere) and white wine (Ugni Blanch, Chardonnay, Narince) were analyzed electrochemically (+0.65 V vs. Ag/AgCl) by HPLC (Aglient 1100 series) on C_{18} silica column (Hichrom 5 C18, 7.75 x 300 mm, 5 µm particle size).The eluent used was methanol / 0.01 N phosphoric acid (30/70 v/v). The use of electrochemical detector (ECD) for wine phenols under isocratic conditions has not been reported yet. The evaluation of phenolic acids content in white and red wines brought about the importance of variety characteristics of grapes used in wine production. Changes in phenolic acids contents during two years production of Cabernet Frank, Cabernet Sauvignon and Carignan wines were determined to be caused by aging mechanisms.

Key words : Wine phenols, Electrochemical detector, HPLC

Introduction

Phenolic compounds of wine (phenolic acid and flavonoids) have an important role due to their some medical properties such as: antioxidant activity;antiinflammatory action ;inhibition of platelet aggregation;antimicrobial activities (Acar,1998;Bocco *et al.*,1998;Clifford *et al.*, 1996;Frankel and Andrew,1995) and certain organoleptic attributes (color,astringency) closely associated with these compounds(Ayala *et al.*,1997;Gomez–Cordoves *et al.*,1995; Rommel, *et al.*, 1996).

Over the past few years it has been accepted that a moderate red wine consumption is a factor benefical to human health. People of France and Italy, the two major wine–producing European countries, eat a lot of fatty food but suffer less from fatal heart strokes than people in North–America or in the northern regions of Europe, where wine is not consumed on a regular basis. This fact is known as "French paradox". It was demonstrated that phenolic molecules

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behave as radical scavengers and antioxidants and in such a way protect cholesterol in the LDL species from oxidation, a process thought to be at the origin of many fatal heart attacks (Lopez *et al.*, 2001; Natella *et al.*, 2001; Teissedre *et al.*,1995).

The types and concentrations of the phenols present depend mainly upon grape variety, ecologycal conditions, grape ripening and the techniques employed in producing the must and in the winemaking itself (Auw *et al.*, 1996;Canbas 1996; Heredina and Guzman-Choza, 1994, Oszmianski *et al.*, 1986).

Phenols of different grape varieties are chemically distinct, so their antioxidant activities are not the same.Phenols have high chemical activity and undergo complex transformations–which are not yet fully known– during initial crushing of berries, fermentation and aging of wines (Gomez-Cordoves and Gonzalez-San Jose 1995;Heredina and Guzman-Choza 1994; Oszmianski *et al.*, 1986; Ricardo-da Silva *et al.*, 1993).

Understanding the importance of these compounds many traditional techniques for their determination such as paper chromatography, thin–layer chromatography and column chromatography have now been superseded by high-performance liquid chromatography (Garcia-Vingera and Bridle 1995;Hertog *et al.*,1992a,Hertog *et al.*,1992b,Rebelo *et al.*, 2000).

Recently a HPLC system equipped with different detector (DAD,UV,FAD) has been used to separate phenolic compounds (Gil *et al.*,1995;Lamuela-Raventos and Waterhouse 1994; Vrhovsek *et al.*, 2001). Since similar chemical characteristics of polyphenols are the cause of many problems in separation the evaluation of their electrochemical properties came up to date.

The present study was designed to identify and determined some phenolic acids (gallic acid, p-hydroxybenzoic acid, syringic acid, 2,3-di hydroxybenzoic acid, ferulic acid, p-coumaric acid and vanillic acid) in red and white wines produced from different grape varities (*Vitis vinifera var.* Cabernet Sauvignon, Cabernet Franc, Merlot, Syrah, Carignan, Grenache, Ugni blanch, Chardonay, Narince, Bogazkere,) some of which were evaluated in two years by a HPLC system equipped with an electrochemical detector.

Materials and Methods

As materials were used grapes with *Vitis vinifera* origin var : Cabernet Sauvignon, Cabernet Franc, Merlot, Syrah, Carignan, Grenache, Ugni Blanch, Chardonnay, Narince and Bogazkere. Grapes were hand-harvested (at 20-25 brix) from a local vineyard (Menderes) in Izmir. All grapes were transported to the Food Engineering Department and crushed within 24 hours of harvest. During fermentation were used two different yeasts named Fermivin (*Sacchromyces cerevisiae 7013* INRA) and Fermirouge (*Sacchromyces cerevisiae 7000* INRA) supplied from Gist-Brocades Company. Pectollytic enzyme Rapidase-ex-color (100 Unit) were obtained also from Gist-Brocades. Gelatine used as a fining agent was supplied by Merck Chemical Company.

Processing White grapes of different origin were crashed, destemmed and pressed immediately in a hydroylic press (Com. Fad. Type G). Portions of juices (3L glasses) were collected, sulfited (50 ppm SO_2) and waited for 6 hours.After that juices were inoculated with Fermivin (2%) add pectolytic enzyme Rapidase-ex-color (2g/hL) allow to ferment to dryness at 27 ^{0}C.

