Vitis **57**, 61–68 (2018) DOI: 10.5073/vitis.2018.57.61-68

Antioxidant activity, phenolic compounds and colour of red wines treated with new fining agents

G. D. Dumitriu[1)], N. López de Lerma[2)] V. V. Cotea[1)] and R. A. Peinado[2)]

[1)] Viticulture and Oenology Department, University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Iași, România

[2)] Agricultural Chemistry Department, University of Córdoba, Córdoba, Spain

Summary

Nowadays the clarification and stabilization of red wines is generally done with conventional fining agents, like bentonite and activated coal, which pose a major challenge to environmental security and wastes management. This stimulated the use of many new techniques in order to discover alternative fining agents that don't have negative influence on color, phenolic compounds and quality parameters. The aim of this research is to determine, how alternative fining agents, in different doses, affect antioxidant activity and colour parameters of 'Cabernet Sauvignon' red wines. Experimental material is from North-East Romania and was fined with mesoporous materials, bentonite and activated coal. Discriminant analysis classified 'Cabernet Sauvignon' wines according to the different fining agents based on total polyphenolic compounds and total antioxidant activity. Alternative fining agents, as mesoporous materials, have less impact on the colour and phenolic content of red wines in contrast to activated coal and bentonite treatments that can conduct to unsatisfying characteristics. Mesoporous materials are preferable and could be an exceptional adsorbent for polyphenolic compounds.

Key words: red wines; alternative fining agents; mesoporous materials; antioxidant activity; colour.

Introduction

Colour and limpidity are among the most important attributes associated with high quality red wines. Such sensory characteristics are appreciated by consumers and contribute actively to the purchasing decision. Furthermore, red wines contain a number of polyphenolic compounds which determine other relevant sensorial aspects, such as astringency, bitterness and mouthfeel. These compounds shape the antioxidant properties of wines, influencing their potential health effect (Dumitriu *et al*. 2015) by having free radical scavenging activity as well as a protective activity against arteriosclerosis, coronary heart disease (Burns *et al*. 2000) or inhibiting cancer cell growth (Zhang *et al*. 2005). The content of phenolic composition in wine depends on the variety, soil, climate, as well as on the oenological practices applied for winemaking, clarification, stabilization treatments, aging and storage conditions (Kostadinovic *et al*. 2012).

Wine stabilization and clarification is normally obtained after winemaking due to chemical and physical phenomena that determine the precipitation of unstable compounds and the sedimentation of the hazy particles. The natural clarification process, in addition to being slow, may not be enough for proper clarity and stability of the wine. This negative aspect in wines implies that they are of poor quality which is refused by consumers. The process is usually improved by adding various agents that will interact with specific wine components and led to a better limpidity in less time and improved stability of the wines (Sims *et al*. 1995). Clarified wine should remain clear and not include any undesirable effects, such as removal of favorable flavors or addition of undesired components.

Frequently used fining agents in the wine industry such as bentonite, gelatine, isinglass, casein, egg albumin and polyvinylpolypyrrolidone can lead to considerable decrease of some phenolic compounds, to the removal of haze formation proteins or other quality parameters of wine.

Bentonite is a popular fining agent which consists of montmorillonite clay that swells in water and produces a sheet like structure on which cation exchange, hydrogen bonding and adsorption can occur (Marchal and Jeandet 2009). It is commonly used in the wine industry as a fining agent that absorbs positively charged wine ingredients. This technique eliminates unwanted components such as proteins, but also compounds that are beneficial to health, taste and even molecules which are contributing to wine aroma. A recent publication highlighted the mechanisms that play a role in the colour intensity decrease from wines fined with bentonite (Stankovic *et al*. 2012). Furthermore, protein based fining agents can determine some declines in astringency and bitterness of wine due to its interaction with tannins (Oberholster *et al*. 2013).

Activated coal is another commonly used fining agent. Its adsorptive characteristics have been proven to reduce the content of ochratoxin A (Sierka 2013), pesticides (Sen *et al*. 2012) and volatile smoke taint in wine (Fudge *et al*. 2012).

Correspondence to: Dr. N. López de Lerma, Agricultural Chemistry Department. Building Marie Curie, Campus de Rabanales, Agrifood Campus of International Excellence ceiA3, University of Córdoba, 14014 Córdoba, Spain. E-mail: b92lolem@uco.es

© The author(s).

BY-SA This is an Open Access article distributed under the terms of the Creative Commons Attribution Share-Alike License (http://creative-commons.org/licenses/by-sa/4.0/).

Overall there are many fining agents available for use in the wine industry each with their own advantages and limitations. Although various studies regarding the use of fining agents (bentonite and activated coal) have already been conducted, only a limited number of experiments that includes mesoporous materials as alternative fining agents are published. Among them some new naturally occurring adsorbent materials were studied, such as biosorbents (Aksu 2001), clays (Banat *et al.* 2000), zeolites (Kuleyin 2007, Kamble *et al.* 2008) and resins (Abburi 2003). An alternative to the porosity and size selectivity of zeolites represents the use of a mesoporous silicate (MCM-41, SBA-15 and KIT-6) as an adsorbent for the removal of undesirable particles. Mesoporous silicates give a number of potential facilities for nanoporous materials (Sing 1985), as adsorbents including larger pore volume and diameter, high surface area, regular well-ordered structure, non-cytotoxic properties and good thermal stability at wide temperature ranges. These properties provide great versatility to those materials for different technological applications, particularly in mass transfer processes such as adsorption and immobilization of substances (Bui and Choi 2009).

The hexagonal structure, MCM-41, was the most studied member of M41S family of mesoporous materials. MCM-41 combines a myriad of attractive properties including highly ordered pore systems with tunable pore diameters, large pore volumes, high hydrocarbon sorption capacities, high BET surface areas and thermal stability, as well as a high density of surface silanols (Qu *et al.* 2006).

