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ORIGINAL RESEARCH PAPER

INFLUENCE OF SULFITATION ON THE DYNAMICS OF THE ACTIVITY OF OXIDIZING ENZYMES IN WHITE GRAPES

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Abstract: Sulfur dioxide is used to control the browning of the wine, but it is known as an irritating agent of the digestive mucous membrane, of the lungs, and, at the same time, it destroys the thiamine, therefore the admissible quantities in vinification have been reduced. The research focused on the influence of different SO_2 doses on the oxidizing enzymes found in grapes, in the must, and in the young wine. During the alcoholic fermentation the activity of the oxidizing enzymes dropped by 74.3 - 87.5% and the laccase was deactivated when doses of 50 - 75 $mg.L^{-1}$ SO_2 were used. The activity of the polyphenoloxidase (tyrosinase and laccase) gradually decreased during the alcoholic fermentation. Using the same doses of SO_2, after the alcoholic fermentation, there has been noted a reduction of 56.6 - 71.8% of the activity of the peroxydase in wine compared to the first day of the alcoholic fermentation.

Keywords: *sulfur dioxide, deactivation, laccase, peroxidase, tyrosinase*

INTRODUCTION

The oxidation of regular wines or of those of a higher quality is considered a flaw and has to be avoided. The excessive oxidation of the white wines depends on the chemical components and on the health state of the grapes, on the way they are processed, on the fermentation conditions of the must, the conditioning, stabilization and preservation of the wines. The main transformations are caused by the oxidative processes, especially by the chemical and enzymatic oxidation of the phenolic compounds, the catechine and proantocyanides having a decisive role. The speed of oxygen combination is amplified very much when the oxidation is produced in an enzymatic way, in which case the process being determined by the existence in the must and wine of the enzymes from the order of oxyreductases [1]. The oxidation is a serious problem during the storage of the drinks, such as the white wine, whose color becomes unstable, which in turn diminishes its trading period. It is one of the main problems which is encountered during the vinification of the grapes, which on one hand affects negatively the sensory properties of the wine (the loss of color, of taste, fragrance, the increase of the tartness level) [2]. The main oxyreductases responsible for the oxidation during the grape processing are: the polyphenoloxydase (PPO), the laccase and the peroxydase (POD).
A simple way to achieve it is the use of SO_2 and that of flocculants or absorbent agents (casein, active charcoal, P.V.P., etc). Consequently, the protection of the must against the enzymatic oxidation has to be done immediately, with the help of SO_2 (the sulfitation of the must) [3].
SO_2 is the most effective way of directing the evolution of the oxyreduction process of the wine, of antioxidizing protection of the grapes, of the musts and wines [4]. The sulfur dioxide forms a protection barrier between the oxygen in the air and the wine or the mixture resulting from the pressing of the grapes. Being easily oxidized, it monopolizes the oxygen and protects the mixture resulted from the pressing of grapes from oxidation [5].
The wine for which there have not been used doses of SO_2 during its making have, naturally, the greatest drawbacks in what regards the oxidation, coming to the brown cassation and to the diminishing of the freshness of the wine. Its excessive use may drastically compromise the quality of the wine and may lend unpleasant tastes and fragrances to the wine or may favor its clouding during its storage [6]. In recent years there has been discussed the reduction of or, even, the replacing of the sulfur dioxide [7].
The purpose of this study has been that of quantifying the activity of the oxidizing enzymes of the *Riesling, Sauvignon, Şarba* and *Băbească* white grapes from the "Dealu Bujorului" vineyard, the oxidizing enzymes activity in the grapes musts, during the alcoholic fermentation and for the new wine by using different doses of SO_2.

MATERIALS AND METHODS

The research has been done at the Research and Development Institute in Viticulture and Vinification, "*Dealurile Bujorului*" vineyard, in the eastern part of Romania, during 2007-2008. The "*Dealurile Bujorului*" vineyard has a temperate-continental climate with a lot of rains at the end of summer, drought periods in July and August and sunny