Red grapes were crushed, destemmed and used for skin fermentation treatments. The crushed grapes were sulfited (25 ppm SO_2), inoculated with Fermirouge (2%), supplied with pectolytic enzyme Rapidase-ex-color (4g/hL) and allowed for skin fermentation lasting 5 days at 25^{0}C.The pomace was stirred and pushed down twice daily.After that alcohol fermentation was allowed to dryness in glass vessels. As a fining agent for white wines were used bentonite (150 g/hL) and gelatine (200 g/hL) for red ones were used only gelatine (250g/hL). After filtration (Seitz, D.6800 – Manheim, plate filter) and bottling wines were stored at 15^{0}C.

Chemicals Standart substances of gallic acid, p-hydroxybenzoic acid, syringic acid,2,3-di hydroxybenzoic acid, ferulic acid, p-coumaric acid, vanillic acid were supplied from Sigma Chemial Company. Solvents used for chromatography were methanol and phosphoric acid of HPLC ultragradient grade supplied by J.T. Baker and Riedel-de Haen AG respectively. Membranes (0.45 μm pore size) used for filtration of the samples were obtained from Sartorius AG (16555 Minisart©).

Apparatus The liquid chromatographic system (Aglient 1100 series) supplied by SEM Company (Izmir/Turkey) was equipped with an electrochemical detector (Aglient 1049 A Programmable Electrochemical Detector), a pump (Aglient 1100 series G1310A isocratic pump), a manual injector (HP 1100 series G1328A Rheodyne 7725I) with 20 μl loop and a chromatographic data processing software (Aglient ChemStation for LC Rev. A. 06. 03 [509]). The separation was performed using an octadecyl (C18) column (Hichrom 5 C18, 7.75 x 300 mm, 5 μm particle size).

Wine Samples White and red wines from different grape varieties of *Vitis vinifera* var. (Cabernet Sauvignon, Cabernet Frank, Merlot, Syrah, Carignan, Grenache, Ugni Blanch, Chardonnay, Narince, Bogazkere) were analyzed. Samples were opened, protected against sunlight and stored at 4°C. The samples were filtered to a 0.45μm membrane filter (Sartorius AG 16555 Minisart©).

Chromatographic Conditions The operating conditions were carried out at 21± 4°C.Separation of phenolic compounds was performed with a flow rate of 1 mL/min for 80 min. Amperometric detection was carried at 0.65 V (vs. Ag/AgCl, 0.5 μA fullscale) in the electrochemical cell. The solvents used and their proportions were: methanol and 0.01 N phosphoric acid (30/70 v/v). Both solvents were degassed (ELMA LC 30/H ultrasonic bath) before use.Each compound was tentatively identified by retention time under the same conditions.Quantitative determinations were carried out by the external standard method based on peak areas.

Statistical Analyses Significant differences among averages were obtained at 95% of significance level. As a confidence interval was taken 1.96. By using Post-Hoc test LSD was performed. For correlation analyses was used Spearman rank R test. Using multivariate exploratory techniques PCA (with cases and variables). Principal component analysis permits visualization of the original arrangement of wines in an n-dimensional space, by identifying the directions in which most of the information is retained. It is therefore possible to explain differences in the various wines by means of thee factors obtained from the generalized correlation matrix of the data sets and at the same time to determine which variables contribute most to such differentiation.

Results

The organic solvent selected for experiment was methanol (MeOH) due to the high solubility of phenols in this solvent. The retention behaviour of phenols was studied in the presence of an acid which prevented ionisation of the hydroxyl groups.The elution order and the retention chracteristics were: gallic acid (t_R =10.874 min); p-hydroxybenzoic acid (t_R =10.947 min);vanillic acid (t_R = 27.255 min);syringic acid (t_R=7.635min);2,3-di hydroxybenzoic acid (t_R = 38.028 min); p-coumaric acid (t_R = 47.927 min) and ferulic acid (t_R = 52.017 min). The concentarations of different phenolic acids are given on Table 1.

Table 1. Concentrations (ppm) of some phenolic acids; gallic acid (P1), p-hydroxybenzoic acid (P2), syringic acid (P3), 2,3-di hydroxybenzoic acid (P4), ferulic acid (P5), p-coumaric acid (P6), vanillic acid (P7) in white and red wines; Macabet 98 (W1), Narince 98 (W2), Chardonnay 98 (W3), Ugni Blanc 98 (W4), Cabernet Frank 98 (W5), Cabernet Frank 99 (W6), Cabernet Sauvignone 98 (W7), Cabernt Sauvignone 99 (W8), Carigna 98 (W9), Carigna 99 (W10), Bogazkere 99 (W11), Grenache 98 (W12), Syrah 98 (W13), Merlot 98 (W14).

Wines	Phenolic Acids (ppm)						
	P_1	P_2	P_3	P_4	P_5	P_6	P_7
W_1	5.040	4.526	16.740	0.330	0.289	0.370	0.594
W_2	14.133	29.378	6.690	0.688	*ND	3.138	3.006
W_3	13.806	12.392	9.480	1.054	*ND	6.938	0.609
W_4	14.241	7.356	*ND	0.085	*ND	*ND	3.709
W_5	28.503	24.131	19.562	0.060	4.123	*ND	1.988
W_6	27.726	22.169	4.676	2.452	2.223	0.820	12.204
W_7	25.384	11.471	3.156	1.540	3.965	*ND	1.815
W_8	32.772	16.699	*ND	*ND	3.654	0.236	9.527
W_9	16.794	23.293	2.440	4.264	*ND	1.089	0.922
W_{10}	14.984	39.094	2.347	5.89	1.237	4.299	5.104
W_{11}	19.260	37.032	*ND	*ND	1.034	*ND	1.558
W_{12}	15.028	6.652	0.998	0.029	0.117	1.235	2.952
W_{13}	22.376	7.968	0.579	0.072	0.089	0.555	3.761
W_{14}	14.344	6.792	2.116	0.165	*ND	1.093	5.432

*ND stands for "not detected".

