The Study of Catalytic Degradation of Malvidin-3-Glucoside from Red Wines, using Molecular Absorption Spectrophotometry

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The presence of oxygen and heavy metals naturally found in red wines, leads to the depreciation of their qualities, due to the malvidin-3-glucoside degradation. In this paper, the kinetic of the malvidin-3-glucoside degradation, in synthetic wine model solutions, in the presence of different concentrations of iron and copper, at a temperature of 70°C and two pH values of the wines (2.9 and 3.4 respectively), was studied using the molecular absorption spectrophotometry method.

Keywords: malvidin-3-glucoside, wine qualities degradation, iron, copper

Anthocyans are the pigments responsible for the color of the young red wine, having usually a concentration in wine, between 350 mg/L up to 1500 mg/L [1, 2].

The color of these pigments depends on their chemical structure and chemico-physical conditions of the environment: *p*H, temperature, sulfur dioxide, light, catalytic metals [3]. The molecules of anthocyans are not very stable and therefore their concentration in wine decreases in time. This diminution is given on one hand by the combining reactions with different components of wine, for example tannins, and on the other hand is due to the degradation reactions [2; 4].

The anthocyans could be transformed into the following compounds, depending on the experimental conditions of degradation process:

-calcone, in alkaline environment [1];

-malvone, under peroxides action;

-dihydroflavonols, in the presence of the alcohol [5].

Thermal degradation of the anthocyans takes place according to the reaction scheme presented in figure 1.

Depending on the anthocyan type, various products of degradation are obtained, but 2,4,6-trihydroxy-benzaldehyde is common for all the anthocyans that undergo a thermal degradation process because is formed from A cycle. The other formed products depend on the substitutes of the lateral B cycle of anthocyans. In the malvidin-3-glucoside case, the obtained product is siringic acid.

Oxidative degradation of the anthocyans

The molecular oxygen cannot react directly with the organic compounds due to its electronic configuration, but in the presence of catalytic metals such as iron and copper, in the solution, oxygen is transformed into peroxides.

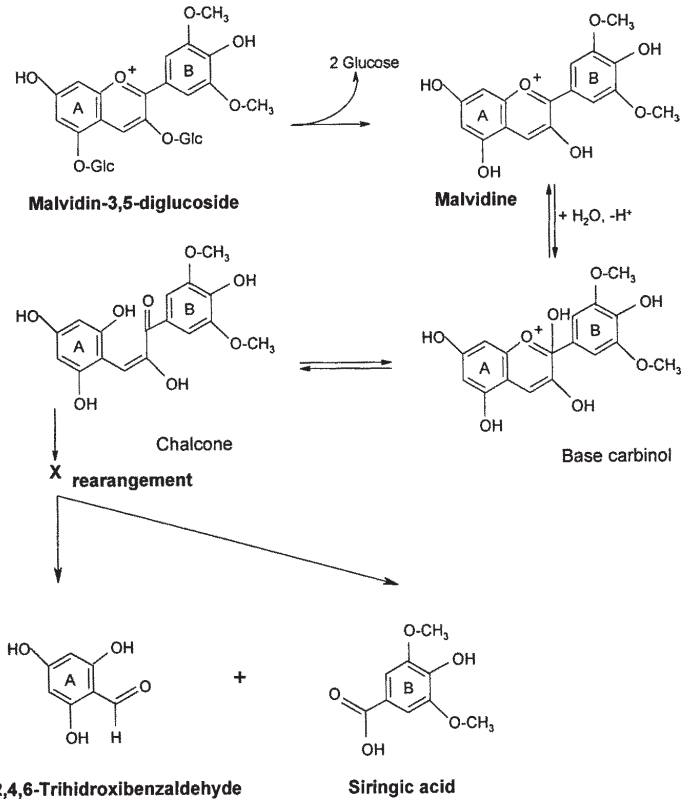

Fig.1. The mechanism of the thermal degradation of malvidin-3-glucoside [6]

