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INFLUENCE OF NANOPOROUS MATERIALS ON THE CHEMICAL COMPOSITION OF MERLOT AND CABERNET SAUVIGNON WINES

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Abstract

The variation of the content of 12 phenolic compounds from 5 months aged Cabernet Sauvignon and Merlot wine samples treated with siliceous and aluminosiliceous porous materials was analysed through a HPLC method. The standard physical-chemical analyses (alcoholic concentration, total acidity, volatile acidity, relative density, reductive sugars, total dry extract, non-reductive extract, free SO_2, total SO_2, pH) were registered. The used nanomaterials: SBA-15, Al-MCM-41, KIT-6 were synthetized in the laboratory and were structurally characterized through the BET and SEM methods. The obtained results are in accordance to literature findings. The experimental results are proof that the maturation of wine, specifically Cabernet Sauvignon and Merlot, in the presence of porous materials modifies the physical-chemical composition as well as the phenolic content of wines.

Key words: adsorption, nanomaterial, phenolic compound, wine

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1. Introduction

Red wines are alcoholic beverages, their composition being influenced by many and diverse factors corresponding to the specific production area, such as grape variety, soil and climate, culture, yeast, winemaking practices, transport and storage.

Every winemaker knows the important role played by polyphenols in the visual and gustative quality of red wines and their importance during aging. Consumers are increasingly demanding highly coloured wines, with a good structure and roundness, meaning that polyphenol content should be as high as possible to ensure colour intensity and stability during aging. The exploration of compositions and structures for micro and mesoporous materials in view of specific applications in the oenological area as sorption and separation support, has led to considerable results, reported in literature (Cotea et al., 2011; Luchian et al., 2011).

Studies were continued by focusing on the time-based influence of these innovative materials with different samples structures of Cabernet Sauvignon and Merlot samples. The wines were bottled and stored for 5 months in contact with the micro- and mesoporous materials and then analyses were performed.

The synthesis of nanoporous materials is an active segment of research. The ordered mesoporous silica materials contain a homogeneous distribution of mesopores (2 nm $<$ d_p $<$ 50 nm) which are characterized by a very narrow pore size distribution. The pore size and pore volume of these materials make them suitable potential matrices to incorporate and then release a large variety of molecules. Highly ordered hexagonal mesoporous silica structure SBA-15 has been synthesized by using commercially available block-copolymer surfactants in strong acid media (Zhao et al., 1998). SBA-15 possesses a hexagonal array of mesopores, approximately 6.0 nm

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in diameter. The textural properties of the mesoporous MCM-41, which have been extensively studied, are determined by the regular array of hexagonally shaped pores. The introduction of aluminum and others transitional metals in the structure of the mesoporous material leads to an increase of acidity and of specific catalytic activity. Creation of Al-O(H)-Si bridges generates Brönsted acidity (Corma et al., 1994, Corma et al., 1995).

In literature it was reported the synthesis of other mesoporous materials with larger pores, KIT-6, with Ia3d cubic type structure and a network of interconnected channels. The siliceous material KIT-6, has numerous applications in adsorption and catalysis, thanks to unique 3-D structures (Xiaoying et al., 2002).

In this study, in the treatment of wine samples we used a natural zeolite - clinoptilolite, which was collected from Mârşid, Romania (obtained from volcanic eruptions). Clinoptilolite is a natural zeolite with a complex formula: $(Na, K, Ca)_{2-3}Al_3(Al, Si)_2Si_{13}O_{36}\cdot12H_2O$, comprising a microporous arrangement of silica and alumina tetrahedra. Natural zeolites are aluminium silicate minerals with high cation exchange capacities (CECs) and high selective adsorption properties (Copcia et al., 2010; Apreutesei et al., 2008).

The aim of this research is to investigate the impact of SBA-15, AlMCM-41, KIT-6 and clinoptilolite materials on the chemical composition and phenolic compound content of Merlot and Cabernet sauvignon red wines, harvested in 2013. The used methods for wine analysis are in accordance with OIV specifications (OIV, 2013).

2. Materials and method

2.1. Chemicals

Amphiphilic nonionic triblock copolymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, molecular weight 5800, Aldrich) as structure directing agent (SDA), tetraethylortosilicate (TEOS 98%, Merck) as silica source, hydrochloric acid (37%, Merck) were used in the synthesis of mesaporous material SBA-15.