Gallic acid was present in all white wines and it's highest level (14.241ppm) was found in Ugni blanc wine. Among the phenolic compounds of all white wines the highest quantity of p-hydroxybenzoic acid (29.378 ppm) was detected in Narince wine (Figure 1)

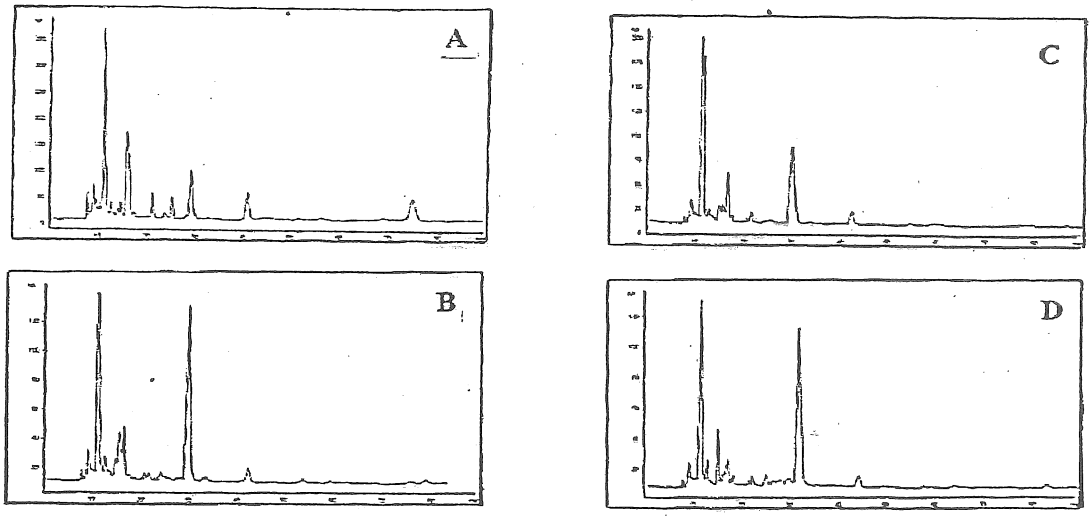

Figure 1: HPLC profiles at 0.65 V of four white wines; Macabet 98(A), Narince 98(B), Chardonnay 98 (C), Ugni Blanch 98 (D). Hichrom 5 C18, 7.75 x 300 mm, 5 µm particle size. Eluent : 0.01 N phosphoric acid - methanol (70:30 v/v). Flow rate : 1 mL/min.

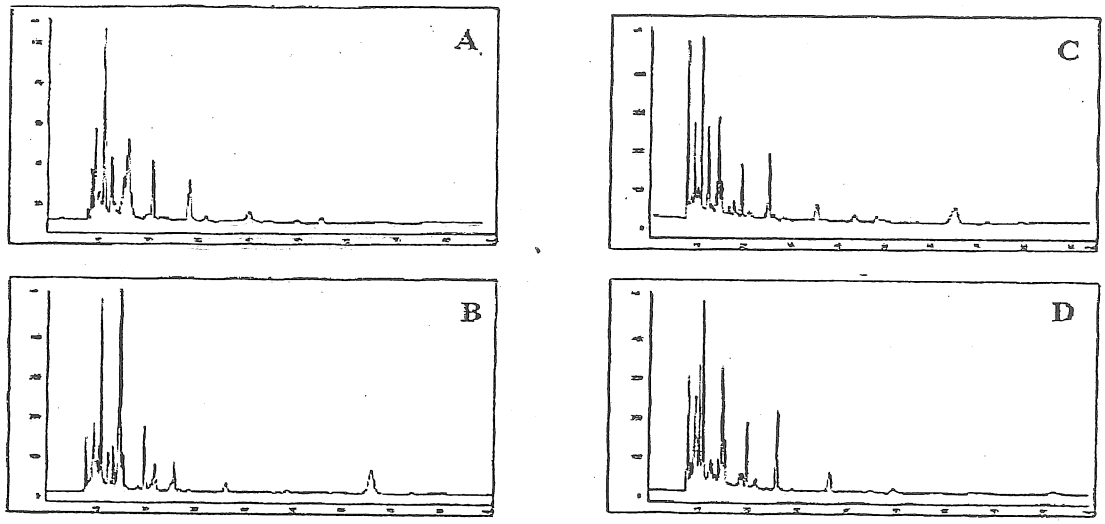

Figure 2. HPLC profiles at 0.65 V of four red wines; Bogazkere 99 (A), Grenache 98 (B), Syrah 98 (C), Merlot 98 (D). Other conditions as in Figure 1.

Considering the another derivate of benzoic acid (2,3-dihydroxybenzoic acid) it was found to be lower than p-hydroxybenzoic acid. It's highest level was found to be 1.054 ppm which is in agreement with previous studies (Jurg 1996;Sims *et al.*, 1995; Singleton and Trousdale 1983). Opposite to the other phenolic acids, the maximum level of syringic acid (16.740 ppm) was determined in Chardonnay wine.Ferulic acid was not detected in Narince,Chardonnay and Ugni Blanc (Table 1) and was found to be present only in small concentations in Macabet wine (0.289 ppm).