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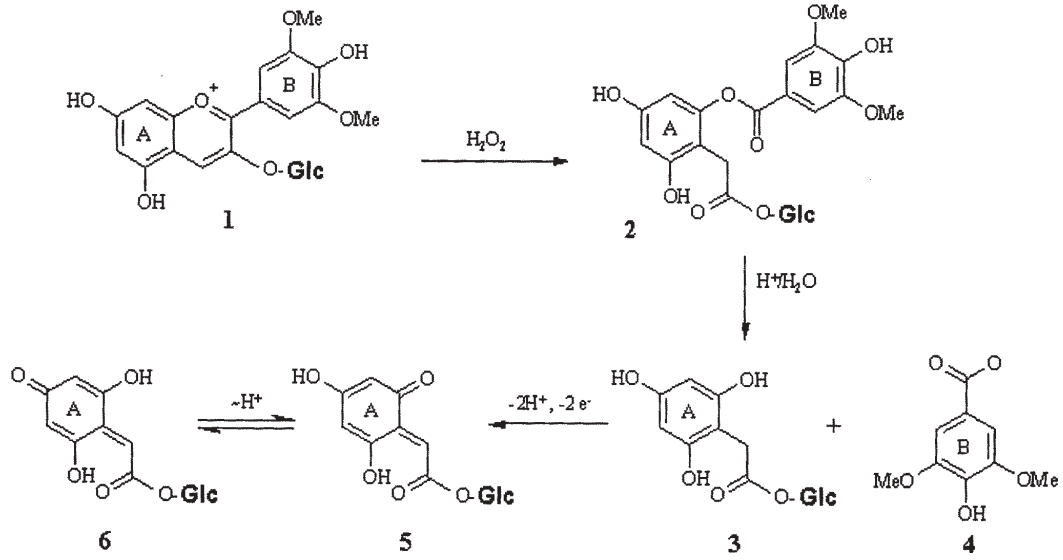

Fig. 2, The hypothesis of the oxidative and thermal degradation mechanism of the malvidin-3-glucoside [8]
1: malvidin-3-glucoside; **2**: malvona-3-glucoside; **3**: glucoside- 2,4,6- trihydroxy-phenyl-acetic;
4: siringic acid; **5**: 8-β-D-glucopyranosil-2,4-dihydroxy-6-oxociclohexa 2,4-dienyliden acetate;
6: 8-β-D-glucopyranosil-2.6-dihydroxy-4-oxo-ciclohexa-2.5 dienyliden acetate

Malvidin-3,5-diglucoside can undergo a nucleophilic attack at C_2 of the flavylium cation, stimulating a breaking of the pyrylium cycle leading to esters of o-benzoiloxy-phenyl acetic acid (malvones), through the Bayer - Villiger oxidation [7].

The malvones are easily hydrolyzed in acid media and can lead to siringic acid, glucoside-2,4,6- trihydroxy-phenyl-acetic acid or coumarone, depending on the *p*H value.

The cascade of the oxidation reactions continues with the formation of 8-β-D-glucopyranosil-2,4- dihydroxy 6-oxociclohexa 2,4-dienyliden acetate. This compound, being a quinonic form, which comes from the degradation of malvidin-3-glucoside, was named malvidone [8].

Experimental part

Two stock-solution of malvidin-3-glucoside of 300 mg/L (0.6 mM/L) were prepared using malvidin-3-glucoside powder (90 % purity), and a solvent solution of synthetic wine that contains 12% ethanol, 5g/L tartaric acid and ultra-pure water provided by Milli Q Water Equipment (Millipore). The former stock-solution had a *p*H value of 2.9 and the latter 3.4 (adjusted with NaOH 1 N solution).

These 300 mg/L malvidin-3-glucoside stock solutions, with 2.9 and 3.4 respectively *p*H values, were used afterwards, in order to study the anthocyans degradation in wine, in the presence of iron and copper, at 70°C, for 24 h.

Work solutions of 150 mg/L were prepared from the malvidin-3-glucoside stock solutions.

Iron and copper solutions, with known concentrations, in the synthetic wine were prepared (for iron the concentrations of the solutions were: 0.56, 5.6, 11.2 , 22.4 mg/L, and for the copper the concentrations of the solutions were: 0.63 , 1.26, 6.3 , 10 mg/L).

The malvidin-3-glucoside degradation was studied by the molecular absorption spectrometry, using a UV-VIS spectrophotometer, type Kontron Uvikon 922 (Italy), monitoring the absorption spectra scanned between 250 nm and 700 nm.

According to the literature [9], the degradation products of malvidin-3-glucoside have absorption maxims of 290 nm and 335 nm respectively.

Anthocyans, usually present an absorption maximum at 520 nm.

A spectral scavenging between 250 nm and 700 nm, for all the studied solutions (the initial solutions, the incubated solutions (70°C), at different *p*H values, in the presence of iron and copper) was made according to the above mentioned method. Meanwhile, a spectral scavenging of the reference samples was made.

By comparing the absorbance at 290 nm, 335 nm and 520 nm corresponding to the initial samples, containing iron and respectively copper, the final samples, after a certain reaction time, and the reference samples, the catalytic influence of these two metals on the malvidin-3-glucoside color degradation can be observed.

