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THE DETERMINATION OF OXIDATION BEHAVIOR OF WHITE WINES PRODUCED FROM LOCAL AND EUROPEAN GRAPE VARIETIES USING SPECTROPHOTOMETRIC METHOD

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Abstract: The article deals with the oxidation processes of experimental wines produced from indigenous grape varieties *Legenda*, *Viorica* and European grapes *Chardonnay*, *Sauvignon*. The browning processes in wine are corelated with oxidation of hydroxycinnamates (hydroxycinnamic acids and their tartaric esters, HCAs) – the most important group of phenolic compounds in white wines. The potential degree of wine colour changes has been appreciated using *Polyphenols Oxidative Medium test* (*POM-test*). The oxidative crocin bleaching (CBA – Crocin Bleaching Assay) has been studied using the method of competition kinetics. The comparative antioxidant capacity of wines has been determined with peroxy radicals 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH).

Key words: *antioxidant capacity, crocin oxidative bleaching, flavonoids, hydroxycinnamates, phenolic compounds, POM-test, wine oxidation*.

Introduction

The biggest part of Moldovan wines are produced from European grape varieties, that are adapted to local growing conditions. These grape varieties are: *Chardonnay*, *Sauvignon*, *Cabernet-Sauvignon*, *Merlot* etc. At the same time, recently, in Republic of Moldova, much attention has been given to the goal of using the potential of old local grape varieties (*Feteasca Alba*, *Feteasca Regala*, *Feteasca Neagra*, *Rara Neagra*) and new grape varieties (*Viorica*, *Legenda, Riton)*. This kind of grape varieties are used by winemakers for producing high quality wines, single grape variety or blended. In this context, it is necessary to study the physico-chemical and organoleptic properties of them, polyphenol metabolism during processing of grapes, winemaking and wine storage.

The importance of phenolic compounds is well known [1]: these chemical substances are responsible for oxidative browning, process that are catalysed by enzyme polyphenol oxidase (PFO) or by ions and transition metals (preponderant Fe and Cu). Phenolic compounds are oxidized by the polyphenol oxidase (PPO) during the alcoholic fermentation, while the oxidation of phenolic compounds by ions and transition metals occurs after alcoholic fermentation, or under certain conditions, after wine bottling.

In addition, hydroxycinnamic acids can be the chemical precursors of 4-vinylphenol, that is further reduced to 4-ethylphenol by the enzyme vinyl phenol reductase of spoilage yeast *Brettanomyces*. This volatile substance, 4-ethylphenol, is responsible for such wine fault like smell of barnyards, fecal and gamey horse aromas.

The (HCAs) determines largely the colour of white wines, the antioxidant properties and some aromas after the alcoholic fermentation. In this regard, it is important to evaluate the phenolic profile and antioxidant capacity of the wine, the oxidation behaviour of phenolic compounds.

According to many scientific studies, (HCAs) and their derivatives have unique biological features. Hydroxycinnamic acids have antioxidant and anti-inflammatory properties, the ability to prevent renal failure, cardiovascular diseases, oxidative stress, insulin resistance, weight gain, dyslipidemia, improve liver functions [2]. Recent data support their beneficial application as preventive and/or therapeutic agents in several oxidative stress related diseases, such as atherosclerosis and cancer [3].

Thus, it's very important to provide conditions for producing high quality and healthy white wines. In this context, the determination of oxidation behaviour of (HCAs), in order to preserve them in wine, is the important task of enology. The development of effective and reliable methods for rapid testing the selective oxidation of (HCAs) is relevant. The currently techniques for appreciation the white wines oxidation are based on the qualitative and quantitative monitoring of chemical composition – liquid chromatography (HLPC), gas chromatography/mass spectrometry (GS/MS), based on colour change characteristics, that reflect the total oxidative reactions – UV-VIS spectrophotometry [4]. It is known that the levels of specific antioxidants, like polyphenols and redox mechanisms may be evaluated by electrochemical methods [5, 6], for example cyclic voltammetry [7]. The lack of a simple and accessible method for studying the oxidation of (HCAs) was the motivation for the research.

Materials and methods

The wines produced from 2 European grape varieties *Chardonnay* (Ch), *Sauvignon Blanc* (S) and 2 local grape varieties *Viorica* (V), *Legenda* (L) have been selected for research. The wines have been produced in 2017 at micro-winery of Enology department, Technical University of Moldova using general technologies of white winemaking. The sulphur dioxide (SO_2) has been added in grape crusher (50 – 75 mg /kg). The grapes have been destemmed and crushed at roller crusher. The grape must has been macerated for 2 hours at 12 – 14°C. In the grape must during maceration there have been added enzymes Ultrazym® 100G (Novozymes A/S, Denmark) (0,5 – 1 g/dl). From the grape variety Legenda, the samples also have been taken directly from the grape press without maceration (L1), after 4 hours of maceration (L2) and after 2 hours of maceration (L3). For all wines the post-fermentation period lasted for 40 days (14 – 16°C).

The wine samples have been filtered through the filter of 0,45 μ for spectrophotometric investigations (absorption spectra, total polyphenol index – IPT, phenolic compounds, the test of oxidation behaviour – POM-test, antioxidant capacity of wines etc.). The spectrophotometric analyses have been done at single beam spectrophotometer PG T70 (PG Instruments, UK) and double beam spectrophotometer Specord 250 Plus (Analytik Jena, Germania). These spectrophotometres have in their software application the function for calculating of derivatives.