Gallic acid content of Cabernet Franc, Cabernet Sauvignon and Carignan for both years were found to be quite higher than that of other phenolic acids.In the content of p-hydroxybenzoic

acid was observed slight increases (1.962 ppm) in Cabernet Frank for 1998;decreases in Cabernet Sauvignon (5.228ppm) and Carignan (15.801ppm), respectively (Figure 2).
The situation concerning syringic acids content in these wines,is exactly the opposite of that found for the p-hydroxybenzoic acid.There was observed quite increases of syringic acids in all wines of 1998 year.Quantities of 2,3-dihydroxybenzoic acid of Cabernet Franc was found to be less in 1998 (2.396 ppm) than that in 1999.The same correlation was observed for Carignan.The presence of this compound in Cabernet Sauvignon (1.549 ppm) was detected only in one year. The concentrations of ferulic acid in Cabernet Franc and Cabernet Sauvignon for both years were found to be the same as for syringic acid.The presence of this compound in Carignan was detected only in 1999 (1.237ppm).The quantities of ferulic acid found were quite higher than that reported in previous works (Mayen *et al.*, 1995a; Mayen *et al.*, 1995b).
Results concerning p-coumaric acid of Cabernet Franc,Cabernet Sauvignon and Carignan are in accordance with the statement of decreases in quatities of 1998 wines. The highest quantity of vanillic acid was detected in Cabernet Franc wine (12.204 ppm) of 1999.The highest quantities of phenolic acids are as follows:gallic acid (22.376 ppm) in Syrah, p-hydroxybenzoic acid (37.032 ppm) in Bogazkere,syringic acid (2.116 ppm) in Merlot, 2,3-dihydroxybenzoic acid (0.165 ppm) in Merlot, ferulic acid (1.034 ppm)in Bogazkere, p-coumaric acid (1.235 ppm) in Grenache, vanillic acid (5.432 ppm) in Merlot (Figure 3).

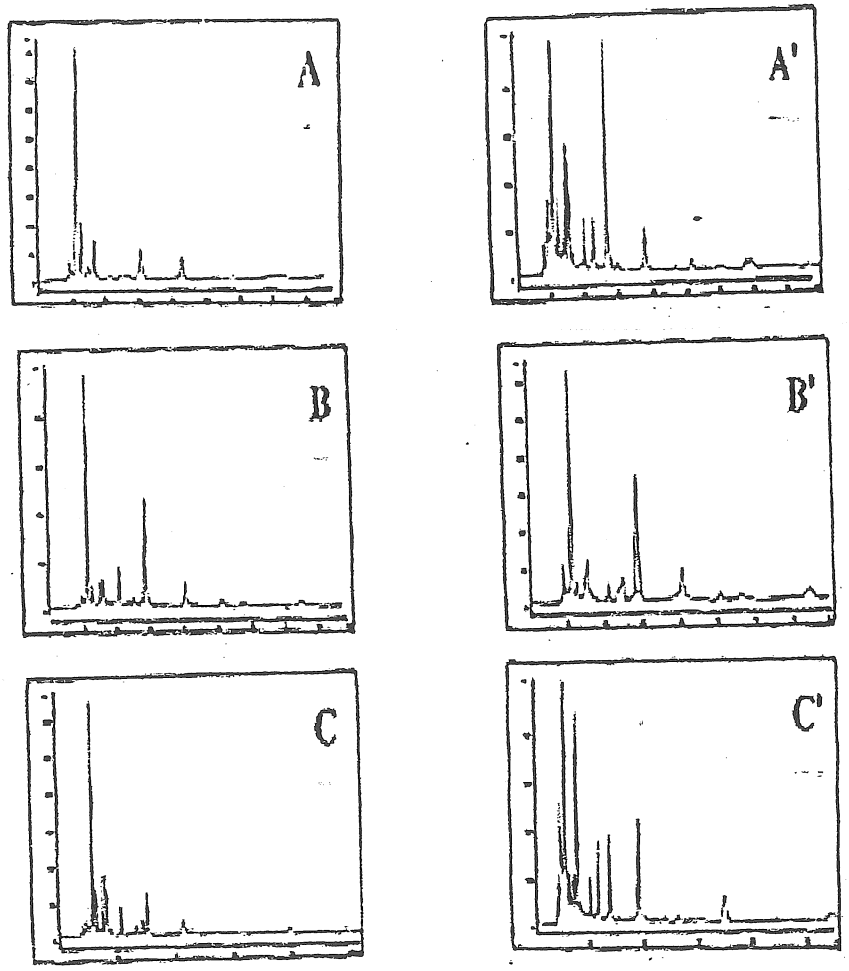

Figure 3: HPLC profiles at 0.65 V of three red wines; Cabernet Franc 98(A), Cabernt Franc 99(A'), Cabernet Sauvignone 98 (B), Cabernt Sauvignone 99 (B'), Carignan 98 (C), Carignan 99 (C'). Other conditions as in Figure 1.

The evaluation of all mean values showed the importance of grape variety origin for formation and quantities of different phenolic acids (Table 2).