Results and discussions

Iron presents a catalytic effect on the malvidin-3-glucoside degradation explained through the decrease of the absorbance at 520 nm. Thus, at 70°C, the absorbance of the malvidin-3-glucoside solutions decreases proportionally with the increasing of iron concentration in the samples. If there are smaller iron concentrations in the samples (as tests 1; 2 and 3, with iron content between 0.56 mg/L and 11.2 mg/L), the *p*H plays an important role, meaning that in acid solutions the decreasing of the absorbance is more accentuated, so the degradation effect is stronger.

Oxygen determines a colour degradation of the malvidin-3-glucoside solutions. Thus for reference sample with oxygen, the absorbance at 520 nm is smaller than that of reference sample with low oxygen content, prepared in nitrogen atmosphere. A lower *p*H value determines a more accentuated color decreasing of the malvidin-3-glucoside solutions (table 2). Also, oxygen presence determines an increasing of the absorbance at 290 nm and 335 nm.

The absorbance at 290 nm for malvidin-3-glucoside containing samples increases proportionally with iron concentration. The absorbance increasing is justified by the issue of new malvidin-3-glucoside degradation compounds, which present a maximum absorption at 290 nm, as it was reported in literature. If lower iron

Table 1

THE EVOLUTION OF THE MALVIDIN-3-GLUCOSIDE AND DEGRADATION COMPOUNDS SPECTRA AT 290, 335 AND 520 nm, IN THE PRESENCE OF DIFFERENT IRON CONCENTRATIONS, AFTER 24 h AT 70°C

ABSORBANCE	The sample	290 nm			335 nm			520 nm		
		t = 0	t = 24 h	Evolution, %	t = 0	t = 24 h	Evolution, %	t = 0	t = 24 h	Evolution, %
pH=3,4	The sample no.1 with 0,56 mg/L iron	0,3259	0,3924	*20,4*	0,1311	0,1516	*15,6*	0,1348	0,0784	*-41,8*
	The sample no. 2 with 5,6 mg/L iron	0,3279	0,4683	*42,8*	0,135	0,181	*34,1*	0,1322	0,0613	*-53,6*
	The sample no. 3 with 11,2 mg/L iron	0,3242	0,5142	*58,6*	0,126	0,2038	*36*	0,1326	0,0534	*-59,7*
	The sample no.4 with 22,4 mg/L iron	0,3252	0,5639	*73,4*	0,1278	0,2044	*59,9*	0,1312	0,0228	*-82,6*
pH=2,9	The sample with 0,56 mg/L iron	0,2497	0,3539	*41,7*	0,0914	0,1238	*35,4*	0,2272	0,076	*-66,5*
	The sample with 5,6 mg/L iron	0,2571	0,4059	*57,9*	0,0979	0,1401	*43,1*	0,2276	0,0447	*-80,4*
	The sample with 11,2 mg/L iron	0,2689	0,4252	*58,1*	0,094	0,1635	*54,7*	0,2253	0,0484	*78,5*
	The sample with 22,4 mg/L iron	0,2575	0,449	*74,4*	0,0961	0,1979	*75,9*	0,2184	0,043	*-80,3*

Table 2

THE EVOLUTION OF REFERENCE SOLUTIONS AT 290, 335 AND 520 nm, AFTER 24 h AT 70°C

pH	Absorbance	290 nm			335 nm			520 nm		
		t = 0	t = 24 h	Evolution %	t = 0	t = 24 h	Evolution %	t = 0	t = 24 h	Evolution %
pH=2,9	Reference sample with oxygen	0,2826	0,3453	*22,2*	0,1044	0,127	*21,6*	0,2387	0,0957	**-59,9**
	Reference sample without oxygen	0,2973	0,3511	*18,1*	0,1208	0,1422	*17,7*	0,252	0,1152	**-54,3**
pH=3,4	Reference sample with oxygen	0,2406	0,3077	*27,9*	0,1009	0,1177	*16,6*	0,1003	0,0545	**-45,7**

Table 3

THE EVOLUTION OF THE MALVIDIN-3-GLUCOSIDE AND ITS DEGRADATION COMPOUNDS SPECTRA AT 290, 335 AND 520 nm, IN THE PRESENCE OF DIFFERENT COPPER CONCENTRATIONS, AFTER 24 h AT 70°C