The preparation of test samples and analysis of the basic parameters of wine has been done using automatic distiller Gibertini DEE and hydrostatic scale Densimat CE with module Alcomat-2 (Gibertini Elettronica, Italy), (the volumetric content determination of ethyl alcohol and dry residue), distillers GlassChem VA-1 (the determination of volatile acidity), and GlassChem SO_2 (GlassChem, South African Republic) (the determination of all SO_2 forms),

electronic titrators Titrette (Brand, Germany). The measurements of the pH values have been performed on pH Meter WTW Inolab 7110 (WTW, Germany). The used reagents (H_2O_2, AAPH) were produced by Sigma-Aldrich.

For the deposition of phenolic substances there have been used bentonite Bento Zero (Dal Cin, Italy), as well as polyvinylpolypyrrolidone (PVPP Vason Enologica, Italy). All necessary solutions have been prepared on distilled water (GFL 2004, Germany).

The deposition of sediment has been done using the centrifuge Universal 320 R (Hettich, Germany). The IPT has been determined by measurement of wine absorption at 280 nm in quartz cuvette (0,1 cm), with recalculation for the cuvette 1 cm.

The POM-test, proposed by Muller-Spath [8] for the oxidation behaviour determination of white wines, consists in the artificial oxidation of wine in the presence of certain concentrations of H_2O_2 within 60 minutes at 60°C. Herewith, there is optical absorption changes of samples at 420 nm in cuvette 1cm. Calculations are made according to the formula:

$$POM\ test\ (\%) = 100 * \frac{A_{420}(wine + H_2O_2) - A_{420}(wine)}{A_{420}\ (wine)}$$

The comparative antioxidant capacity of wines has been determined using the method Crocin Bleaching Assay (CBA) [9, 10]. The absorbance capacity of crocin (water-soluble carotenoid) has been measured at 443 nm. The generation of the radicals and its reaction with substrates have been performed in cuvettes held in thermostat at 40°C. Crocin has been extracted from commercial saffron (*Crocus Sativus L.*) (Aromatica SRL, Italia) and it has been purified according to Ordoudi and Tsimidou [11]. The concentration of the extract has been determined using spectrophotometric analysis. In reactant solutions with added wine it is ensured the crocin concentration of 10^{-6} M. The concentration of total phenolic compounds, flavonoids and cinnamic compounds has been performed at the spectrophotometer according to Somers and Verette [12].

Results and discussion

The absorption spectra of the studied wines in UV-vis region are measured at 260 – 280 nm. This value is based on the characteristic absorption of the benzene cycles of the majority phenols at 280 nm. Hydroxycinnamates (C6 – C3 phenolic compounds) have the maximum absorption with bathochromic shift at wavelength 300 – 350 nm.

The (HCAs) are the major non-flavonoid phenolic compounds in white grape and wine and thus their absorption spectra differ from the absorption spectra of red wine to balance of the maximum absorption at wavelengths at 260 – 280 and 300 – 350 nm. The visual analysis offers a first information about (HCAs) content in the complex of total phenols. However, original spectra do not show the fine differences between studied samples, oxidized wine and unoxidized wine. This is possible if we study the second order derivative spectra. The minimum interdependences $d^2A/d\lambda^2$ show the exact positions of the obvious and latent maximum. The final spectra, the algebraic sum of individual spectra, can distinguish them and have no coincidence of maximum values with the values presented in the specialty literature. The second order derivative spectra are more sensitive at quantitative and quality changes of wines and allow to find out the differences using spectrophotometry, a method more accesible than chromatography.

The absorption spectra UV-vis of experimental wines *Legenda* without preliminary treatment (L1, L2, L3) and their second order derivative spectra are shown in the "Figure 1". The second order derivative spectra in original forms are similar and highlight the essential differences in different spectral ranges. The major differences have been revealed at wavelength 260 – 280 nm, 300 – 315 nm and to a lesser extent at 330 – 345 nm. The sample with the less level of browning (L1-0) had a minimum of second derivative spectra at 269 nm and it had nearby the inflection point (281 nm), while the most oxidized wine (L2-0) has the minumum value at wavelength of inflection point (L1-0), where the (L1-0) has the minumum.

The wine (L3-0) with intermediate browning have 2 minimum values in these positions (270 and 279 nm). This fact allow us to consider wavelength 269 – 270 nm suitable to maximum of unoxidized polyphenols, while the products of its oxidation correspond to wavelength with bathochromic shift 279 – 281 nm. The differences in the range 300 – 315 are less expressed where the second order derivative spectra (L1-0) have extreme points.