Table 2. The significance of wine (grape) origin for phenolic acids concentrations.

Phenolic acids	Wine	Significance (p)
2,3-dihydroxybenzoic acid	Cabernet Sauvignon	p=0.048
p-hydroxybenzoic acid	Carignane	p=0.049
	Bogazkere	p=0.043
p-coumaric acid	Chardonnay	p=0.041
	Ugni Blanc	p=0.041
	Cabernet Frank	p=0.029
	Cabernet Sauvignon	p=0.026
	Bogazkere	p=0.036
	Syrah	p=0.044
ferulic acid	Cabernet Sauvignon	p=0.044
	Carignane	p=0.042
	Bogazkere	p=0.048
	Grenache	p=0.047
	Merlot	p=0.044
gallic acis	Cabernet Frank	p=0.036
	Cabernet Sauvignon	p=0.030
	Grenache	P=0.041
	Merlot	P=0.036

Evaluation of data concerning the years effect over phenolic acids showed the significance of year for quantities of p-hydroxybenzoic (p=0.538) and vanilic acid (p=0.009) (Figure 4).
These results were confirmed by correlation analyses, also (p-hydroxybenzoic r=0.631; vanilic acid r= 0.637). Considering the colour of wines the most significant effect was determined for gallic acid (p=0.574).This result was supported by correlation analyses (r=0.618) with significance of p=0.018 (Figure 5).

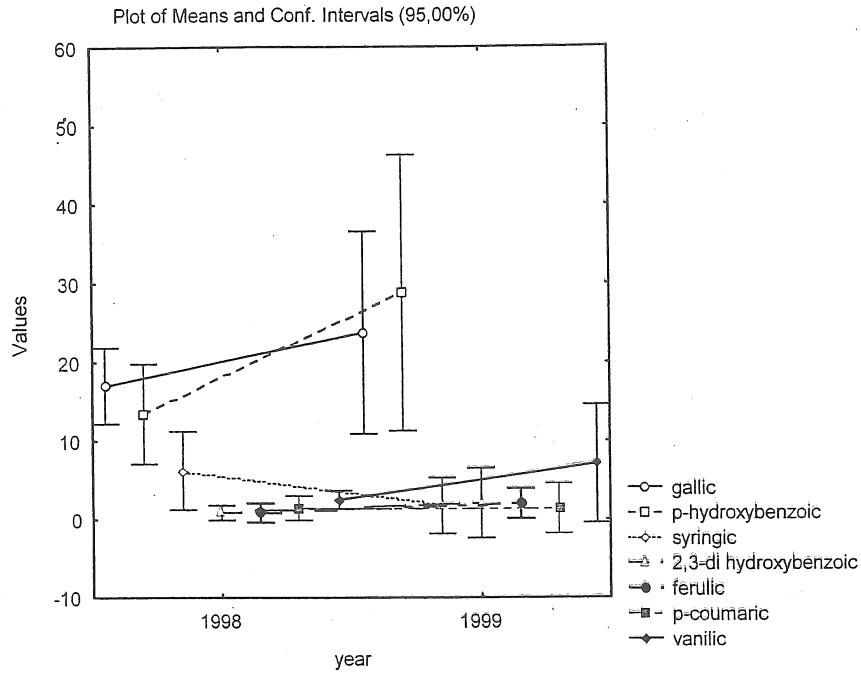

Figure 4. Phenolic acids concentrations (with standart deviations) of wines during two year.

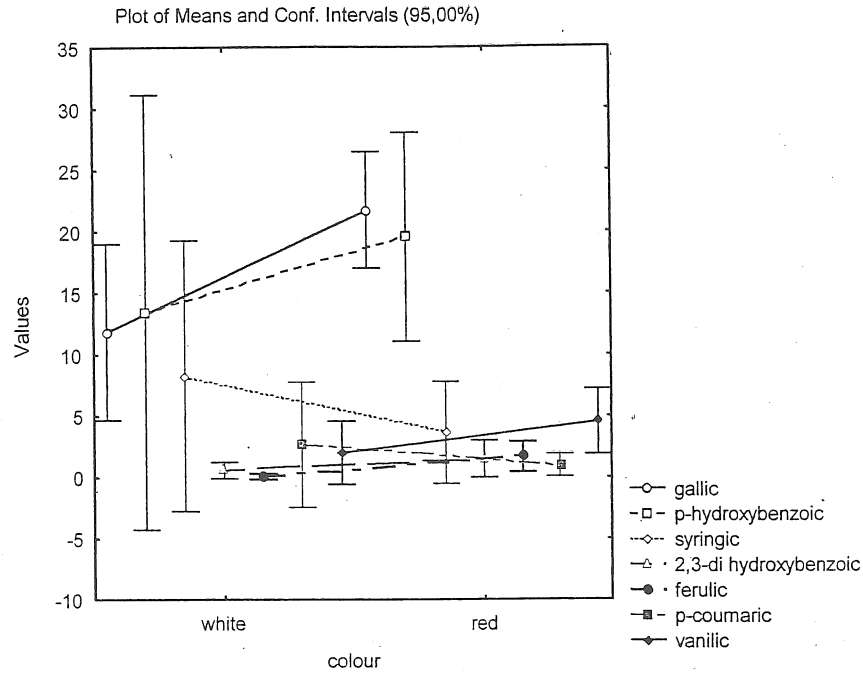

Figure 5. Phenolic acids concentrations (with standart deviations) of white and red wines.