Zhao *et al.* (1998) developed SBA-15 mesostructured silica, which consists of parallel cylindrical pores with axes arranged in a hexagonal unit cell. SBA-15 exhibits thicker pore walls, which provide high hydrothermal stability (Khodakov *et al.* 2005), and is suitable for use in aqueous media. Compounds that are present in silica gel, like silanol groups (Si-OH), are known as responsible for the adsorption of organic molecules. Also, the OH groups that are placed on the SBA-15 internal surface contribute in a high level to the total surface area of the mesoporous material (Saini *et al.* 2010).

Recently, KIT-6 as a member of mesoporous silica, has captivated great interest thanks to three-dimensional cubic *Ia3d* symmetry structure with interpenetrating continuous network of chiral channels (Liu *et al.* 2002). Some of these materials have recently tested as a way to prevent protein haze in with wines (Dumitriu *et al.* 2018) but to our knowledge there is no studies about their impact as fining agent in red wines. The purpose of this work is to evaluate the effect of these different fining agents on the phenolic compounds, antioxidant activity and colour parameters of 'Cabernet Sauvignon' red wines.

Material and Methods

Nanomaterials: All nanomaterials (KIT-6, MCM-41 and SBA-15) were purchased from ACS Material (Advanced Chemicals Supplier), LLC 18 Vernon Street Medford, USA. The characteristic of the nanomaterial used are present in Tab. 1.

Table 1

Types and characteristics of materials. SSA: specific surface area, PD: pore diameter, PS: particle

Material	SSA ($m^2 \cdot g^{-1}$)	PD (nm)	PS (μm)	Dosage ($g \cdot L^{-1}$)	Sample code
KIT-6	600-800	8-10	10-100	2	KIT 2
				4	KIT 4
				6	KIT 6
				10	KIT 10
MCM-41	850-850	3.4-5.0	100-1000	2	MCM 2
				4	MCM 4
				6	MCM 6
				10	MCM 10
SBA-15	600	7-10	1-2	2	SBA 2
				4	SBA 4
				6	SBA 6
				10	SBA 10
Bentonite	-	-	-	1	B 1
				1.5	B 1.5
				2	B 2
Activated coal	-	-	-	1	CA 1
				1.5	CA 1.5
				2	CA 2

Winemaking conditions: Experimental material used is 'Cabernet Sauvignon' grapes from North-East Romania, harvested in 2014. The red wine was done by classical winemaking technology, with a maceration-fermentation process at temperature of 10-12 °C, for 14 d and was inoculated with selected yeast. After that, must was pressed and the wines obtained were transferred in tanks for the finishing of alcoholic and malolactic fermentation. Spontaneous clarification with fining agents was performed. Three types of nanomaterials (KIT-6, MCM-41 and SBA-15) at different doses (2 $g \cdot L^{-1}$, 4 $g \cdot L^{-1}$, 6 $g \cdot L^{-1}$ and 10 $g \cdot L^{-1}$) were added directly to wine samples (see Tab. 1 for codes). In addition, positive controls using bentonite (B) at different doses (1 $g \cdot L^{-1}$, 1.5 $g \cdot L^{-1}$ and 2 $g \cdot L^{-1}$) and activated coal (CA) also at different doses (1 $g \cdot L^{-1}$, 1.5 $g \cdot L^{-1}$ and 2 $g \cdot L^{-1}$) were carried out. Bentonite was first dissolved in hot water; the mixture was agitated and left at room temperature for 24 h. Then hydrated bentonite was added to the red wine (Lambri *et al.* 2012). Also, a control variant was obtained without any addition of materials. All samples were performed in three repetitions.

The red wines were stirred 24 h in hermetic glass flask and the resulting variants were centrifuged at 5,000 rpm and 4 °C for 10 min and straightaway analyzed.

Analytical parameters: The general wine parameters were analyzed (pH, titratable and volatile acidity, respectively, alcohol strength) by using the analytical methods recommended by the OIV (2015). Turbidity was measured with a nephelometer (HANNA instruments, HI 93703 C). A specific calibration was made and after that the turbidity index of the wines were measured.

The samples were filtered through a HA-0.45 μm paper (Millipore, Milford, MA). The absorbance at 420, 520 and 620 nm was determined in a Perkin-Elmer Lambda 25 spectrophotometer and the colour intensity and tonality were calculated according to Glories (1984). Also, chroma or saturation (c*) and hue (h*) was determined.

Determination of antioxidant activity by discoloration of ABTS$^{\bullet +}$: Total antioxidant activity was measured with the blue/green chromophore ABTS$^{\bullet +}$ method described by Re *et al*. (1999). The ABTS$^{\bullet +}$ was produced by oxidation of 7 mM ABTS with 2.45 mM potassium persulphate in conditions of darkness for 12-16 h. The resulting ABTS$^{\bullet +}$ solution was diluted in 20 mM phosphate buffer at pH 7.4 to obtain an absorbance at 734 nm of 0.7. A volume equal to or greater than 900 µL of this test mixture was reacted with a volume equal to or less than 100 µL of wine samples, previously filtered through HA-0.45 µm papers (Millipore, Milford, MA), for 6 min. After this, the absorbance at 734 nm of the reaction mixture was measured against a blank of distilled water instead of wine. Antioxidant activity was measured in terms of the proportion of ABTS$^{\bullet +}$ inhibited: % inhibition = (A_{734} blank – A_{734} sample) x 100/ A_{734} blank. This percentage of inhibition should fall in the range 20-80 %.