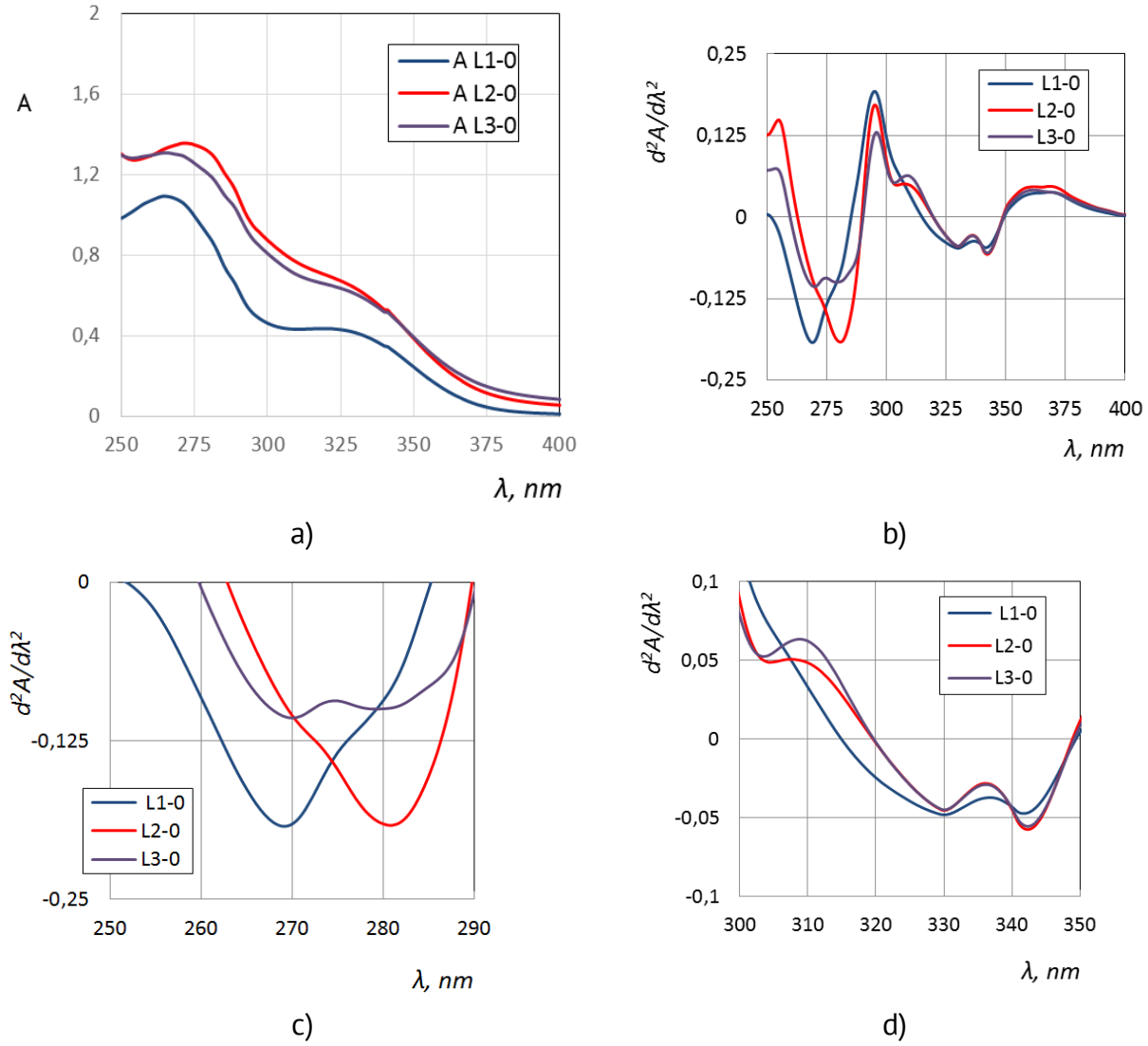

Figure 1. The absorption spectra of experimental raw wines Legenda with different level of oxidation (L1-0, L2-0 and L3-0 – a), and its second order derivative spectra (b, c, d). The wines are undiluted, the cuvettes path length – 0,1 cm.

The more oxidized samples (L2-0, L3-0) are characterized by minimum – maximum wavelength at 304 and 309 nm respectively. The samples (L2-0 and L3-0) have more

expressed minimum values than (L1-0) at 342 nm, because they have a higher content of oxidized polyphenols. These differences can be used for monitoring the oxidative processes in wines produced from *Legenda* grape variety.

The significant differences according to level of oxidation have been observed and in the visible region. These differences generally are quantitative, not qualitative. *Legenda* wines have no obvious or latent maximum values in the visible region. The absorbance at 420 nm for oxidized samples (L2-0, L3-0) is 0,538 and 0,567 (the layer thickness = 1cm) and 0,164 for unoxidized sample (L1-0). *Viorica*, *Chardonnay* and *Sauvignon* wines in UV-vis region have absorption spectra with 2 distinct regions 250 – 300 nm and 300 – 350 nm "Figure 2".