Positive corelation was determined between ferulic and gallic acids (r=0.808) with significance of p=0.050 and between vanilic and gallic acid (r=0.554) with significance of p=0.040.Statistical treatment of red wines (Cabernet Frank, Cabernet Sauvignon, Carignane) produced and analysed in two years period showed the importance of differences in vanilic acid (p=0.025) during years. Considering the grape varieties differences only three of the phenolic acids (gallic acid: Cabernet Frank p=0.029;Cabernet Sauvignon p=0.024;Carignan p=0.028;2,3-dihydroxybenzoic acid: Cabernet Sauvignon and Carignan p=0.048; ferulic acid: Cabernet Sauvignon and Carignan p=0.042) were found to be affected from varieties. Positive correlation for the three red wines was determined between vanilic acid and year (r=0.869) with significance of p=0.025. Except syringic acid in all phenolic acids were seen definite increases in phenolic acid contents in the second year (1999).

The projection of the variables (phenolic acids) on the factor plane of the first two factors (60.20% x 20.24%) could be seen in Figure 6. The evaluation of cases (wine types) on the factor plane of the first two factors (60.20% x 20.24%) could be found in Figure 7.

The definite separation were observed between two years wines. The wines of 1999 are located in the upper part, whiles of 1998 ones are distributed in the lower part of coordinate.

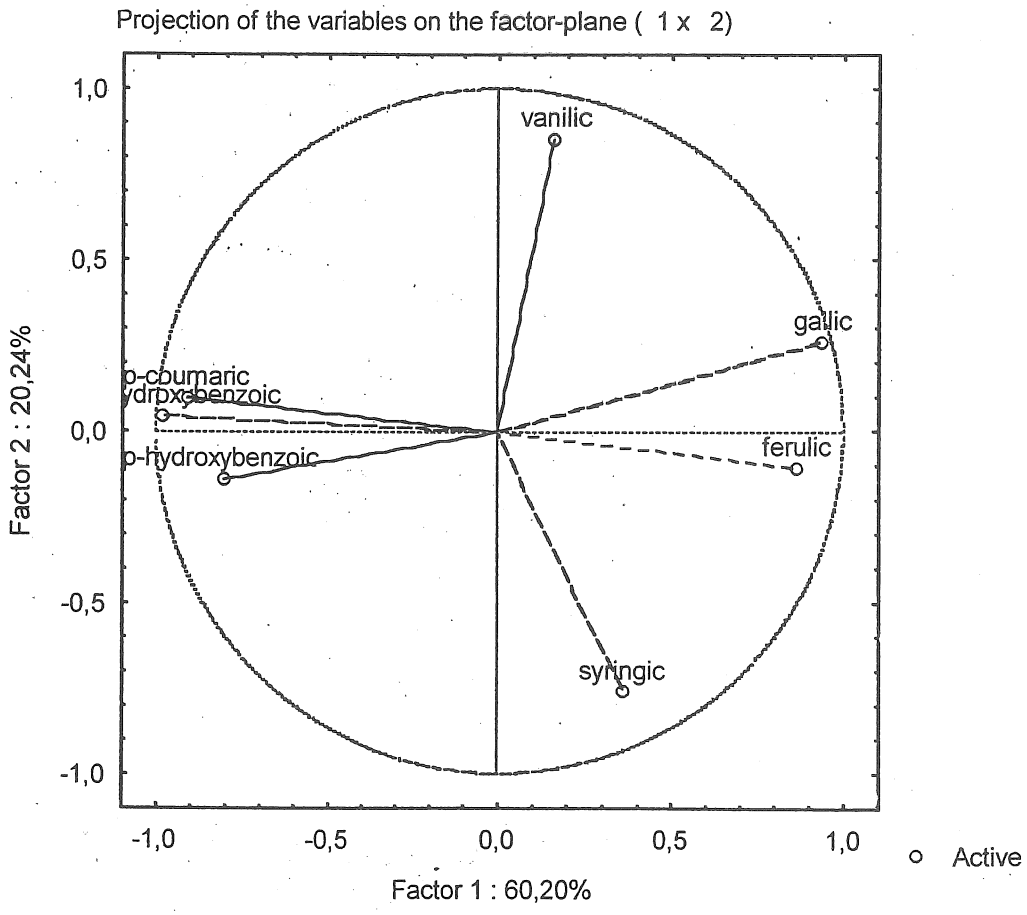

Figure 6. PCA of all phenolic acids in wines.