Enzymatic determination of total phenolic content: The total phenolic content of the wines and of their respective phenolic fractions was determined with the enzymatic method described by Stevanato *et al*. (2004). A volume of 2.9 mL of a 0.1 M solution of potassium phosphate at pH 8, containing 2mM hydrogen peroxide, 3mM 4-aminoantipyrine and 0.33 µM horseradish peroxidase, was reacted with 0.1 mL of sample, previously filtered through Millipore HA-0.45 µm papers (Millipore, Milford, MA). After 10 min of reaction, the absorbance at 500 nm of the reaction solution was measured against a blank of distilled water.

Statistical analysis: The overall data set was processed using the software package Statgraphics Centurion XVI StatPoint Technologies, Inc. (Warrenton, VA, USA). Sample results were expressed as the mean values and the standard deviations. A homogeneous group's analysis was done to study if there were significant differences among the samples in the determined parameters. The simple regression analysis was employed to determine the correlation coefficients among means. The data set, which was composed of values obtained from total antioxidant activity and total polyphenolic compounds analysis, was used for discriminant analysis.

Heat maps method was used for visualizing the data set. Samples were organized as an matrices. At the beginning, the heat map analysis reorders the rows and columns by bringing together samples with similar profiles. After that, each sample from the data matrix is attributed a specific color according to its value, and so allowing the identification of patterns graphically. By using correlation-based distances and the Ward method of agglomeration a dendrogram was created. All computations were done with R 3.0.33 software and the packages "gplots" were used.

Results and Discussion

Enological parameters: Selected nanomaterials are composed of silica and include porous systems with various sizes and topology. SBA-15 and MCM-41 show 2D hexagonal pore structures, while SBA-15 has larger pore diameters and thicker walls than MCM-41. KIT-6 has a 3D connected cylindrical system of pores. Transmission electron microscopy (TEM) confirmed the textural and morphological characteristics of the materials (Tab. 1). According to the pore diameter, SBA-15 and KIT-6 possesses the largest pore diameter (7-10 nm) and MCM-41 shows the smallest pore diameter (3.4-5.0 nm).

Turbidity was measured with a turbidimeter and results were expressed in Nephelometric Turbidity Units, or NTU. According to Ribéreau-Gayon *et al*. (2012), white wine is considered brilliant when NTU is 1.1; for rosés, when NTU is 5.8; and for reds, when NTU is 8.0. Wine turbidity is an important clarity indicator. In alcoholic beverage, the presence of suspensions contributes to the turbidity. Loss of clarity can come from various sources: microbial growth and production of polysaccharides; precipitation of chemical compounds, denaturation and complex formation between macromolecules, such as polyphenolics, proteins, and polysaccharides, or other.

Fig. 1 shows the turbidity of the untreated wine (Control) and of the wine treated with the different materials. Results put in evidence that fining agents increased the limpidity of wines in different manners, in agreement with the results reported by several studies (Oberholster *et al*.

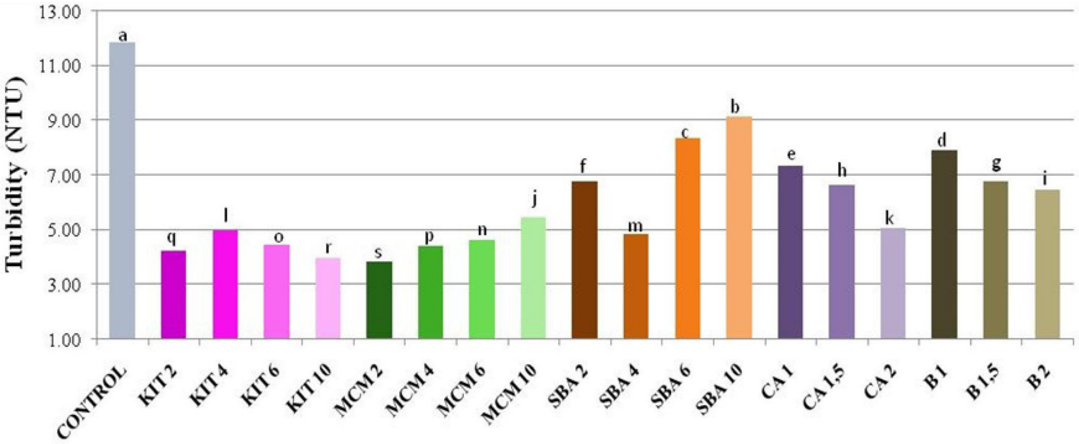

Fig. 1: Turbidity of 'Cabernet Sauvignon' wines after treatment with nanomaterials, activated coal (CA) and Bentonite (B) expressed as nephelometric turbidity units (NTU). The numbers after the fining material indicate the dose used (g·L^{-1}). Different letters indicate significant differences at 95 % confidence level.

Table 2

Chromatic parameters, total and volatile acidity of 'Cabernet Sauvignon' wines after the treatment with nanomaterials, activated coal and bentonite. The numbers after the fining material indicate the dose used (g·L^{-1}). Different letter indicate significant differences at 95 % confidence level