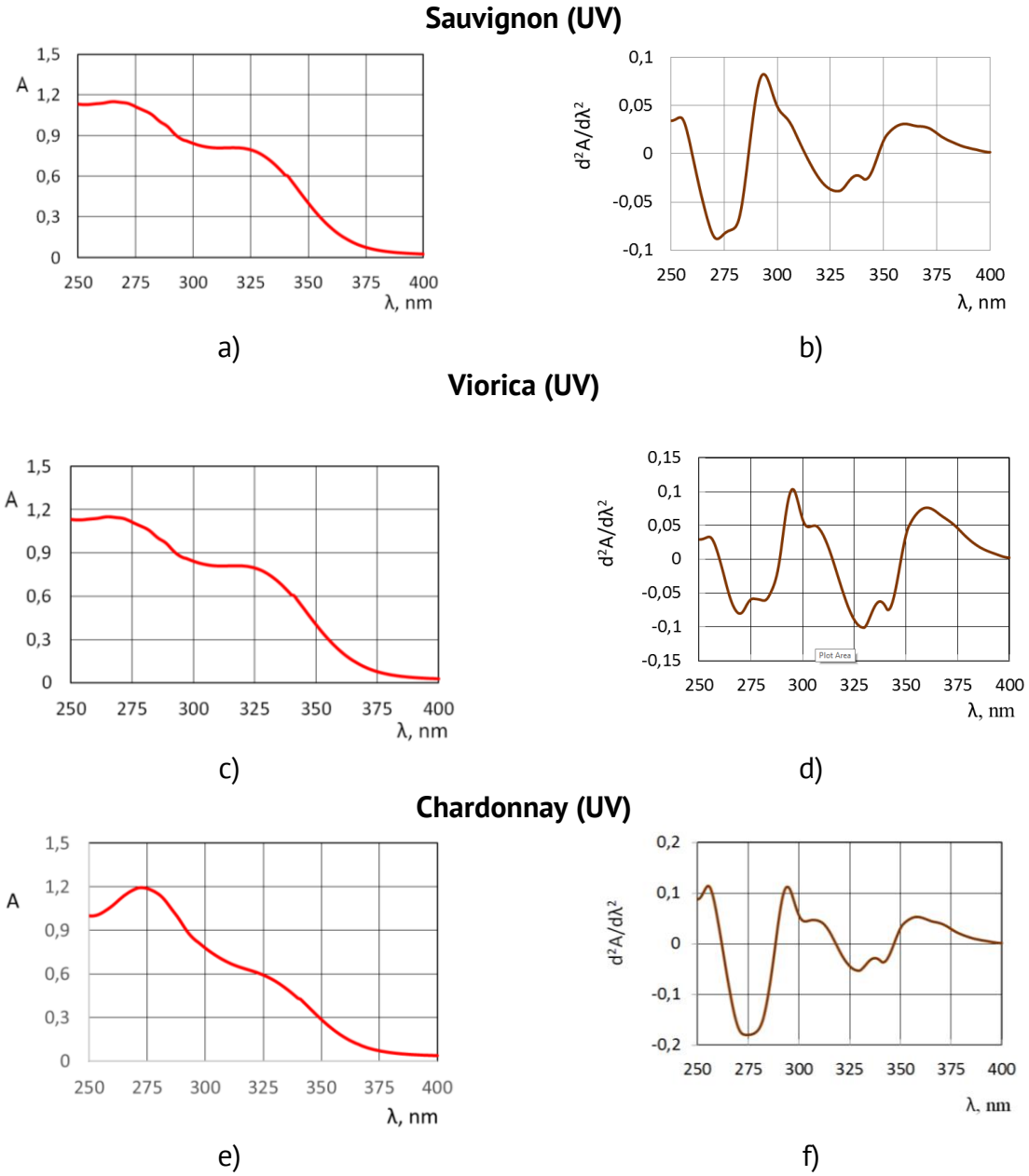

Figure 2. The absorption spectra of experimental raw wines Sauvignon (a), Viorica (c), Chardonnay (e) and its second order derivative spectra (b, d, f). The wines are undiluted, the cuvettes path length – 0,1 cm.

They are similar to *Legenda* wine spectra. In the case of *Sauvignon* wine it prevails the first minimum value (272 nm), the second minimum is masked and is presented by an inflection point. Absorption spectra show an increased (HCAs) content in *Viorica* wine. The quantitative differences are more evident in second order derivative spectra. The same groups of minimum values in 300 – 350 nm region have been observed. In the interval between 250 – 300 nm, 2 minimum values 270 nm and 281 nm are evident for *Viorica* wine. At Chardonnay wine the both minimum have the very close values and its superposition gives a single band with λ_{min} = 274 nm.

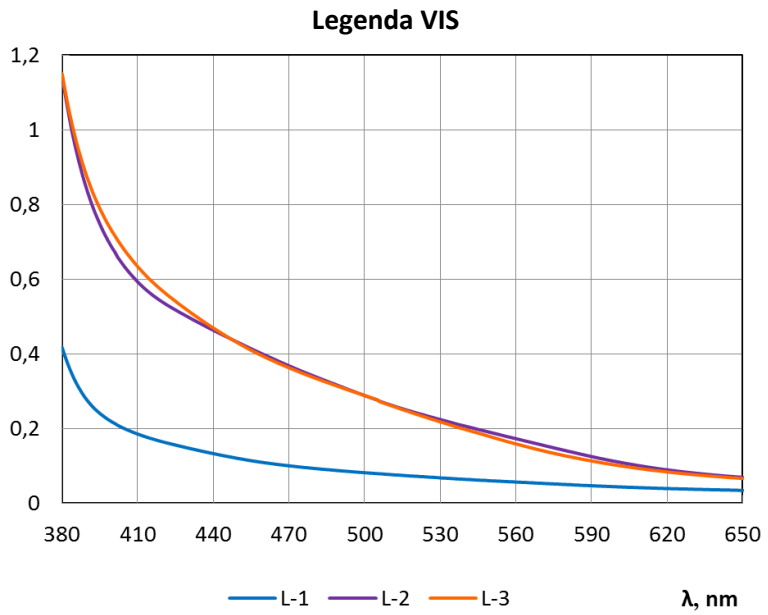

Figure 3. The absorption spectra of Legenda wine samples in the visible region, (undiluted wines, cuvettes path length 1 cm).