autumns. The varieties of grapes used in the analysis *Riesling, Sauvignon, Şarba* and *Băbească* were harvested at technological maturity.
The grapes which were sulfitated with different doses of SO_2 (0, 25, 50 and 75 $mg.L^{-1}$) were crushed-unclustered in the pressing-unclustering device type Leonida. The resulting mixture was pressed in the hydraulic press type Ticus-40. The alcoholic fermentation has been done at 17-21°C in 10-litre demijohns. During the alcoholic fermentation there have been taken samples daily for enzymatic and physical-chemical determinations. The type of flavored grapes *Şarba* followed the same microvinification technology as the other white wines with the addition of a procedure of a short maceration-fermentation on pomace in order to facilitate the extraction of the flavor compounds specific to this flavored type of grapes.
The resulting musts and wines were analyzed for the total polyphenols content by reaction with Folin-Ciocâlteau reagent, and were expressed as $mg.L^{-1}$ of galic acid. The laccase and tyrosinase activities were quantified by using the method described by Dubernet *et al.* [8]. The peroxidase activity was evaluated by using the method described by Ciopraga *et al.* [9].
At the same time the browning index (BI) and the polyphenoloxidase index (PPOI) were calculated as mentioned by Mantis (1980) and Leglise *et al.* (1969) quoted by Ioniță *et. al.* [10]. Official methods (OIV) have been used to analyze the musts and wines. All determinations were carried out in duplicate, and the relative standard deviations are less than ± 1%.

RESULTS AND DISCUSSIONS

The activity of the oxidizing enzymes of the must

The main physical-chemical parameters of the musts are presented in Tables 1 and 2.

Table 1. *Physical-chemical characteristics of musts*

Grape variety	Sugar [$g.L^{-1}$]	Total acidity [$g.L^{-1}$ H_2SO_4]	Total polyphenols [$g.L^{-1}$ galic acid]	Optical density (OD) at 420 nm
Riesling	228	3.3	0.250	0.310
Sauvignon	223	3.7	0.282	0.340
Şarba	215	3.5	0.263	0.300
Băbească	202	3.2	0.242	0.350

The total content of sugar and the total acidity have close values for all the varieties of grapes that have been studied 202 – 228 $g.L^{-1}$ for sugar and 3.2 – 3.7 $g.L^{-1}$ H_2SO_4, respectively.
The activity of the laccase has been of 3 $OD_{520\ nm}.min^{-1}$ for the *Riesling* variety and of 5 $OD_{520\ nm}$ for the *Sauvignon* variety. The activity of the tyrosinase has been of 7 $OD_{420\ nm}.min^{-1}$ for the *Băbească, Şarba* and *Riesling* varieties and 6 $OD_{420\ nm}.min^{-1}$ for the *Sauvignon* varietes. The activity of the laccase is lower than that of the tyrosinase by 3 $OD_{520nm}.min^{-1}$ for the *Riesling* grapes and, respectively, by 4 – 5 $OD_{520nm}.min^{-1}$ for the *Sauvignon, Şarba* and *Băbească* varieties. The activity of the peroxydase has been

of 6.85 OD $_{420nm}$.min^{-1} for the *Sauvignon* variety and of 7.23 OD $_{420nm}$.min^{-1} for the *Riesling* variety. The polyphenoloxydase index (PPOI) and the browning index (BI) have been situated between 5.2 and 6.0 and 0.12 and 0.14, respectively.

Table 2. *The oxidizing enzymatic activity of grape musts*

Grape variety	Laccase activity $OD_{520\ nm}$.min^{-1}	Tyrosinase activity $OD_{420\ nm}$.min^{-1}	Peroxidase activity $OD_{420\ nm}$.min^{-1}	PPO index	Browning index
Riesling	3	7	7.23	6.0	0.14
Sauvignon	5	6	6.85	5.4	0.12
Şarba	4	7	7.00	5.2	0.13
Băbească	4	7	6.93	5.7	0.13

At the same time, the content of total polyphenols and the intensity of the color of the musts have recorded normal values for the white grapes varieties.

The activity of the oxidizing enzymes of the must during the alcoholic fermentation

There has been assessed the activity of the oxidizing enzymes in the must during the alcoholic fermentation (after 24, 48 and 72 h). The enzymatic activity of the tyrosinase, laccase and peroxydase decreased during the alcoholic fermentation by 74.3 – 87.5%. The activity of the tyrosinase dropped after 72 h of fermentation by approximately 75 – 87.5% from the oxidizing enzymatic activity registered after 24 h (Figures 1 and 2).