No modifications were brought to the following reagents used for the Al-MCM-41 synthesis: cetyltrimethylammonium bromide ($C_{16}H_{33}(CH_3)_3N^+Br$, Aldrich) for structure directing reagent, tetraethylorthosilicate (TEOS, 98% Merck) as silica source, aluminum isopropoxide (Al–[O–CH–$(CH_3)_2]_3$, Merck) to generate aluminum cations, tetraethylammonium hydroxide (($C_2H_5)_4NOH$ 10%, Merck) as mineralizing agent.

No changes were applied to the tetraethylortosilicate (TEOS 98%, Merck) as silica source, amphiphilic nonionic triblock copolymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, molecular weight 5800, Aldrich) as structure directing reagent, hydrochloric acid (37%, Merck), n-butanol (99.4%, Aldrich) used in the synthesis of KIT-6. Other chemicals necessary for the normal physical-

chemical and HPLC analyses were purchased from Merck. All chemicals were used as received without further purification.

2.2. Grape and wine samples

Grapes of the Cabernet Sauvignon and Merlot varieties were harvested from Târgu Bujor vineyard in September 2013. *Saccharomyces cerevisiae* (Fermol Grand Rouge®, Spindal, 30g/100L) was used for wine fermentation. After stabilization, the wine was mixed with prepared porous materials (about 4 g/L) and stored in glass bottles for 5 months. The samples were then filtered and analysed.

2.3. Synthesis of mesoporous silica SBA-15

The mesoporous silica SBA-15 was synthesized using Pluronic P123 by method found in specific literature (Zhao et al., 1998) with changes. The molar ratio of the components was as follows: $1SiO_2$: 0.017 P123: 5.87 HCl: 194 H_2O. The process to obtain solid powder SBA-15 involves dissolving of P123 (4 g) in acidic solution (HCl, 2M) (150 mL) under stirring, adding drop-wise TEOS – as silica source (9.6 mL). The mixed solution was aged at 45 °C for 8 hours and finally the sol-gel suspension was heated up to 80 °C for 5 hours in a conventional oven. The white solid was filtered off, washed several times with deionized water, dried at room temperature and finally calcined at 550 °C for 6 hours (heating rate of 1^0C/min) in air in a programmable furnace.

2.4. Synthesis of mesoporous silica KIT-6

The synthesis of mesoporous KIT-6 silica was performed according to procedures found in literature (Xiaoying et al., 2002). Typically, 5 g of Pluronic P123 was dissolved in 180 g of distilled water and 9.9 g of HCl solution (35 %) under vigorous stirring at 35 °C. After complete dissolution, 5 g of n-butanol (99.4 %) were added. Following further stirring for 1 h, 10.75 g of TEOS was immediately added. The molar ratio of the gel was:

0.017 P123: 1.3 TEOS: 1.31 BuOH: 1.83 HCl : 195 H_2O

The mixture was left stirring at 35 °C for 24 h and transferred into autoclave, which, in turn, was sealed and maintained at 100 °C for 24 h. The resulting solid product was filtered and dried at 100°C overnight. After a brief ethanol/HCl washing, the final sample was dried at 70 °C and calcined at 550 °C for 6 h in air.

2.5. Synthesis of Al-MCM-41

For the synthesis of mesoporous material Al-MCM-41 (Si/Al = 16), 22.3 mL (1 mol) of TEOS was mixed with 0.68 g (0.033 mol) of aluminium

isopropoxide. The obtained solution was stirred for 30 min at 250 rpm and tetraethylammonium hydroxide solution (10% water) was added with continues stirring for another 30 min at a speed of 250 rpm until the formation of the gel (pH = 11). 7.2 g (0.2 mol) of CTAB was added dropwise (30 mL / h). The gel became suspension. After further stirring for 1 h, the resulting gels of composition: *$1SiO_2$: $0.033Al_2O_3$: $0.2C_{16}TMABr$: $100H_2O$* was transferred into a teflon steel autoclave and heated to 150 °C for 48 h. After cooling, the sample was recovered by filtration. The obtained solid was washed with distilled water plus ethanol, and then dried in air at 70 °C for 1 h and finally calcined in flowing air at 540 °C for 6 h (Stein and Holland, 1996).