	Absorbance			Hue	Chroma	Titratable acidity (g·L^{-1})	Volatile acidity (g·L^{-1})
	420 nm	520 nm	620 nm				
CONTROL	4.05 ± 0.03^{b}	4.69 ± 0.02^{a}	1.55 ± 0.01^{i}	23.16 ± 0.74^{h}	44.33 ± 1.39^{h}	5.75 ± 0.00^{a}	0.56 ± 0.02^{a}
KIT 2	4.05 ± 0.02^{b}	4.52 ± 0.02^{b}	1.57 ± 0.02^{h}	22.82 ± 0.65^{i}	43.83 ± 1.34^{i}	5.65 ± 0.04^{d}	0.55 ± 0.01^{a}
KIT 4	4.00 ± 0.02^{c}	4.39 ± 0.02^{c}	1.59 ± 0.01^{f}	22.50 ± 0.63^{l}	43.38 ± 1.34^{l}	5.50 ± 0.04^{g}	0.55 ± 0.01^{a}
KIT 6	4.00 ± 0.01^{c}	4.52 ± 0.01^{b}	1.58 ± 0.01^{g}	22.60 ± 0.69^{j}	43.52 ± 1.37^{j}	5.63 ± 0.07^{e}	0.55 ± 0.01^{a}
KIT 10	3.96 ± 0.02^{d}	4.39 ± 0.03^{c}	1.58 ± 0.01^{g}	22.59 ± 0.64^{k}	43.49 ± 1.37^{k}	5.65 ± 0.04^{d}	0.55 ± 0.01^{a}
MCM 2	4.09 ± 0.03^{a}	4.52 ± 0.04^{b}	1.63 ± 0.01^{b}	21.88 ± 0.63^{r}	42.54 ± 1.36^{q}	5.73 ± 0.04^{b}	0.47 ± 0.01^{b}
MCM 4	4.00 ± 0.01^{c}	4.39 ± 0.04^{c}	1.62 ± 0.02^{c}	21.90 ± 0.58^{q}	42.52 ± 1.36^{r}	5.65 ± 0.04^{d}	0.47 ± 0.01^{c}
MCM 6	4.09 ± 0.03^{a}	4.52 ± 0.03^{b}	1.62 ± 0.01^{c}	22.00 ± 0.58^{o}	42.67 ± 1.36^{p}	5.63 ± 0.09^{e}	0.47 ± 0.01^{c}
MCM 10	4.09 ± 0.03^{a}	4.69 ± 0.03^{a}	1.64 ± 0.01^{a}	21.79 ± 0.63^{s}	42.41 ± 1.36^{s}	5.70 ± 0.04^{c}	0.47 ± 0.01^{c}
SBA 2	3.92 ± 0.03^{e}	4.52 ± 0.01^{b}	1.54 ± 0.01^{j}	23.21 ± 0.68^{g}	44.34 ± 1.74^{g}	5.55 ± 0.04^{f}	0.54 ± 0.01^{ab}
SBA 4	3.96 ± 0.01^{d}	4.30 ± 0.02^{d}	1.60 ± 0.01^{e}	22.36 ± 0.69^{m}	43.16 ± 1.69^{m}	5.13 ± 0.04^{l}	0.54 ± 0.01^{ab}
SBA 6	4.00 ± 0.02^{c}	4.39 ± 0.02^{c}	1.61 ± 0.01^{d}	22.23 ± 0.68^{n}	42.99 ± 1.46^{n}	5.05 ± 0.01^{m}	0.54 ± 0.01^{ab}
SBA 10	3.96 ± 0.02^{d}	4.30 ± 0.05^{d}	1.63 ± 0.01^{b}	21.98 ± 0.72^{p}	42.68 ± 1.46^{o}	5.05 ± 0.04^{m}	0.54 ± 0.01^{ab}
Activated Coal 1	2.82 ± 0.01^{i}	3.92 ± 0.01^{h}	0.97 ± 0.01^{n}	30.56 ± 0.63^{b}	57.27 ± 1.53^{c}	5.20 ± 0.02^{k}	0.53 ± 0.01^{b}
Activated Coal 1,5	2.27 ± 0.02^{j}	3.34 ± 0.02^{i}	0.76 ± 0.01^{o}	31.91 ± 0.64^{a}	62.46 ± 1.74^{b}	5.75 ± 0.04^{a}	0.53 ± 0.01^{b}
Activated Coal 2	1.91 ± 0.01^{k}	2.77 ± 0.03^{j}	0.63 ± 0.01^{p}	30.27 ± 0.65^{c}	63.35 ± 1.78^{a}	5.73 ± 0.02^{b}	0.53 ± 0.01^{b}
Bentonite 1	3.41 ± 0.03^{f}	4.22 ± 0.03^{e}	1.18 ± 0.02^{k}	27.92 ± 0.74^{f}	51.93 ± 1.53^{f}	5.35 ± 0.01^{i}	0.53 ± 0.01^{b}
Bentonite 1,5	3.19 ± 0.03^{g}	4.15 ± 0.05^{f}	1.09 ± 0.02^{l}	29.09 ± 0.75^{e}	53.92 ± 1.53^{e}	5.36 ± 0.01^{h}	0.53 ± 0.01^{b}
Bentonite 2	3.05 ± 0.01^{h}	4.05 ± 0.02^{g}	1.03 ± 0.02^{m}	29.92 ± 0.74^{d}	55.81 ± 1.51^{d}	5.32 ± 0.04^{j}	0.53 ± 0.01^{b}

2013). 'Cabernet Sauvignon' wine samples fined with mesoporous materials present better clarification when using KIT-6 and MCM-41, decreasing with 66.4 %, respectively 67.6 % when compared with the control wine. Bentonite treatments ranged between 33.1 % and 45.5 %, while activated coal recorded values among 37.9 % and 57.2 %. Nanomaterials used at the highest doses do not seem to be suitable for red wine fining due to the low reduction of wine turbidity.

pH values varied in function of fining agent applied in the interval 3.23-3.39. Fining agents did not change significantly the alcoholic degree of the wines (results not show), registering a value around 13.2 % (v/v). Fining treatment decreased titratable acidity and in a lesser extent the volatile acidity (Tab. 2). The highest differences in this last parameter were observed in wines treated with MCM-41 nanomaterial independently of the doses used. Bentonite and activated coal showed the same behavior. Regarding titratable acidity, SBA nanomaterial at 6 and 10 g·L^{-1} showed the highest reduction.