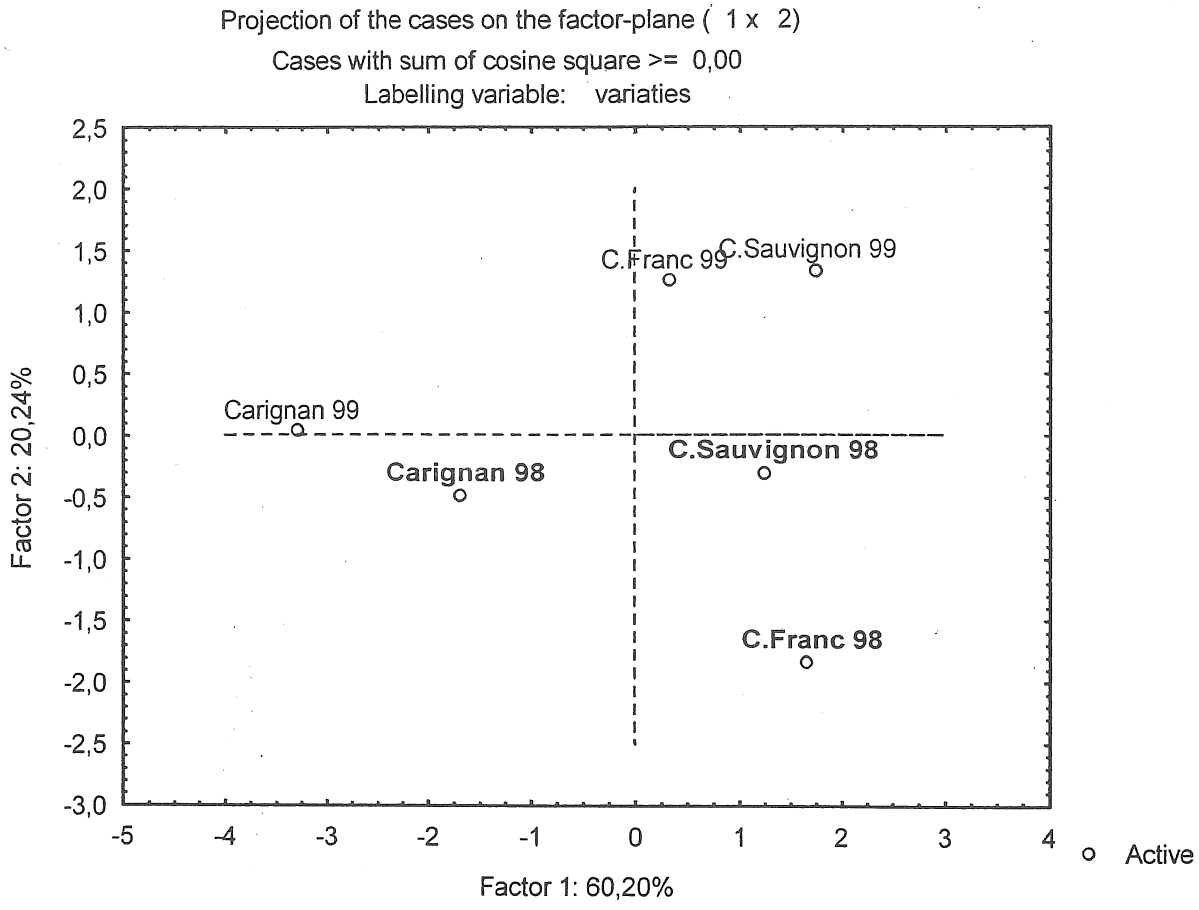

Figure 7. PCA of wines during two years

The definite separation were observed between two years wines. The wines of 1999 are located in the upper part, whiles of 1998 ones are distributed in the lower part of coordinate.

Discussion

The HPLC chromatograms of analysed four white wines obtained under isocratic condition showed that resolution of phenolic compounds accorded with elution order of standards.The concentration of these substances seem to vary considerably, due to diverse factors, such as variety type,vinification techniques.The higher quantity of gallic acid in these wines than to those described in global survey of Semillion, Thomson seedless, Chenin Blanc, Frenc Colombard (Main and Morris, 1994; Mayen *et al.*, 1995) could be explained by considering the differences of variety types. The level of 2,3-dihydroxybenzoic acid (1.054 ppm) was found to be lower than p-hydroxybenzoic acid, which is in agreement with previous studies (Jurg 1996; Sims *et*

al., 1995,Singleton and Trousdale,1983). An explanation of lower concentaration of ferulic acid except variety differences could be the polymerization or condensation reactions that occur after fermentation (Levendood and Boulton, 1997; Singleton and Trousdale, 1983; Timberlake and Bridle, 1976).

Relatively high quantities of p-hydroxybenzoic acid (except it's absence in Ugni Blanc) is so important since it is one of the critical phenolics found in wines responsible for formation of the other phenols in their metabolitic way (Ramos *et al.*, 1993).The microvinification experiments carried out in 1998 and 1999 with Cabernet Frank, Cabernet Sauvignon and Carignan showed that aging for a year could be one of the reason of chances in quantities of phenols.

Phenols undergo variations during the aging process, in part due to the polymerization or hydrolysis of flavonoid compounds some of which are in form of aglycones, chiefly as a result of the hydrolysis of glycosides during fermentation. This fact was observed in previous works, also (Castelari *et al.*,1998; Cheynier *et al.*,1997; Lazlavic *et al.*,1995).

Gallic acid content in Cabernet Franc, Cabernet Sauvignon and Garignan for both years were found to be quite higher than that of other phenolic acids.The reasonable explanation for this could be the fact that gallic acid is augmented in wine by pomace contact involving seeds as well as skins and the hydrolysis of the gallic acid from a bound form, epicatechin gallate. The higher quantities of gallic acid in 1998 years could be explained by that gallic acid as one of the major phenolics of red wines may not polymerize with anthocyanins.This is in agreement with previous studies (Kovac *et al.*,1995; Mayen *et al.*, 1994).The variations of gallic acid differ in wide range depending on different treatment (7.1;15.9;16.1ppm) (Bakker *et al.*,1996;Canbas,1996;Deryaoglu, 1997) different grape varieties (3.55;38.82;71.05;78.21 ppm) (Mayen *et al.*,1995; Shirley and Waterhouse,1996;Teissedre *et al.*,1995);aging (6.78;6.90 ppm) (Gomez-Cordoves *et al.*, 1995; Jurg 1996; Lazlavic *et al.*, 1995).