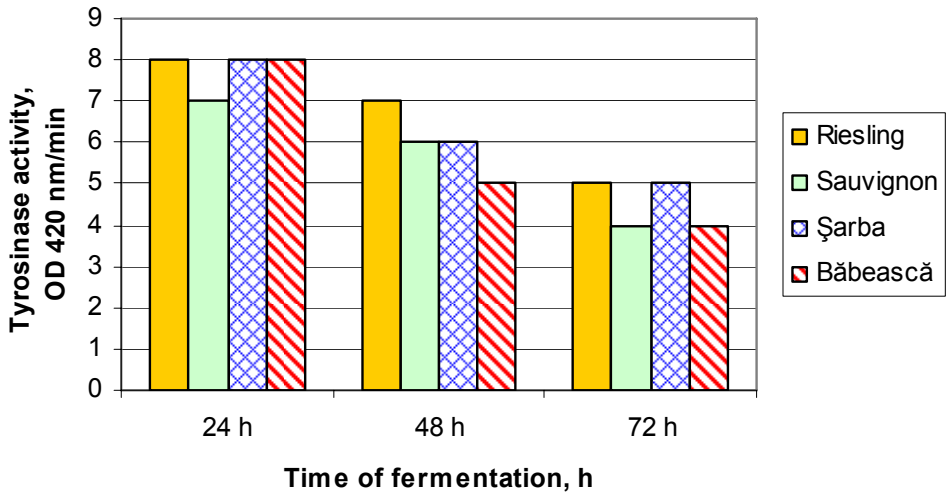

Figure 1. *Tyrosinase activity during the alcoholic fermentation of non-sulfited must*

The activity of the laccase decreased after 72 h of fermentation by approximately 80 – 87.5% from the oxidizing enzymatic activity recorded after 24 h. The laccase was deactivated during the alcoholic fermentation at doses of 50 and 75 mg.L^{-1} SO_2 (Figures 3 and 4).

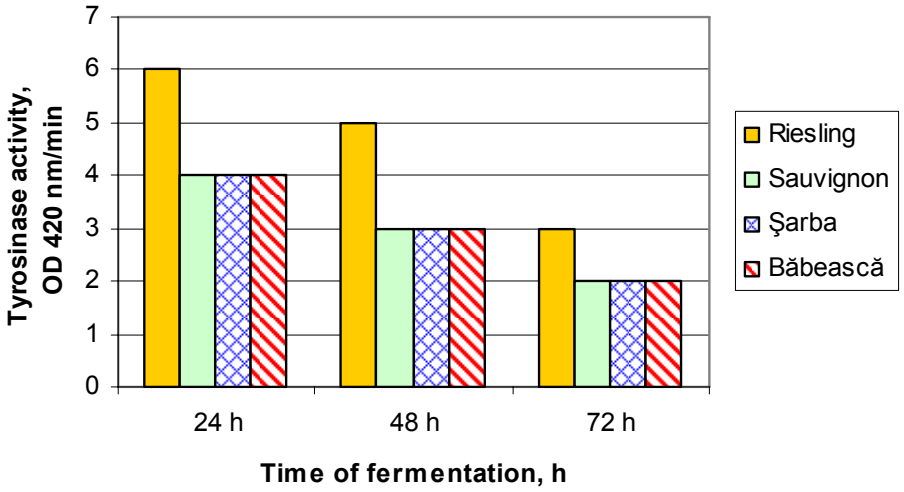

Figure 2. *Tyrosinase activity during the alcoholic fermentation of 50 mg.L^{-1} sulfited must*

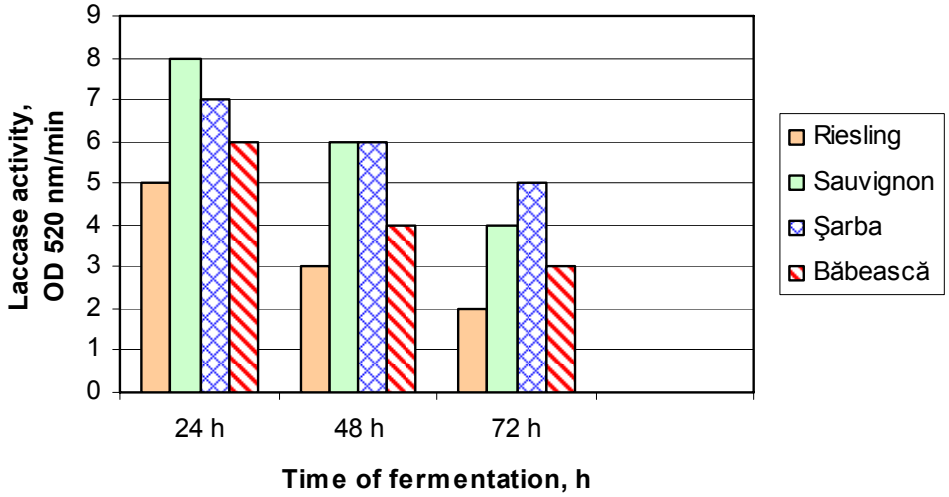

Figure 3. *Laccase activity during the alcoholic fermentation of non-sulfited must*

The activity of the peroxydase dropped after 72 h of fermentation by approximately 74.3 – 80.1% from the oxidizing enzymatic activity recorded after 24 h. The decrease has been directly linked to the increase of the used dose of SO_2 (Figures 5 and 6).
The evolution of the polyphenoloxydase index (PPOI) is given in Figures 7, 8.
There has been noticed an increase of both indices (PPOI and BI) during the fermentation but the values have dropped when high doses of SO_2 have been added (Figures 9 and 10).