Total antioxidant activity and total polyphenolic compounds: The total polyphenolic compounds (TPC) of red wines determined by the enzymatic method varied from 1344.1 ± 86 mg catechin·L^{-1} to 3165.3 ± 106 mg catechin·L^{-1}. In Fig. 2 can be clearly seen that in general the different fining agents applied in-

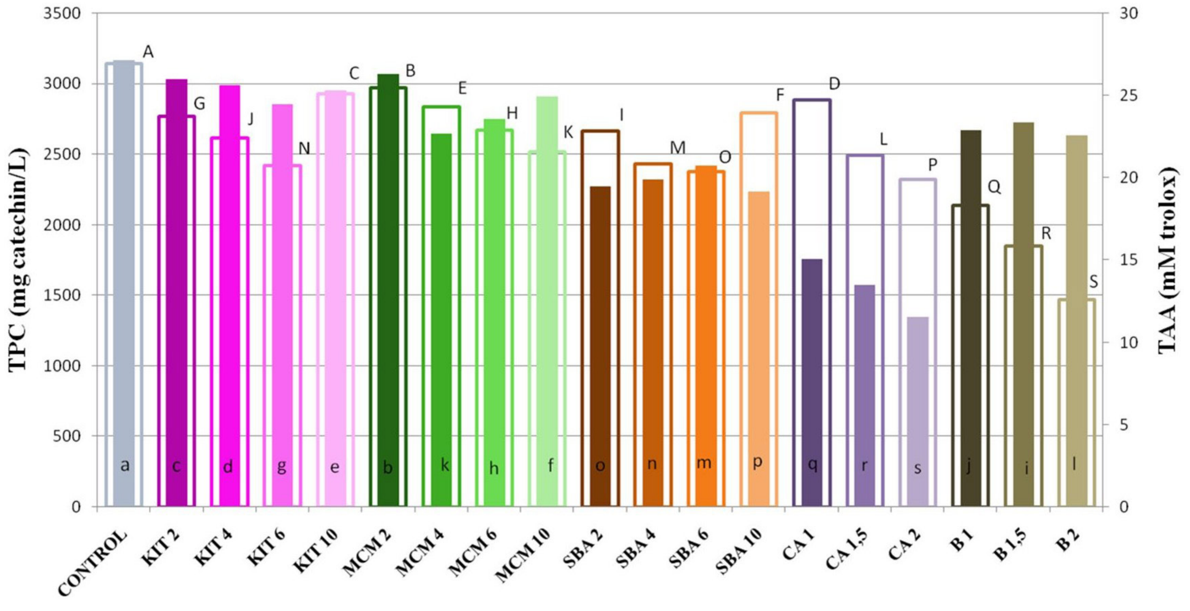

Fig. 2: Total polyphenolic compounds (TPC), expressed as milligram of catechin per liter (full bars), and total antioxidant activity (TAA), expressed as millimolar of trolox (empty bars), of wines after treatment with nanomaterials, activated coal (CA) and Bentonite (B). The numbers after the fining material indicate the dose used (g·L^{-1}). Different small letters indicate significant differences at 95 % confidence level in TPC values. Different capital letters indicate significant differences at 95 % confidence level in TAA values.

duced a decrease of the total polyphenolic content present in wines. A significant content reduction of total polyphenolic compound was observed for SBA-15 and ranged between 23.5 % (SBA 6) and 29.3 % (SBA 10) followed by MCM-41 that ranged from 3.1 % (MCM 2) to 16.4 % (MCM 4) in respect to the control variant. The adsorption on the mesoporous materials can be attributed to the presence of the silanol groups and the availability of electrons of the phenyl rings may enhance the interactions with Si-OH group. For activated coal and bentonite a significant TPC reduction occurred as well, 57.5 % for activated coal at 2 g·L^{-1} and 16.7 % for bentonite at 2 g·L^{-1}.

For the total antioxidant activity (TAA), SBA-15 and KIT-6 were closely ranked according to the obtained results. In both cases, the percentage values decreased from 11.2 % to 24.4 % (SBA-15) and respectively, from 6.9 % to 23.1 % (KIT-6). As can be seen Fig. 2, the most important decreases of antioxidant activity were recorded after treatments with 2 g·L^{-1} of activated coal and 2 g·L^{-1} bentonite, each treatment reducing with 26.1 % and 53.3 % the content when compared with control variant.

A strong correlation between the antioxidant activity and total polyphenolic compounds was found for red wines in this study, with a correlations coefficient of 0.829 (Fig. 3). This can be attributed to a predominant role of polyphenols in the antioxidant action. Many other authors have been also reported a very high degree of correlation between total polyphenols content and antioxidant activity of red wines (Yoo *et al*. 2011). The antioxidant activity of red wines is believed to depend to a great extent on the flavanol content, but is also influenced by the relative amounts of the individual flavanols. Fining agent's nature has a different effect on the phenolic composition, especially in flavanol monomers and dimers. Cosme *et al.* (2007) reported that some fining agents (plant proteins and gelatines) have a selective action, precipitating highly polymerized and highly galloylated tannins from red wines. This can be similar to our results that indicate a selective action of mesopourous materials on individual phenolic composition and concomitantly these individual compounds contribute in various modalities to the red wine antioxidant activity.

Discriminant analysis (DA), as a multidimensional discriminant technique, is implemented for finding a linear combination of features, which characterizes or separates two or more classes of objects. The first discriminant function (F1) accounts for 89.86 % of the total dispersion with a correlation of 0.976, which measures the association among the discriminant scores and the groups. The second discriminant function (F2) accounts for 10.14 % of the total variance with a canonical correlation of 0.836. Fig. 4 shows the distribution of red wines treated with various fining agent at different concentrations using total polyphenolic compounds and total antioxidant activity as parameters. TPC and TAA permits to differentiate among wines subjected to mesoporous materials, bentonite and activated coal. SBA-15 and activated coal were situated in the left part of the coordinate, which is correlated with the highest retained quantity of total polyphenolic compounds and antioxidant activity. The location of MCM-41 and KIT-6 in the right part, which is opposite to the other fining agents, indicates that they retained the lowest amounts of TPC and TAA.