2.6. Clinoptilolite preparation

Clinoptilolite was collected from Mârşid Romania subsoil assets (obtained from volcanic eruptions). It was crushed and sieved to obtain the fractions of 0.1 and 1.0 mm.

Natural zeolite was subjected to an acidic treatment, with the aim of improving its ionic exchange property and also for usage in the wine industry. Oxalic acid was used for this treatment - $H_2C_2O_4$ (1 M concentration), liquid solid ratio of 5:1. After treatment, the samples were filtered, washed with distilled water for a few times and dried at 105 °C for 6 hours. After drying, the samples were calcinated at a temperature of 550 °C (Copcia et al., 2010).

2.7. Characterization

N_2 sorption

The textural properties of SBA-15, AlMCM-41 and KIT-6 were determined with a NOVA 2200 Quanta Chrome Inc.) sorption apparatus. The sample was degassed at 300 °C for 3 hours before the measurement was taken. Pore size distribution was determined based on the Barret-Joyner-Halenda (BJH) adsorption curve.

Scanning Electron Microscopy (SEM) was carried out on a SEM VEGA II LSH TESCAN with EDX detector tip QX2 (Bruker/ Roentex).

X-ray diffraction

The clinoptilolite samples were characterized by analyzing the X rays diffraction (DRX) using a Philips PW 3710 diffractometre with CuKά diffraction. The X rays tube voltage was 40V and the current intensity was 30 mA, the 2θ angle was between 5° and 70° with a step size of 0.02°.

Standard chemical analysis of wine according to OIV methods

Each wine was decarbonated and then analysed for: volatile acidity OIV-MA-AS313-02, total acidity OIV-MA-AS313-01, alcoholic strength by frequency oscillator OIV-MA-AS312-01A,

reducing substances OIV-MA-AS311-01A, pH OIV-MA-AS313-15, total dry matter and non-reducing substances OIV-MAAS2-03B were done according to present standards (OIV, 2013) and specific literature (Ribéreau-Gayon et al., 2006).

Wine phenolic compounds analysis.

Phenolic compounds were identified and quantitatively determined with high-performance liquid chromatograph (HPLC) Shimadzu. This unit is equipped with two chromatographic columns Merck Chromolith Performance RP-18 (Castellari et al., 2002; Bodîrlău et al., 2009).

3. Results and discussion

3.1. Physical adsorption, BET

Fig. 1 exhibits the N_2 adsorption – desorption isotherm at -196 °C for calcined silica-SBA-15. Typical silica-SBA-15 isotherm is of Type IV with a hysteresis loop Type H1, a characteristic of mesoporous solids, according to the IUPAC classification (Kruk et al., 2000; Kruk and Jaroniec, 2001). The specific surface area of calcined silica-SBA-15 was calculated using the multiple point BET method and BET equation - S_{BET}= 775 m^2/g. The pore size distribution curve (inside Fig. 1) was computed based on the BJH model and the pore size estimated from the peak position in BJH curve – D_{BJH} = 6.62 nm. The total pore volume (0.942 cm^3/g) was obtained from the volume of N_2 adsorbed at a relative pressure p/p$_0$ = 0.955.

Fig. 2 represents the adsorption - desorption isotherm of N_2 at a temperature of -196 °C, for the calcined KIT-6 material, synthetized by classical method. The typical isotherm for this material is a type IV, characteristic for mesoporous solids, in accordance with IUPAC classifications. The textural characteristics of the synthetized material are: specific BET surface calculated through BET method is 644,5 m^2/g, pore diameter calculated through BJH method is 8,34 nm, total pore volume at p/p$_0$ = 0.97 is of 0.761 cm^3/g.
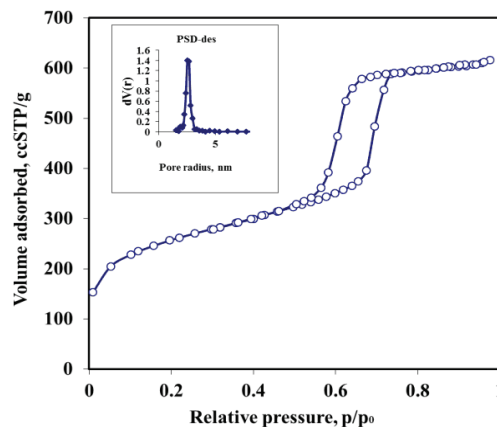

Fig. 1. The N_2 adsorption – desorption isotherm at -196 °C of calcined silica-SBA-15. Inset: the pore size distribution (PSD)

Fig. 3 indicates the allure of the absorption - desorption isotherm of N_2 for the calcined AlMCM-41 material.