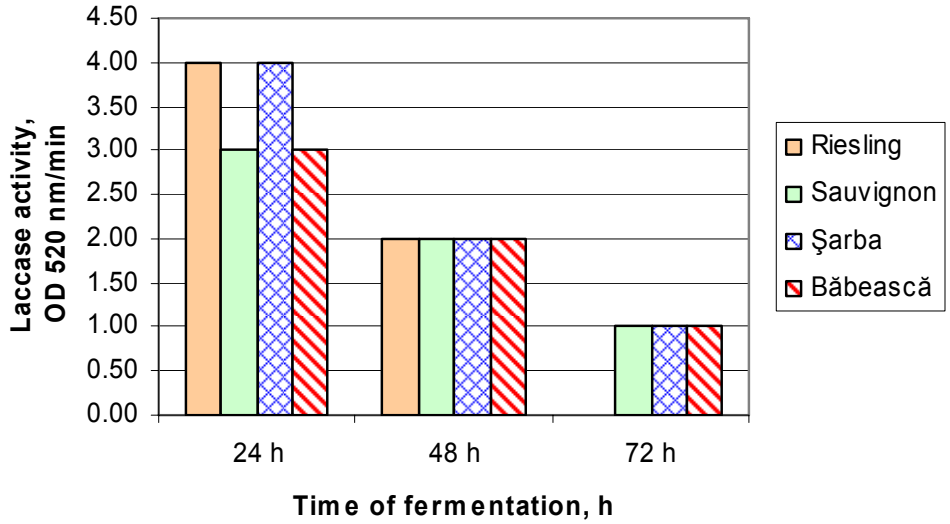

Figure 4. *Laccase activity during the alcoholic fermentation of 50 mg.L^{-1} sulfited must*

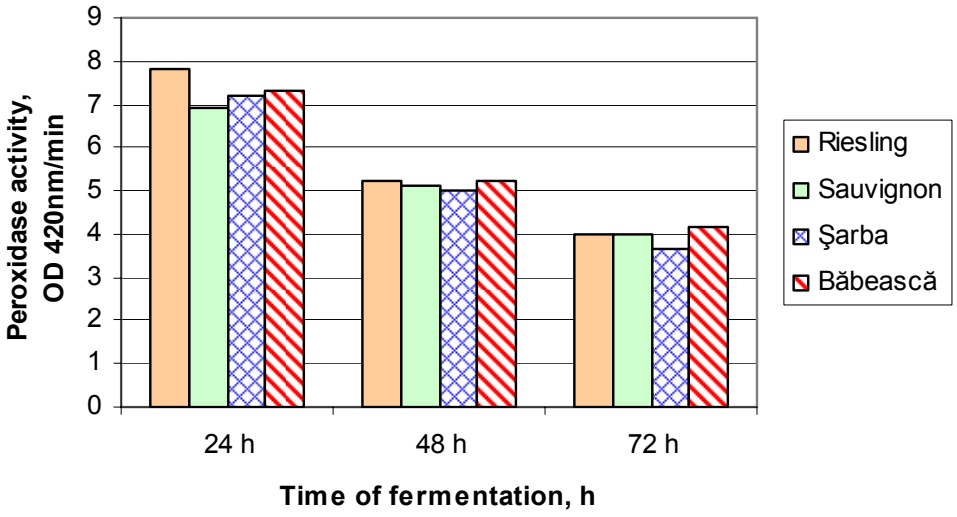

Figure 5. *Peroxidase activity during the alcoholic fermentation of non-sulfited must*