Colour properties: Colour is one of the principal attributes of a wine and it represents a decisive factor for the choice of consumers. In this regard, colour studies can be a helpful tool in oenological practices in the recognition of the typical characteristics of a wine.

Red wine colours are influenced by the content in phenolic compounds and derivative pigments from anthocyanins. Also, the colours depend on the copigmentation phenomena and pH of wine (Boulton 2001). Chemical structure, degree of ionisation and levels of anthocyanins

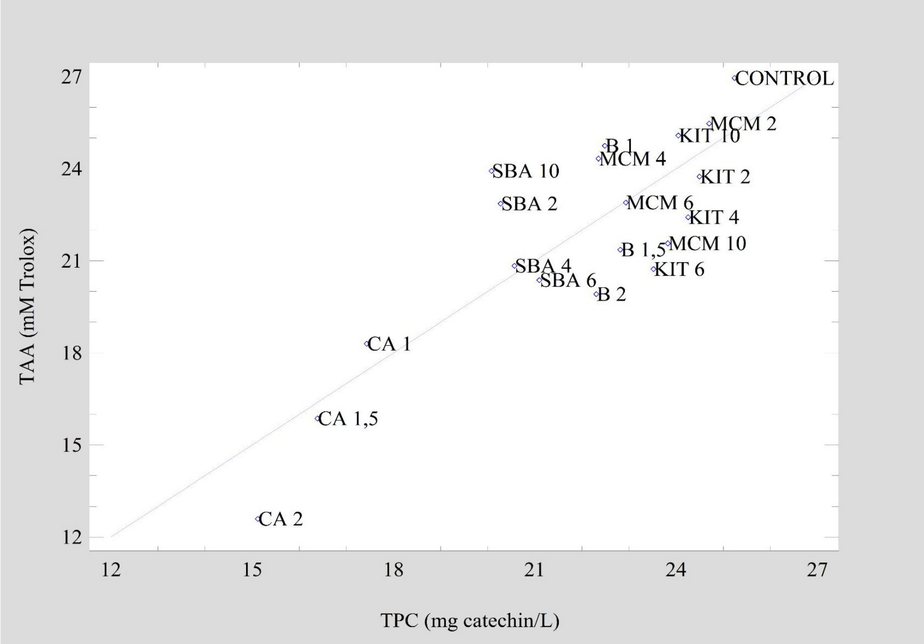

Fig. 3: Simple regression analysis between total polyphenolic compounds and total antioxidant activity of wines after treatment with nanomaterials, activated coal (CA) and Bentonite (B). The numbers after the fining material indicate the dose used (g·L^{-1}).

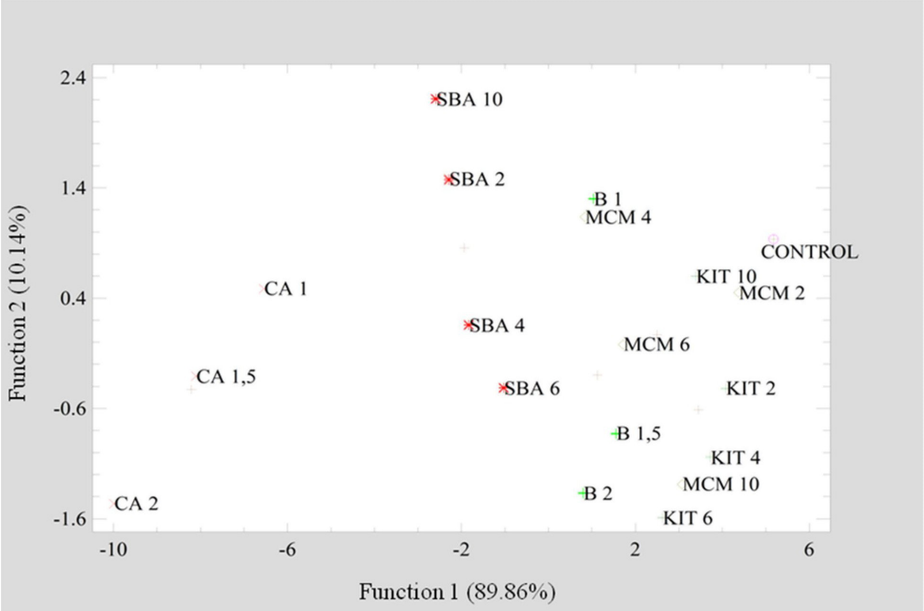

Fig. 4: Discriminant analysis using total polyphenolic compounds and total antioxidant activity as classifying variables of wines after treatment with nanomaterials, activated coal (CA) and Bentonite (B). The numbers after the fining material indicate the dose used ($g \cdot L^{-1}$).

primordially define the chromatic properties of young red wines and affect the formation of derivative pigments.

Tab. 2 shows the chromatic parameters of 'Cabernet Sauvignon' treated wines. The majority of fining treatments applied led to a colour intensity decrease when compared to the control wines. A single exception was observed for the samples treated with MCM 10. Activated coal and bentonite have an important impact on the colour of red wines (Tab. 2) affecting their intensity and quality.

Reduction of colour intensity (0.36 % for MCM 2 up to 4.16 % for SBA 4) was accompanied by increases of tonality (0.58 % for SBA 2 up to 6.85 % for SBA 4) in the wines clarified with mesoporous silica materials. Also, it can be noted that diminution in colour intensity (14.3 % for Bentonite at 1 $g \cdot L^{-1}$ up to 48.4 % for activated coal at 2 $g \cdot L^{-1}$) was accompanied by a tonality decrease (6.2 % for bentonite at 1 $g \cdot L^{-1}$ up to 20.9 % for activated coal at 1.5 $g \cdot L^{-1}$).

Hue and chroma parameters also decreased for variants treated with nanomaterials, and an increase for activated coal and bentonite samples was observed. The contradictory behavior of wine samples fined with different treatments is another proof of the high variance of phenolic profile.