In the visible region, the absorption spectra of samples *Legenda* are quantitatively different. So, according to "Figure 3", oxidized samples (L-2 and L-3) are characterized by significantly large absorption (about 3 times) than non-oxidized sample.

The spectrum of non-oxidized sample of *Legenda* wine (L-1) are quantitatively similar to the spectra of non-oxidized samples wines *Viorica*, *Chardonnay* and *Sauvignon* "Figure 4". In the visible region of spectrum, the using of second derivatives spectra of the studied wines unnecessarily due to their low information content (the gentle course of the spectra, lack of characteristic points clear highs on them and / or inflection points).

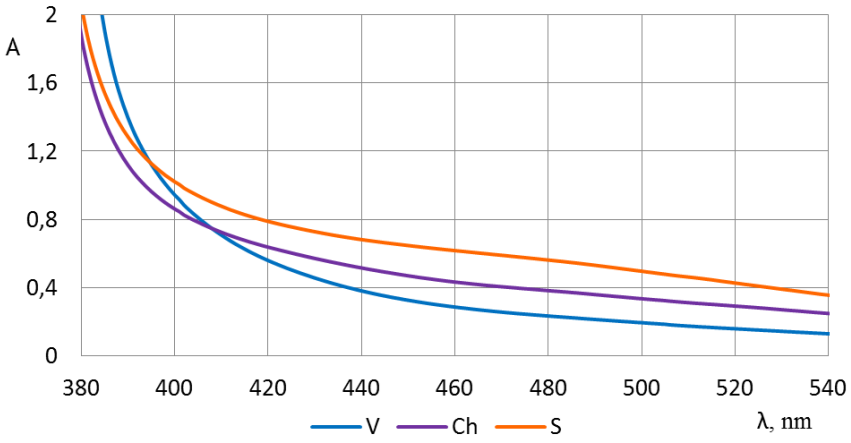

Figure 4. The absorption spectra of Viorica (V), Chardonnay (Ch) and Sauvignon (S) wine samples in the visible region, (undiluted wine, cuvettes path length 5 cm).

The concentration of total phenolic compounds – SFT (Galic Acid Equivalents, mg/l), phenolic cinnamic compounds – SFC (Caffeic Acid Equivalents, mg/l) and phenolic flavonoid compounds – SFF (Catechin Equivalents, mg/l) are presented in "Table 1".

Table 1

The concentrations of main phenolic compounds, the index of oxidation behavior (POM-test) and the parameter of relative antioxidant capacity (K).

Parameter	Wine					
	L1-0	L2-0	L3-0	V	Ch	S
Total phenolic compounds, SFT (Galic Acid Eq., mg/l)	145,1	269,4	236,1	199,3	220,4	68,8
Hydroxycinnamic acids and their derivatives, SFC (Caffeic Acid Eq., mg/l)	29,6	56,2	51,7	67,0	48,1	25,7
Flavonoid compounds, SFF (Catechin Eq., mg/l)	206,2	377,0	319,1	160,1	298,7	43,6
IPT	8,95	12,98	11,94	10,76	11,47	6,59
POM-test (%)	81,8	74,2	42,1	24,0	10,1	67,7
K ($K_o/K_v \div f$ (wine volume)	1,34	5,46	9,14	1,.9	2,07	1,06
R^2	0,8964	0,9960	0,9759	0,9409	0,9793	0,9359

The maceration of (L2-0) and (L3-0) wines have been ensured the better extraction of hydroxycinnamic acids and their derivatives (SFC), total phenolic compounds (SFT) and flavonoid compounds (SFF) than in (L1-0) wine.

The POM-test shows the increased oxidation behavior in (L1-0) wine. This fact can be explained by the presence in the respective samples of unoxidized forms of hydroxycinnamic acids and their derivatives. The smallest value of POM-test have been identified in *Chardonnay* wine samples.

The value of oxidation index (67,7%) for Sauvignon wine can be the consequence of decreased content of phenolic compounds extracted during processing. Our experiments show that wine treatment with bentonite and polyvinylpolypyrrolidone (PVPP) can reduce efficiently the content of oxidized polyphenols with brown tones in the studied wines.

The deposition of part of the phenol complex have been carried out by using bentonite in the concentration range 0.5 ÷ 2 g/l. As a result of wine analysis before and after treatment by bentonite, there has been found out that the sorbent works differently on different groups of phenolic compounds. This is clearly seen in "Figure 5".

As in the case of *Legenda* wine, as well as in the case of *Viorica*, *Chardonnay* and *Sauvignon* wines "Figure 6", bentonites exhibit large sorption properties in relation to flavonoid phenolic compounds of wine, which being bigger molecules than (HCAs), easier precipitate them. With this type of bentonite hydroxycinnamic acids and their derivatives are practically not removed from the wine.

In this way, the use of bentonites to reduce the concentration of phenolic substances in order to provide increased resistance of white wines to oxidative processes, should be

argumented by experimental treatments with quantitative determination of the main groups of phenolic compounds.