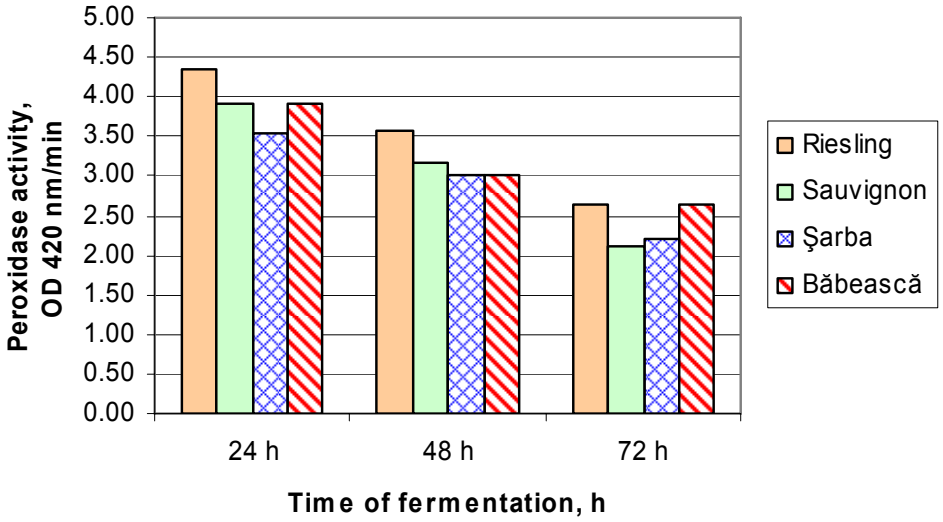

Figure 6. *Peroxidase activity during the alcoholic fermentation of 50 mg.L^{-1} sulfited must*

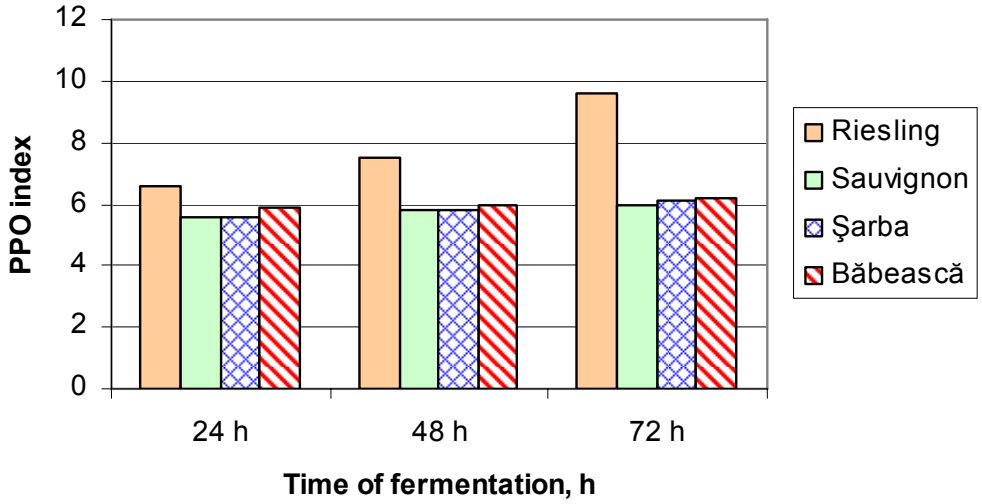

Figure 7. *PPO index during the alcoholic fermentation of non-sulfited must*

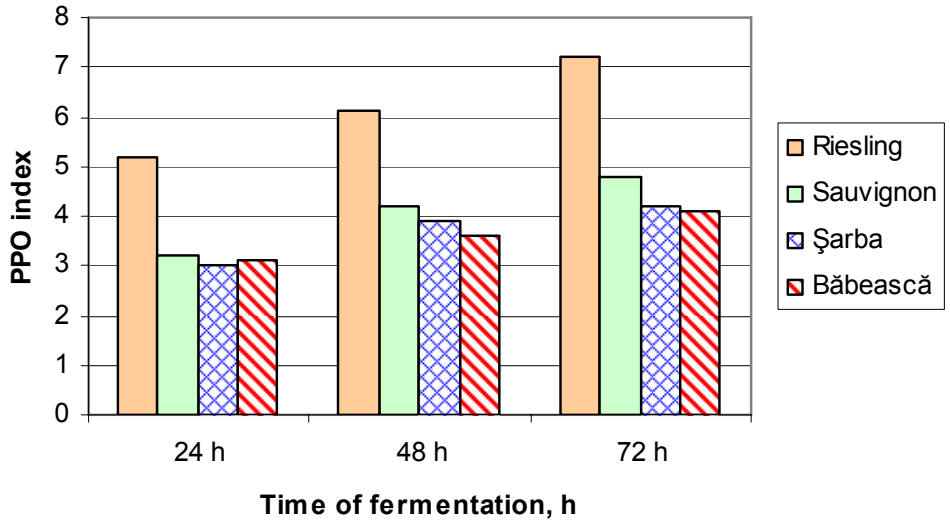

Figure 8. *PPO index during the alcoholic fermentation of 50 mg.L^{-1} sulfited must*