The intensity of yellow, red and blue colours (absorbance at 420 nm, 520 nm and 620 nm) decreased obviously in activated coal and bentonite variants in contrast with nanomaterial variants. However, hue and chroma values increased in wines treated with activated coal and bentonite when compared with mesoporous materials variants (Fig. 5). A hierarchical cluster analysis was used to group

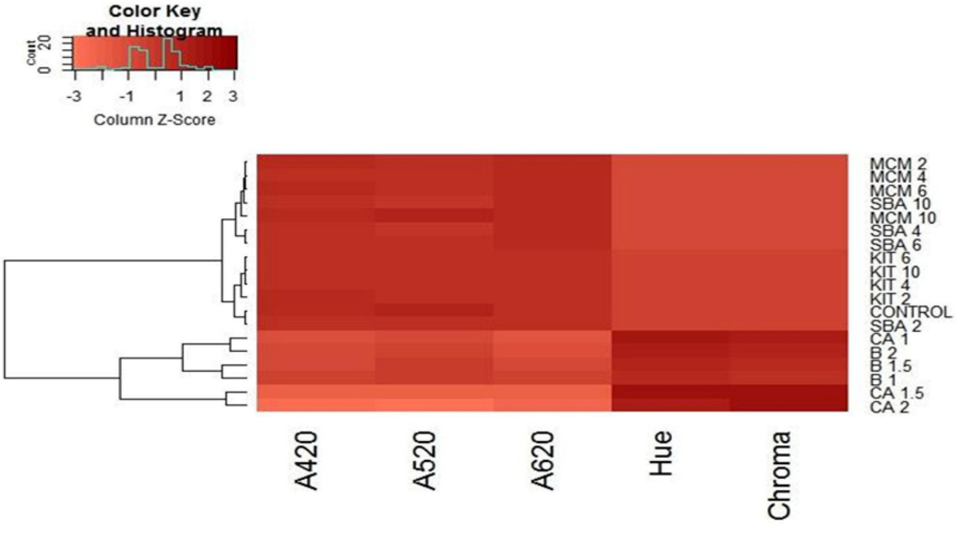

Fig. 5: Heatmap of colour parameters obtained after treatment with nanomaterials, activated coal (CA) and Bentonite (B). The numbers after the fining material indicate the dose used ($g \cdot L^{-1}$).

the wine samples according to their colour properties (Fig. 5). In the clustering heatmap we can notice a clear change of colour profiles that occurred in clarified wines with different treatments. Colour parameters were grouped into two main clusters. Cluster 1 represented activated coal and bentonite variants and cluster 2 includes mesoporous materials and control wines sample.

Conclusions

Nanomaterial treatments diminished wine turbidity in 'Cabernet Sauvignon' whereas KIT-6 and MCM-41 have shown better NTU values. Fining agents produced different impacts on the total polyphenolic compounds and the total antioxidant activity, determining a various influence on the colour of the wine. Among mesoporous materials, SBA-15 reduced significantly the TPC and TAA. Meanwhile, activated coal and bentonite presented the strongest removal of phenolic contents from all studied variants. Alternative fining agents, as mesoporous materials, have less impact on the colour and phenolic content of red wines in contrast to activated coal and bentonite treatments that can conduct to unsatisfying characteristics. Taking into consideration the negative impact of bentonite on the environmental and the elimination of important vital compounds from red wines, mesoporous materials are preferable and could be an exceptional adsorbent for polyphenolic compounds.

Acknowledgements

This paper was carried out under the frame of OIV Research Grant. We are also grateful to the Oenological Research Center-Romanian Academy, Iași Branch.

References

Abburi, K.; 2003: Adsorption of phenol and p-chlorophenol from their single and bisolute aqueous solutions on Amberlite XAD-16 resin, J. Hazard. Mater. B **105**, 143-156.

Aksu, Z.; 2001: A comparative adsorption/biosorption study of mono-chlorinated phenols onto various sorbents, J. Yener. Waste Manage. **21**, 695-702.

Banat, F. A.; Al-Bashir, B.; Al-Asheh, S.; Hayajneh, O.; 2000: Adsorption of phenol by bentonite, Environ. Pollut. **101**, 391-398.

Boulton, R.; 2001: The copigmentation of anthocyanins and its role in the color of red wine: a critical review. Am. J. Enol. Vitic. **52**, 67-87.

Bui, T. X.; Choi, H.; 2009: Adsorptive removal of selected pharmaceuticals by mesoporous silica SBA-15. J. Hazard. Mater. **168**, 602-608.

Burns, J.; Gardner, P. T.; O'Neil, J.; Crawford, S.; Morecroft, I.; McPhail, D. B.; Lister, C.; Matthews, D.; MacLean, M. R.; Lean, M. E.; Duthie, G. G.; Crozier, A.; 2000: Relationship among antioxidant activity vasodialtion capacity and phenolic content of red wines. J. Agric. Food Chem. **48**, 220-230.

Cosme, F.; Ricardo-Da-Silva, J. M.; Laureano, O.; 2007: Protein fining agents: characterization and red wine fining assays. Ital. J. Food Sci. **19**, 39-52.

Dumitriu, D.; Peinado, R. A.; Peinado, J.; López de Lerma, N.; 2015: Grape pomace extract improves the *in vitro* and *in vivo* antioxidant properties of wines from sun light dried Pedro Ximénez grapes. J. Funct. Foods **17**, 380-387.

Dumitriu, G. D.; López de Lerma, N.; Luchian, C.; Cotea, V.; Peinado, R. A. 2018: Study of the potential use of mesoporous nanomaterials as fining agent to prevent protein haze in white wines and its impact in major volatile aroma compounds and polyols. Food Chem. **240**, 751-758

Fudge, A. L.; Schiettecatte, M.; Ristic, R.; Hayasaka, Y.; Wilkinson, K. L.; 2012: Amelioration of smoke taint in wine by treatment with commercial fining agents. Aust. J. Grape Wine Res. **18**, 302-307.