The AlMCM-41 has a specific BET surface of 2098 m^2/g and a total pore volume of 0.691 cm^3/g (at $p/p_0 = 0.94$). The distribution of pores according to rays is quite tight, the diameter of pores being 3.92 nm.

3.2. SEM analysis of mesoporous materials

Fig. 4 shows the SEM images of the calcined sample.

3.3. X-ray diffraction analysis

The analysis of XRD diffractograms of natural and modified clinoptilolite tuff indicates a small change in the peak position and peak intensity caused by the removal of additional species as well as the dealumination of zeolite (hydrolysis of Al-O-Si bonds) as presented in Fig. 5.

Moreover, during the acid or alkaline treatment, the removal of cations Na^+, Ca^{2+}, K^+, Mg^{2+} and Fe^{3+} takes place, as well as the change in pore dimension and pore distribution, corresponding to clinoptilolite in H^+ form, respectively in Na^+ form.
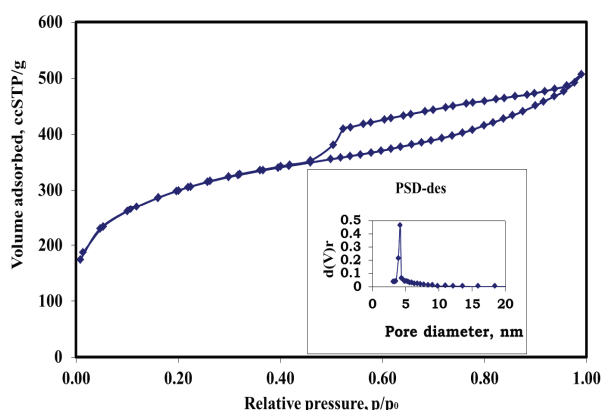
3.4. Standard chemical analysis of wine

Ethanol, relative density, pH, total acidity, volatile acidity, sugars, total dry extract (TDE), total and free SO_2 of the wine sample were analysed by the recommended methods of International Organisation of Vine and Wine (OIV) (Table 1). Analysing the results presented in Table 1, it can be seen that the use of meso- and microporous materials used as wine treatments, influences its physical-chemical composition. Both in the case of Cabernet Sauvignon and Merlot wine samples, the alcoholic concentration changes, as follows: clinoptilolite and Kit-6 lead to its significant increase for Merlot samples, it increases from 11.83% (M) to 12% (M3) respectively 12.15% (M4); for Cabernet Sauvignon samples, it increases from 13.23% (CS) in the control sample to 13.41% (CS3) and 13.39% (CS4).

In the case of total acidity, small variations are registered in the case of samples treated with clinoptilolite and KIT-6 as well (M3, CS3 and CS4). The volatile acidity increases in Merlot samples treated with ALMCM-41 (M2) and clinoptilolite (M3); it decreases in Cabernet sample treated with AlMCM-41 (CS2). Total and free SO_2 have decreasing values in all wine samples. The other indices show no significant changes.
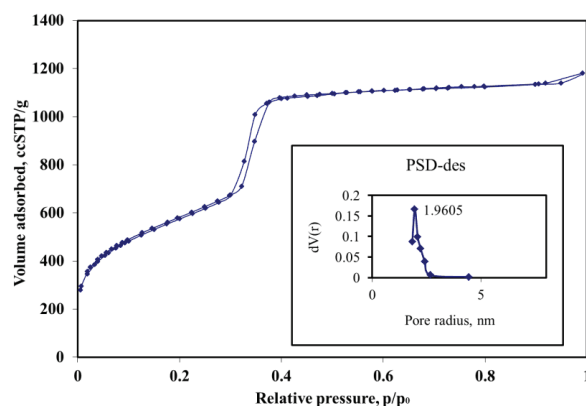