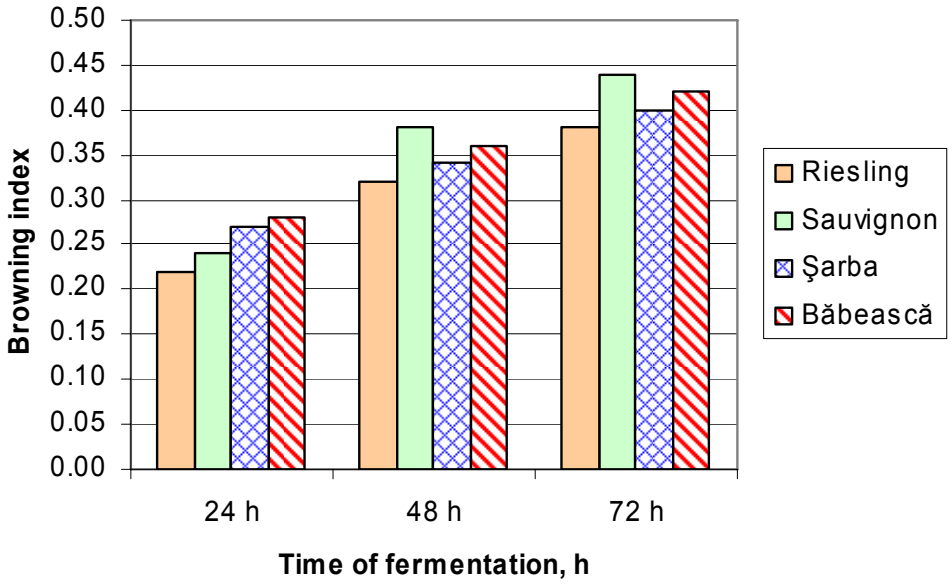

Figure 9. *Browning index during the alcoholic fermentation of non-sulfited must*

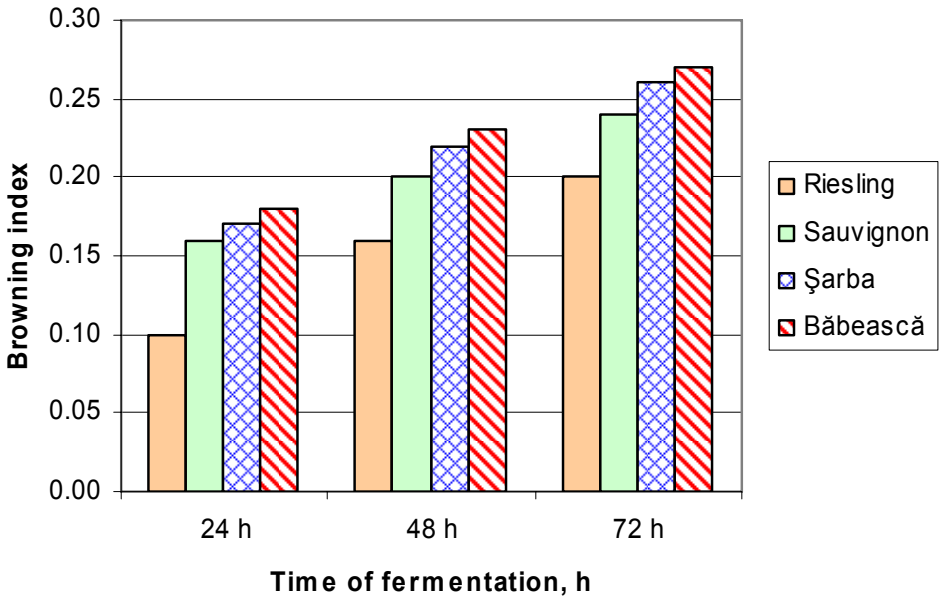

Figure 10. *Browning index during the alcoholic fermentation of 50 mg.L^{-1} sulfited must*

During the alcoholic fermentation the total content of phenolic compounds decreases due to the precipitation and condensation reactions (Figures 11 and 12).