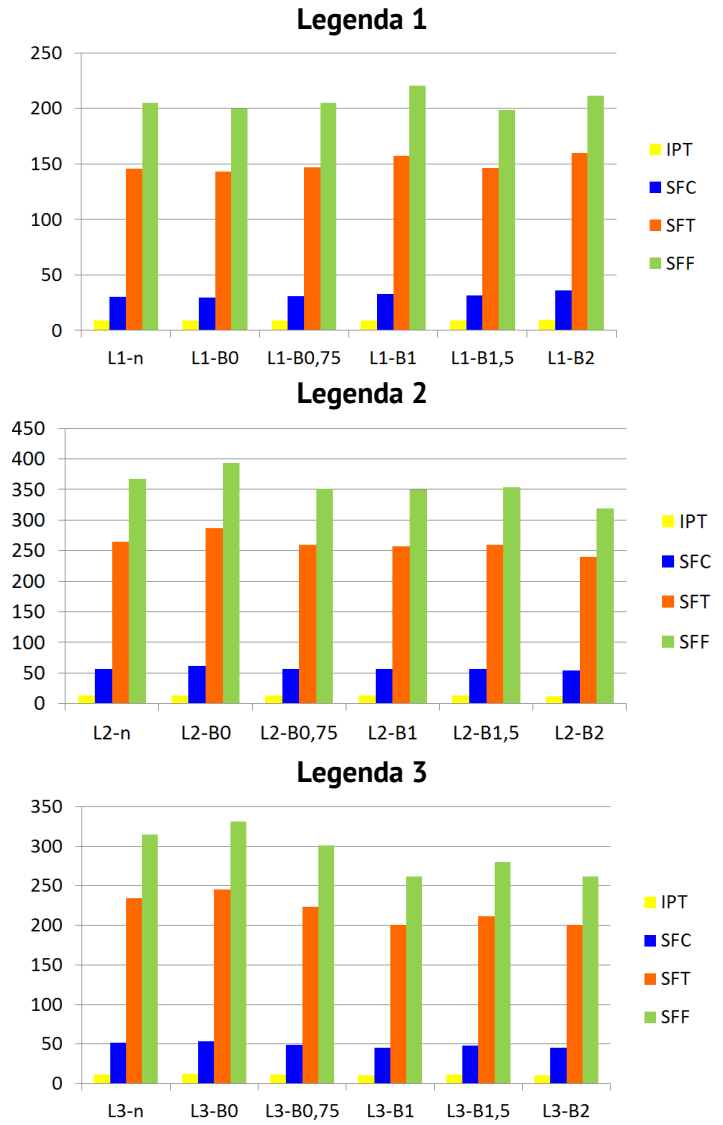

Figure 5. The impact of increasing doses of bentonite on total polyphenol index (IPT) and main groups of phenolic compounds in Legenda wines.

The involvement of wine components in redox reactions with various active forms of oxygen influences the wine capacity to withstand the oxidation during formation, ageing and after bottling. Of course, winemakers can add sulfur dioxide (SO_2) for better wine preservation. Sulfur dioxide is the most preservative used in the wine industry and has been widely applied, as antioxidant and antibacterial agent. However, in recent times, it has been shown that the intake of (SO_2) implicates a wide range of adverse health consequences, such as allergic reactions and cumulative harmful effects. For this reason, reducing the amount of SO_2 in wines is a decisive strategy for the wine industry and one of the current topics on the oenological science. This is also highly recommended by The World Health Organization (WHO).

The total antioxidant capacity of experimental wine samples have been determined by watching the competitive kinetics of crocin bleaching. The antioxidant capacity has been expressed by the interdependance between constant of colour fading speed in absence of

wine addition (Ko) and in the presence of wine addition in different volumes (v), in the reactant mixture (5 ml), in the spectrophotometer cuvettes (Kv). The monitoring of process has been done at 443 nm.

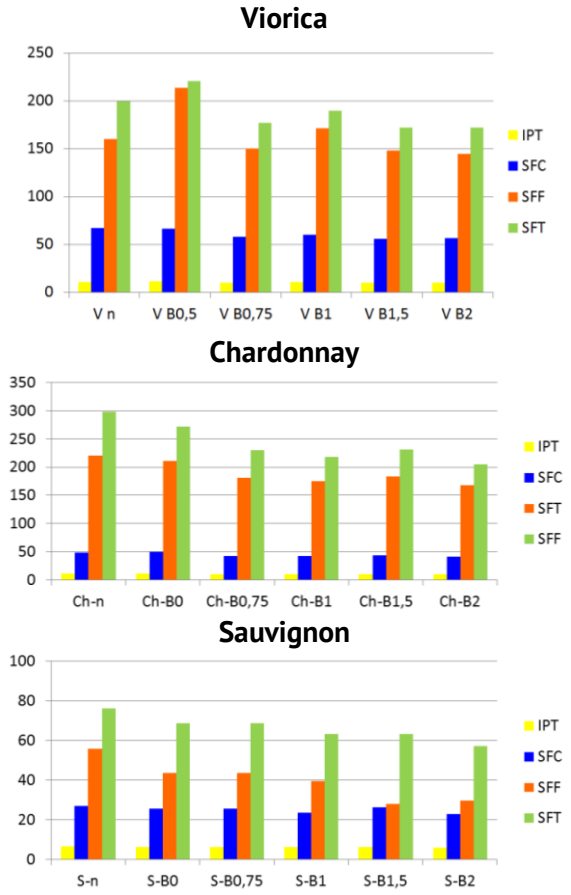

Figure 6. The effect of increasing doses of bentonite on total polyphenol index (IPT) and on the main groups of phenolic compounds in the Viorica, Chardonnay and Sauvignon wines.