Fig. 2. The N_2 adsorption – desorption isotherm at -196 °C of calcined KIT-6. Inset: the pore size distribution (PSD)

Fig. 3. The N_2 adsorption – desorption isotherm at -196 °C of calcined AlMCM-41.Inset: the pore size distribution (PSD)
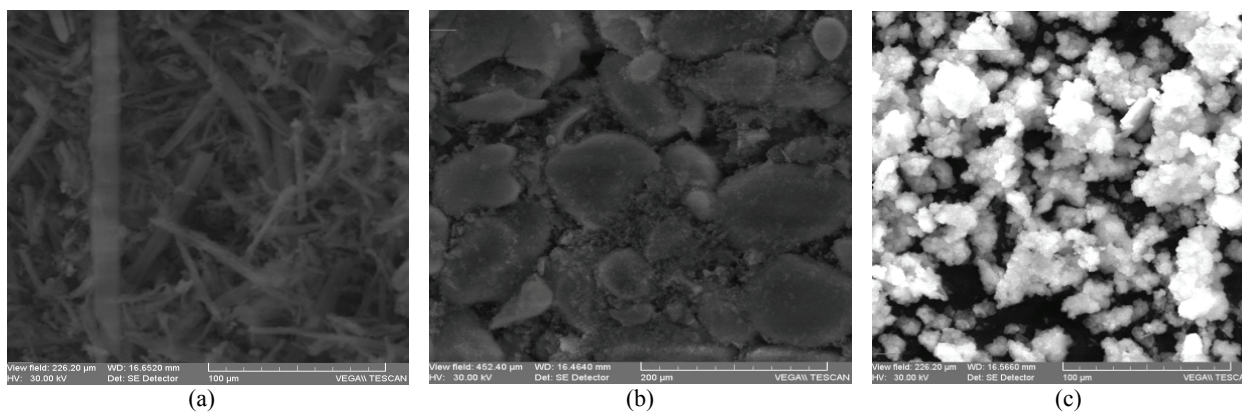

Fig. 4. SEM micrograph of calcined AlMCM-41 (a), SBA-15 (b) and KIT-6(c) samples
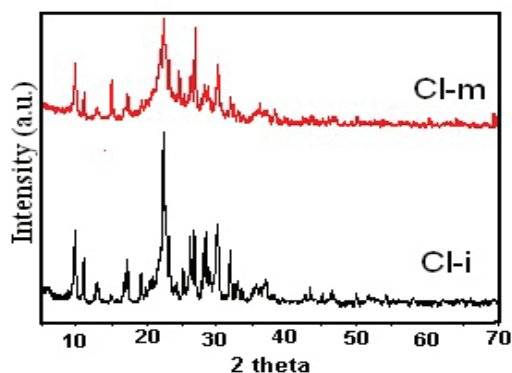
Fig. 5. XRD spectra for natural clinoptilolite tuff samples (Cl-i) and modified (Cl-m)

All analyses were repeated three times and the mean value was registered.

3.5. Wine phenolic compounds analysis

Identification of the phenolic compounds from treated wines was carried out using a HPLC method. In this case wine samples were analysed after a preliminary microfiltration. The separation optimization of these compounds was achieved after experimenting with various gradient profiles and mobile phase flow rates. The HPLC method used to identify the adsorbed species involved an elution program. One of the eluents contains an aqueous solution of 1% methanol acidulated with TFA and the other, an aqueous solution of 50% methanol. The quantitative identification of phenolic components is based on their retention time and spectral characteristic (Table 2). The chromatographic analysis of samples registers a modified phenolic composition compared to the control wine samples. Salicylic acid present in the control sample in Cabernet sauvignon (0.42 mg/L) is under the detection limit in the wine sample treated with SBA-15.

Quercitine is under the detection limit in the control Merlot sample, while in the variants treated with clinoptilolite and KIT 6 the values are of 0.08 mg/L and 0.22 mg/L. Moreover, the rutin trihydrate concentration increases in all treated Cabernet Sauvignon samples and in the Merlot variants where SBA-15 and AlMCM-41 were used. The concentration of caffeic acid is double in the treated Cabernet Sauvignon wine samples. All analyses were repeated three times and the mean value was registered.