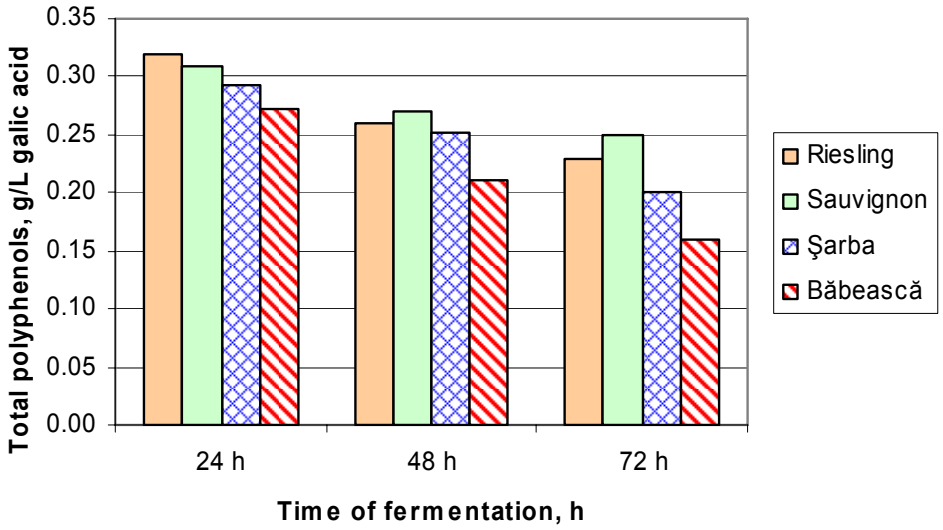

Figure 11. *Total polyphenols content during the alcoholic fermentation of non-sulfited must*

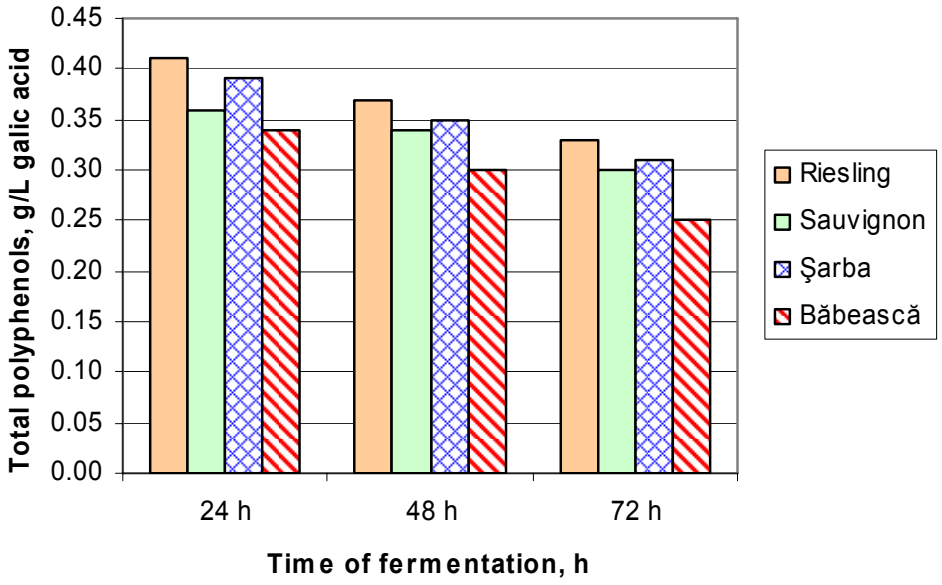

Figure 12. *Total polyphenols content during the alcoholic fermentation of 50 mg.L^{-1} sulfited must*

The same decreasing evolution has been noticed for the intensity of the color (Figures 13 and 14).