The scheme of the reactions is the following:

(1) $R\text{-}N{=}N\text{-}R \rightarrow 2R^{\cdot} + N_2$

(2) $R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$

(3) $ROO^{\cdot} + CH \rightarrow ROOH + C^{\cdot}$

(4) $ROO^{\cdot} + AH \rightarrow ROOH + A^{\cdot}$

(5) $CH + A^{\cdot} \rightarrow C^{\cdot} + AH$

where:

R-N=N-R – radical initiator, AAPH;
$R^{\cdot}$ – primary radicals;
$ROO^{\cdot}$ – peroxy radicals;
CH – crocin (orange);
$C^{\cdot}$ – crocin radical (oxidation product, colorless);
AH – wine antioxidants (phenolic compounds);
$A^{\cdot}$ – antioxidant radicals of wine $ROO^{\cdot}$.

Since the concentrations of A· is significantly lower than concentrations of ROO·, the reaction (5) in the crocin bleaching may be neglected. The ROO· radicals, in the presence of wine antioxidants, have been spent not only in the crocin oxidation (reaction 3), but also in the interaction with wine antioxidants (reaction 4).

As a result, their concentration decrease and the reaction speed (3) decreases proportionally to the concentration of AN. Under the same addition of wine, in the same conditions, the higher total antioxidant activity of the wine, the higher will be the inhibition of crocin oxidation. In the "Figure 7" there are presented the results of the kinetic curves of crocin oxidation in the reaction mixture of various amounts of *Chardonnay* wine. There is also shown the linearization of the kinetic curves A443 ÷ t in coordinates ln (1 / At) ÷ t with high values of correlation coefficients "Figure 8".

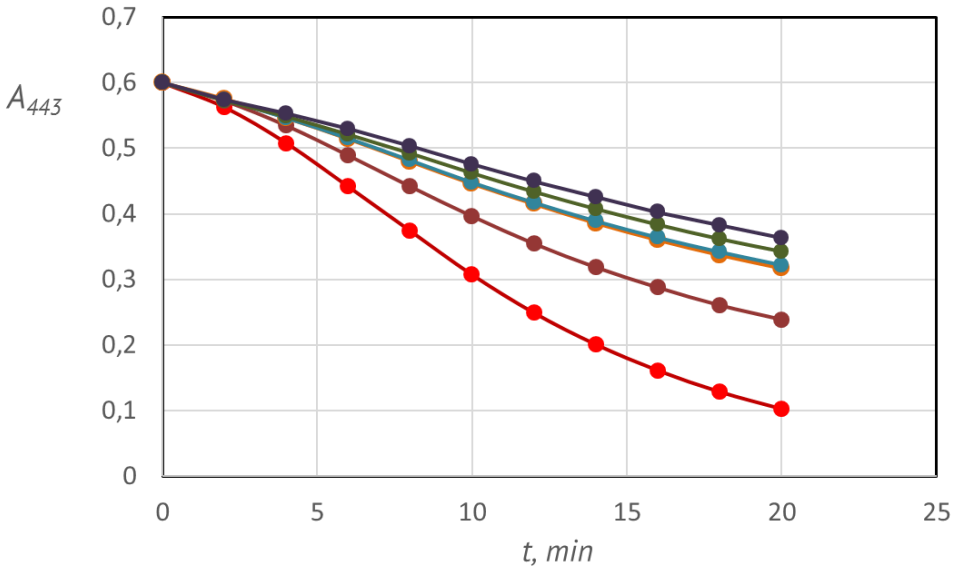

Figure 7. The kinetic curves of crocin oxidation (443 nm) in the absence (Ch-0) and in the presence in the reaction mixture of various amounts of Chardonnay wine: 0,15; 0,30; 0,45; 0,60 and 0,90 ml / 4 ml of the reaction mixture.Thermal initiation of peroxy radicals using (AAPH) in thermostated (at 40° C) cuvettes path lenght (1 cm).

From the slopes of the straight lines, the kinetic rate constants of the reaction between the wine antioxidants and peroxy radicals have been calculated. The higher capacity of wine antioxidants to react with the generated peroxy radicals, the more inhibited is crocin bleaching due to the competition of the crocin and the wine for ROO·. The phenolic radicals that are formed from reaction with ROO· are stable and inert compounds by the delocalization