ABSORBANCE		290 nm			335 nm			520 nm		
		t=0 h	t=24 h	Evolution, %	t=0	t=24 h	Evolution, %	t=0	t=24 h	Evolution, %
pH=3,4	The sample with 0,63 mg/L Cu	0,2413	0,2907	*20,5*	0,0952	0,1173	*23,2*	0,1012	0,0533	*-47,3*
	The sample with 1,26 mg/L Cu	0,2304	0,2922	*26,8*	0,0903	0,1214	*34,4*	0,097	0,0506	*-47,8*
	The sample with 6,3 mg/L Cu	0,2286	0,3377	*47,7*	0,0881	0,1326	*50,5*	0,091	0,058	*-67,6*
pH=2,9	The sample with 0,63 mg/L Cu	0,2699	0,3354	*24,3*	0,104	0,1386	*33,3*	0,2322	0,1165	*-49,8*
	The sample with 1,26 mg/L Cu	0,2688	0,3406	*26,7*	0,0987	0,1491	*51*	0,2321	0,1122	*-51,7*
	The sample with 6,3 mg/L Cu	0,2711	0,343	*26,6*	0,1009	0,142	*40,7*	0,2292	0,1009	*-56*
	The sample with 10 mg/L Cu	0,2623	0,3253	*24*	0,0992	0,1299	*30,9*	0,2232	0,0964	*-56,8*

concentrations of the malvidin-3-glucoside samples (between 0.56 mg/L and 5.6 mg/L) are used, the increasing of the absorbance is more pronounced at a *p*H value of 2.9. If there's a higher iron concentration (test 4, 22.4 mg/L iron), the variation of the absorbance is almost independent of the *p*H value.

At 335 nm, the absorbance proportionally increases with iron concentration, for the malvidin-3-glucoside samples. The *p*H presents a critical influence on the color degradation of the malvidin-3-glucoside solutions.

Table 1 shows that the absorbance at a *p*H value of 2.9 is higher than that obtained at a *p*H value of 3.4. Considering that the absorbance at 335 nm is owed to the malvidin-3-glucoside degradation products, it can be concluded that the catalytic effect of iron is higher at lower *p*H values.

Thus, for similar iron concentration (0.56 mg/L), the absorbance at a *p*H value of 2.9 increases to 126.9 %, in comparison with the absorbance obtained at a *p*H value of 3.4.

If higher iron concentrations (22.4 mg/L) are used, the influence of *p*H is lower. Thus, at a *p*H value of 2.9 the absorbance increases only 26.7 %.

The catalytic effect of copper, at 70°C, is emphasized by the decreasing of the absorbance for malvidin-3-glucoside solutions, measured at 520 nm. For a lower *p*H value, a more accentuated color degradation of the malvidin-3-glucoside solutions, with lower iron concentrations in the samples (samples with copper content between 0.63 mg/L and 1.26 mg/L) can be observed. At higher copper concentrations (6.3 mg/L) and higher *p*H values, a more pronounced decreasing of the absorbance at 520 nm occurs.

In the presence of copper, at 70°C, the absorbance of the malvidin-3-glucoside samples, recorded at 290 nm, gets higher with the increasing of copper concentration. The increasing of the absorbance values is accelerated at higher *p*H values (table 3).

The malvidin-3-glucoside samples that contain copper, at 335 nm, kept at 70°C for 24 h , at a pH value of 3.4, lead to a proportional increasing of the absorbance with the copper concentration.

At a *p*H value of 2.9, the most powerful catalytic effect was observed for a copper content of 1.26 mg/L. The highest value was recorded at 335 nm.

At higher values of copper concentration in the malvidin-3-glucoside solutions, the absorbance at 335 nm records a decreasing. If more than 1.26 mg/L copper is used, an inhibitory effect on the issuing of the malvidin-3-glucoside degradation products is observed (table 3).

Conclusions

In this paper, a kinetic study of the catalytic degradation of malvidin-3-glucoside from red wines, using the molecular absorption spectrophotometry method was presented, in order to point out the depreciation of the wine quality, due to the heavy metals naturally present in red wines. Among these heavy metals, the iron and copper have a more common presence:

- iron presents a catalytic effect at 70°C, either at a *p*H of 2.9 or 3.4; due to this effect, iron presence determines a decreasing of the absorbance for the malvidin-3-glucoside solutions at 520 nm, and an increasing of the absorbance at 290 nm and respectively 335 nm (wavelength characteristic to the absorption of the malvidin-3-glucoside degradation compounds), comparing to the reference samples;
- the malvidin-3-glucoside degradation is proportional with iron concentration in solution;
- at similar temperature and degradation periods, the malvidin-3-glucoside degradation is more pronounced at lower *p*H values;
- iron presents a better catalytic effect than copper, in the same experimental conditions: temperature, time and concentration (expressed in mM/L).

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