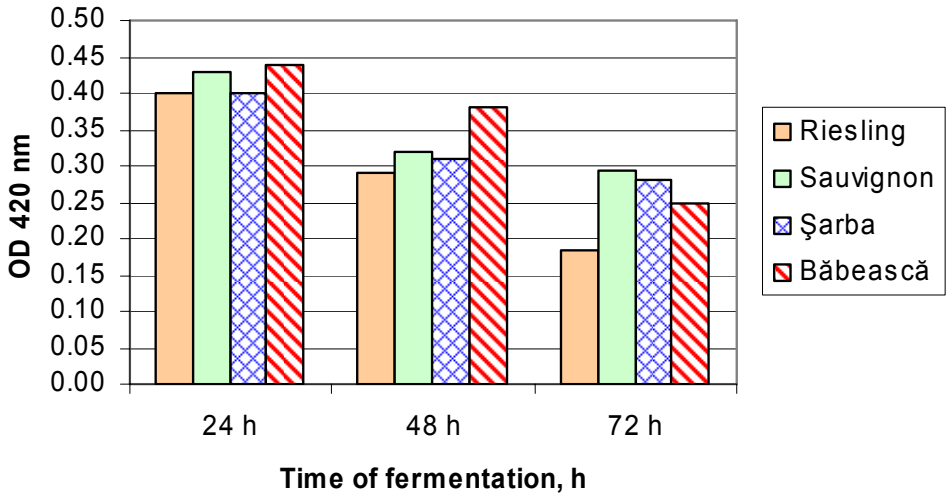

Figure 13. *OD 420 nm during the alcoholic fermentation of non-sulfited must*

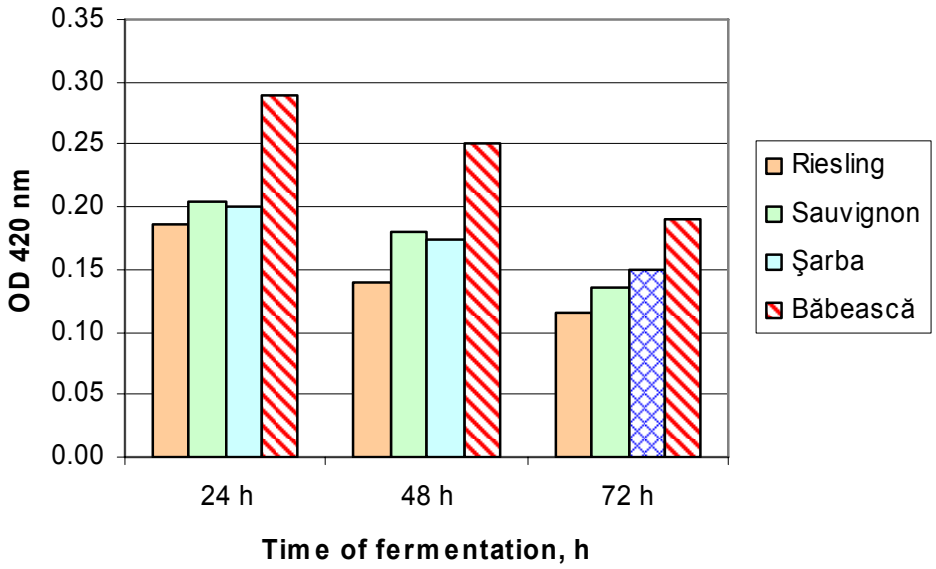

Figure 14. *OD 420 nm during the alcoholic fermentation of 50 mg.L^{-1} sulfited must*

The activity of the oxidizing enzymes of the wines

The alcoholic degree in young wines has been specific to each grape variety depending on the quantity of sugar accumulated in the grapes. It has been of 11.7% alcoholic vol. for the *Băbească*, 13.1% alcoholic vol. for the *Riesling* (depending on the accumulated sugar quantity in the grapes). The total acidity has been situated within normal limits for all varieties (3.8 – 5.1 g.L^{-1} H_2SO_4). The volatile acidity has been situated between 0.18 – 0.31 g.L^{-1} H_2SO_4, and the total extract between 21 – 25.5 g.L^{-1}. The pH of the wines that have been studied has had values between 3.08 – 3.24, free SO_2 between 2 – 22 g.L^{-1}, and total SO_2 between 3 – 51 g.L^{-1}, respectively.
The activity of the oxidizing enzymes is lower in wines than in musts. The activity of the tyrosinase has been zero at doses of 50 and 75 mg.L^{-1} SO_2. The activity of the laccase has been 0 for doses of 50 – 75 mg.L^{-1} SO_2 for all the studied grape varieties and for the *Riesling* even for the 25 mg.L^{-1} SO_2 dose. The activity of the peroxydase in the wine drops by 56.6 – 71.8% compared to the first day of the alcoholic fermentation.

CONCLUSIONS

There has been noticed that during the alcoholic fermentation the activity of the oxidizing enzymes has decreased by 74.3 – 87.5% and the laccase has been deactivated when there have been used doses of 50 and 75 mg.L^{-1} SO_2.
The activity of the polyphenoloxydase (tyrosinase, laccase) has decreased during the alcoholic fermentation and there has not been detected the activity of the polyphenoloxydase in the wine, when different quantities of SO_2 (25, 50 and 75mg.L^{-1}) have been added.

The activity of the oxidases is lower in wines than it is in musts. The activity of the tyrosinase has been zero for added doses of 50 and 75 $mg.L^{-1}$ SO_2. The activity of the laccase has been zero for doses of 50, 75 $mg.L^{-1}$ SO_2 for all the studied varieties of grapes and for the *Riesling* even for the dose of 25 $mg.L^{-1}$ SO_2.The activity of the peroxydase in the wine drops compared to the first day of alcoholic fermentation by 56.6 – 71.8%.

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