Glories, Y.; 1984: La couleur des vins rouges. Mesure, origine et interprétation. Partie I. Connaiss. Vigne Vin **18**, 195-217.

Kamble, S. P.; Mangrulkar, P. A.; Bansiwal, A. K.; Rayalu, S. S.; 2008: Adsorption of phenol and o-chlorophenol on surface altered fly ash based molecular sieves, Chem. Eng. J. **138**, 73-83.

Khodakov, A. Y.; Zholobenko, V. L.; Bechara, R.; Durand, D.; 2005: Impact of aqueous impregnation on the long-range ordering and mesoporous structure of cobalt containing MCM-41 and SBA-15 materials, Micropor. Mesopor. Mat. **79**, 29-39.

Kostadinović, S.; Wilkens, A.; Stefova, M.; Ivanova, V.; Vojnoski, B.; Mirhosseini, H.; Winterhalter, P.; 2012: Stilbene levels and antioxidant activity of Vranec and Merlot wines from Macedonia: Effect of variety and enological practices. Food Chem. **135**, 3003-3009.

Kuleyin, A.; 2007: Removal of phenol and 4-chlorophenol by surfactant modified natural zeolite, J. Hazard. Mater. **144**, 307-315.

Lambri, M.; Dordoni, R.; Silva, A.; De Faveri, D. M.; 2012: Comparing the impact of bentonite addition for both must clarification and wine fining on the chemical profile of wine from Chambave Muscat grapes. Int. J. Food Sci. Tech. **47**, 1-12.

Liu, X. Y.; Tian, B. Z.; Yu, C. Z.; Gao, F.; Xie, S. H.; Tu, B.; Che, R. C.; Peng, L. M.; Zhao, D. Y.; 2002: Room-temperature synthesis in acidic media of large-pore three-dimensional bicontinuous mesoporous silica with Ia3d symmetry. Angew. Chem. Int. **41**, 3876-3878.

Marchal, R.; Jeandet, P.; 2009: Use of enological additives for colloid and tartrate salt stabilization in white wines and for improvement of sparkling wine foaming properties. In: M. Moreno-Arribas, M. Polo (Eds): Wine chemistry and biochemistry, 128-158: Springer.

Oberholster, A.; Carstens, L. M.; Du Toit, W. J.; 2013: Investigation of the effect of gelatine, egg albumin and cross-flow microfiltration on the phenolic composition of Pinotage wine. Food Chem. **138**, 1275-128.

OIV; 2015: Compendium of International Methods of Wine and Must Analysis. O I V (Off. Int. Vigne Vin), Paris, France.

Qu, F.; Zhu, G.; Huang, S.; Li, S.; Suan, J.; Zhang, D.; Qius, S.; 2006: Controlled release of captopril by regulating the pore size and morphology of ordered mesoporous silica. Micropor. Mesopor. Mat. **92**, 1-9.

Re, R.; Pellegrini, A.; Pannala, A.; Yang, M.; Rice-Evans, C.; 1999: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. **26**, 1231-1237.

Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D.; 2012: Traité d'Œnologie, Tome 2 - Chimie du Vin. Stabilisation et Traitements. 6eme éd. Dunod, Paris.

Saini, V. K.; Andrade, M.; Pinto, M. L.; Carvalho, A. P.; Pires, J.; 2010: How the adsorption properties get changed when going from SBA-15 to its CMK-3 carbon replica. Sep. Purif. Technol. **75**, 366-376.

Sen, K.; Cabaroglu, T.; Yilmaz, H.; 2012: The influence of fining agents on the removal of some pesticides from white wine of *Vitis vinifera* L. cv. Emir. Food Chem. Toxicol. **50**, 3990-3995.

Sierka, R. A.; 2013: Activated carbon adsorption and chemical regeneration in the food industry. NATO science for peace and security series C: Environ. Secur. **119**, 93-105.

Sims, C.; Eastridge, J.; Bates, R.; 1995: Changes in phenols, color, and sensory characteristics of Muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. Am. J. Enol. Vitic. **46**, 155-158.

Sing, K. S. W.; Everett, D. H.; Haul, R. A. W.; Moscou, L.; Pierotti, R. A.; Rouquerol, J.; Siemieniewska, T.; 1985: Reporting physisorption data for gas/solid systems-with special reference to the determination of surface area and porosity, Pure Appl. Chem. **57**, 603-619.

Stanković, S.; Jović, S.; Zivković, J.; Pavlović, R.; 2012: Influence of age on red wine colour during fining with bentonite and gelatin. Int. J. Food Prop. **15**, 326-335.

Stevanato, R.; Fabris, S.; Momo, F.; 2004: New enzymatic method for the determination of total phenolic content in tea and wine. J Agric Food Chem. **52**, 6287-6293.

Yoo, Y. J.; Prenzler, P. D.; Saliba, A. J.; Ryan, D.; 2011: Assessment of some Australian red wines for price, phenolic content, antioxidant activity, and vintage in relation to functional food prospects. J. Food Sci. **76**, 1355-1364.

Zhang, Y.; Vareed, S. K.; Nair, M. G.; 2005: Human tumor cell growth inhibition by non-toxic anthocyanidins, the pigments in fruits and vegetables. Life Sci. **76**, 1465-1472.

Zhao, D.; Huo, Q.; Feng, J.; Chmelka, B. F.; Stucky, G. D.; 1998: Nonionic triblock and star diblock copolymer and oligomeric surfactant syntheses of highly ordered, hydrothermally stable, mesoporous silica structures, J. Am. Chem. Soc. **120**, 6024-6036.

Received July 27, 2017
Accepted March 6, 2018