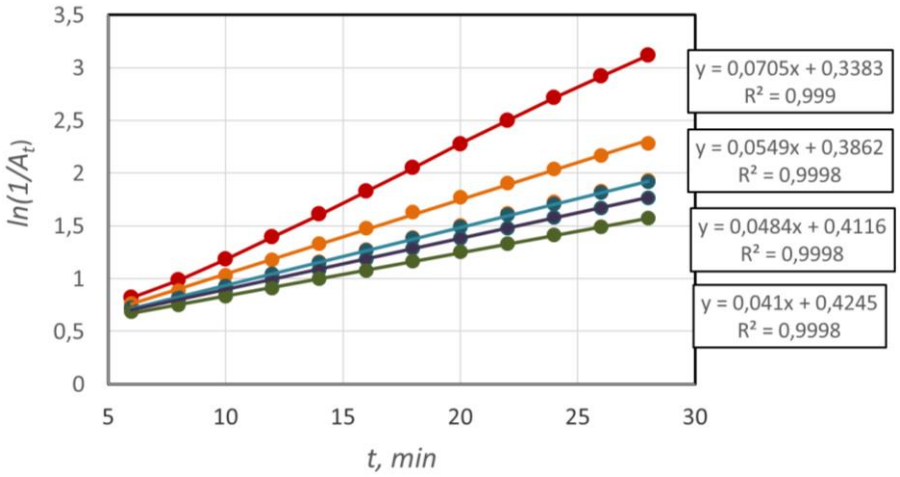

Figure 8. The linearization of the kinetic curves for the crocin bleaching with various Chardonnay wine supplements in the coordinates ln (1 / At) ÷ t.

of the electron density of the unpaired electron. The efficiency of inhibition depends on the nature of the phenolic antioxidants and their content.

The interdependencies Ko/Kv for all studied wines show more expressed antioxidant capacity at *Sauvignon*, *Viorica*, *Legenda* (L1-0) wines and unexpected expressed antioxidant capacity at *Legenda* (L2-0) and (L3-0) wines "Figure 9".

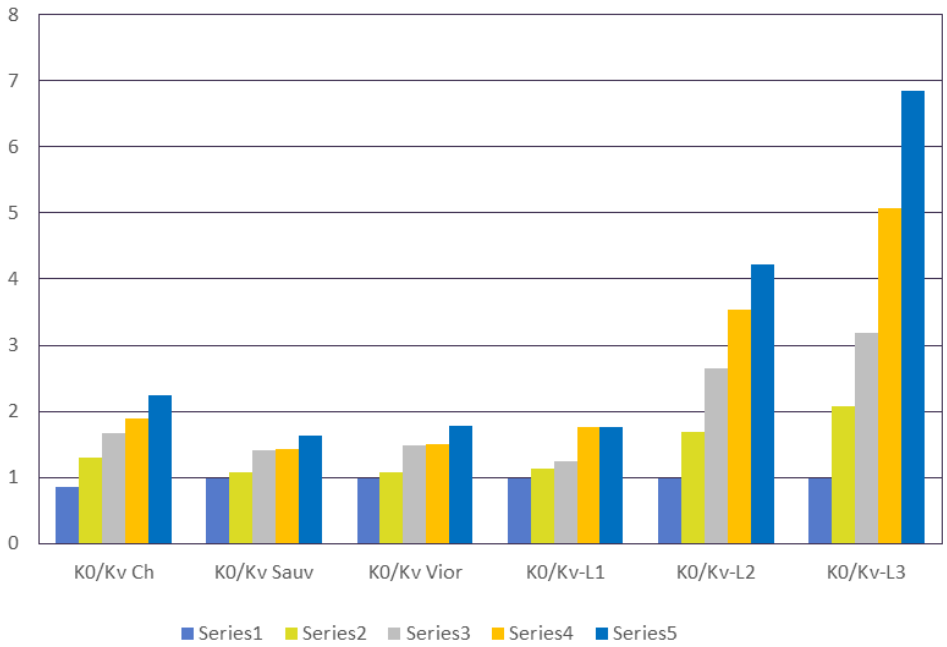

Figure 9. The comparative total antioxidant properties of Chardonnay, Sauvignon, Viorica, Legenda 1, Legenda 2 and Legenda 3 wines depending on their concentration in the reaction mixture, by reaction with crocin in the presence of radicals initiator AAPH.

Series 1 – without wine supplements; Series 2-0.15 ml / 4 ml of reaction mixture; Series 3-0.30 ml / 4 ml of reaction mixture, Series 4-0.45 ml / 4 ml of reaction mixture, Series 5-0,60 ml / 4 ml of the reaction mixture.

The close correlation between antioxidant capacity and content of SFT, SFC and SFF have not been found, although the trend has been barely observed. The oxidation behavior of browning (L2-0) and (L3-0) wines can be explained due to high content of compounds mentioned before and possible antioxidant capacity of some products of browning, although there has not been identified direct connection between the antioxidant capacity and browning degree of Maillard products in hydrophilic medium.

The strict elucidation of these interdependencies requires the complex investigations to determine the influence of different wine antioxidant compounds, to reveal the possible effect of endogenous antioxidants of wine, the redox transformations that are catalized by transition metals, enzymes.

The constants of linear dependencies Ko/Kv on the wine concentration in the reactant mixture have been determined with high values of correlation coefficient R^2 "Table 1".

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Conclusions

1. The oxidation of hydroxycinnamic acids and their derivatives in experimental white wines *Legenda*, produced by using different technologies, are correlated by quantitative spectral changes in UV region, that are visible in second order derivative spectra.
2. The POM-test offers the possibility to predict the risk of white wine browning.
3. The using of bentonite as sorbent of phenolic compounds in order to reduce the risk of wine oxidation at storage requires preliminary study of the removal level of various wine phenolic fractions.
4. The total antioxidant capacity of white wines *Legenda*, *Viorica*, *Chardonnay*, *Sauvignon* can be determined from dependencies of the oxidation rate of crocin bleaching by peroxy radicals that are formed at thermal decomposition of AAPH at 40°C.

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