Table 1. Traditional analysis of Cabernet Sauvignon and Merlot treated wines

Variants	Physical-chemical characteristics									
	Alcohol strength (%)	Total acidity (g/L $C_4H_6O_6$)	Volatile acidity (g/L $C_2H_4O_2$)	Relative density (g/cm³)	Reductive sugar (g/L)	EST (g/L)	EN (g/L)	Free SO₂ (mg/L)	Total SO₂ (mg/L)	pH
Merlot										
M	11.83	5.54	0.40	0.9923	1.99	19.80	17.81	37.56	95.47	3.42
M1(SBA-15)	11.69	5.54	0.46	0.9925	2.01	21.90	19.89	30.66	85.47	3.44
M2 (Al-MCM-41)	11.77	5.54	0.54	0.9925	2.05	22.22	20.15	35.61	91.35	3.42
M3 (Clinoptilolite)	12.00	5.78	0.61	0.9920	1.65	20.30	18.65	28.18	80.21	3.43
M4 (KIT-6)	12.15	5.69	0.45	0.9921	1.79	20.90	19.14	25.7	82.06	3.41
Cabernet Sauvignon										
CS M	13.23	5.48	0.46	0.9910	2.48	21.40	18.92	28.89	70.30	3.17
CS 1(SBA-15)	13.38	5.48	0.45	0.9912	2.42	21.90	19.48	23.23	70.61	3.28
CS 2 (Al-MCM-41)	13.33	5.43	0.39	0.9913	2.42	21.60	19.18	17.96	60.08	3.23
CS 3 (Clinoptilolite)	13.41	5.43	0.45	0.9914	2.47	21.90	19.43	18.89	68.13	3.27
CS 4 (KIT-6)	13.39	5.64	0.46	0.9912	2.42	20.90	18.48	24.46	70.08	3.26

EST(g/L)-Total dry extract; EN(g/L)-Non-reductive extract

Table 2. Polyphenolic constituents (mg/L) in Cabernet Sauvignon and Merlot treated samples

	Gallic acid	Protoca-techic acid	Gentisic acid	Cate-chin	Vani-llic acid	Caffeic acid	Cloro-genic acid	Sali-cylic acid	Epi-cate-chin	Sina-pic acid	trans Resve-ratrol	Rutin trihy-drate	Quer-citin
M	7.36	1.51	5.48	6.42	1.15	1.64	1.11	0.67	1.56	0.03	0.17	1.65	0
M 1	7.33	1.58	5.33	7.23	1.23	1.61	1.15	0.58	1.49	0.03	0.18	2.72	0
M 2	7.34	1.69	5.14	7.42	1.41	1.59	1.08	1.05	0.83	0.06	0.18	2.85	0
M 3	7.32	2.16	5.30	9.25	1.58	1.50	0.99	0.81	9.41	0.08	0.18	1.52	0.08
M 4	7.10	2.11	5.02	7.57	1.07	1.20	0.81	0.13	1.06	0.06	0.13	1.18	0.22
CS	10.60	5.79	37.69	8.36	2.67	0.99	4.25	0.42	4.91	0.08	0	1.54	0.08
CS 1	10.5	5.68	39.32	8.31	2.70	1.90	7.37	0	5.85	0.4	0.18	2.35	0.18
CS 2	10.46	5.64	38.15	8.19	2.64	1.78	7.38	4.51	2.24	0.27	0.12	1.73	0.16
CS 3	9.99	5.46	39.37	1.08	2.81	1.98	7.24	4.62	2.25	0.45	0.18	2.33	0.32
CS 4	9.91	5.29	38.73	0.29	2.67	1.97	7.15	4.36	2.28	0.45	0.17	2.24	0.96

4. Conclusions

SBA-15, AlMCM-41, KIT-6 materials, all synthetized in the laboratory, were characterized from a structural and morphological point of view and are in accordance with the data in specific literature. Clinoptilolite, natural zeolite, was treated with oxalic acid, in order to bring it in H form and was then analysed by X-ray diffraction.

The physical-chemical characteristics of treated wines are changed, a higher influence being observed in the clinoptilolite and KIT-6.

The chromatographic analysis shows that the applied treatments modify selectively the phenolic compounds' content in wines matured for 5 months.

Acknowledgments

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