The increase of p-hydroxybenzoic acid would be explained by the hydrolysis of some co-pigments (p-hydroxybenzoic acid and anthocyanins or tannins) formed after fining. In available literatures the content of p-hydroxybenzoic acid is not more than 20 ppm (Mayen *et al.*, 1995a; Mayen *et al.*, 1995b). Quantities of syringic acid in literature vary depending on grape varieties (5.25;5.91;9.09;12.90;13.18;14.79 ppm) (Gil *et al.*,1995; Mayen *et al.*, 1995a); applied heating temperature (3.39;8.04;10.20 ppm) (Auw *et al.*,1996; Oşzmianski *et al.*, 1986; Rommel *et al.*, 1990).The quantities of ferulic acid found are quite higher than that reported in previous works (Mayen *et al.*, 1995a, Mayen *et al.*, 1995b).

Results concerning p-coumaric acid of Cabernet Franc,Cabernet Sauvignon and Carignan are in accordance with the statement of decreases in quatities of 1998 wines.The concentrations of this compound in literature vary among the different grape varieties (0.4;1.3;1.8 ppm) (Gil *et al.*, 1995;Mayen *et al.*, 1995b) and durations of must fermentation (0.396; 0.702 ppm) (Deryaoglu *et al.*,1997;Mayen *et al.*,1994).The highest quantity of vanillic acid was detected in Cabernet Franc wine of 1999 (12.204 ppm).According to data availabale in literature vanillic acid increase during fermentation (Mayer *et al.*, 1995a) and its content fluctuated depending on grape varieties and vinification procedures in a wide range (1.89-16.78 ppm) (Auw *et al.*, 1996; Cheynier *et al.*, 1993; Deryaoglu *et al.*, 1997).

The HPLC chromatograms of Bogazkere, Grenache, Syrah, Merlot wines and their quantities expressed in emphasized the differences between different red wines in their phenolic content. Close inspection of these data lead us to conclude that the patterns of phenolics substances are considerably influenced by the genetics (i.e.,variety) of the grapevine.Evaluation of phenols in all wines (white and red) showed the considerable differences between them.This fact emphasized the hypothesis that phenol concentrations depend not only upon grape variety, climatic conditions, grape ripenig but also on technique employed during vinification.

The results obtained by HPLC analyses and evaluated by LSD, correlation analyses are confirmed by PCA analyses.One of the major groups were formed with following parameters: p-coumaric;2,3-dihydroxybenzoic;p-hydroxybenzoic acid. So near to this group were found total phenols.Separately from these clusters could be accepted vanilic acid.Gallic and ferulic acids could be separated in another group.The intensities are found to be similar.

Conclusion

Detection and quantification of some phenolic acids (gallic acid, p-hydroxybenzoic acid, syringic acid, 2,3-di hydroxybenzoic acid, ferulic acid, p-coumaric acid, vanillic acid) under isocratic condition present in white and red wines by using electrochemical detector were performed. Evaluation of phenolic acids content in four white wines (Macabet, Ugni Blanc, Chardonnay, Narince) and seven red wines (Bogazkere, Cabernet Sauvignon, Cabernet Franc, Merlot, Syrah,Carigna,Grenache) emphasizes the importance of variety characteristics of wines. The differences in phenolic acids of Cabernet Franc, Cabernet Sauvignon and Carignan wines in two years production manner showed the possible aging effect over phenolics.Overall observation of wines (white and red) indicates the effects of variety differences on phenols and some microvinification factors such as must fermentation, yeast strain, SO_2 quantities and fining agent type which are different in white and red ones.

Özet

Kırmızı (Cabernet Sauvignon, Cabernet Franc,Merlot,Syrah,Carignan,Grenache,Bogazkere) ve beyaz şaraplarda (Ugni Blanch, Chardonnay, Narince) bulunan bazı fenolik asitlerinin (gallik,p-hidroksibenzoik, siringik, 2,3-dihidroksibenzoik, ferulik, p-kumarik and vanillik asit) C_{18} silica kolonlu (Hichrom 5 C18, 7.75 x 300 mm,5 µm particle size) HPLC (Aglient 1100 series) sisteminde elektrokimyasal olarak (+0.65 V vs. Ag/AgCl) tayin edilmiştir. Solvent olarak metanol ve 0.01N fosforik asit (30/70 v/v) kullanılmıştır.Taranan literatürlerde şarap fenollerinin elektrokimyasal dedektörlü HPLC sistemi ile izokritik koşullarda henüz analiz edilmedigi ortaya çıkmıştır. Kırmızı ve beyaz şaraplardaki fenolik asitlerin degerlendirilmesi sonucu şarap üretiminde kullanılan üzüm çeşitlerinin önemi ortaya çıkmıştır.Cabernet Sauvignon, Cabernet Franc ve Carignan şaraplarının iki yıllık üretimi sırasında fenolik asitlerde meydana gelen degişmeler yıllandırmadan kaynaklabilecegi belirlenmiştir.

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