THE INFLUENCE OF OENOLOGICAL TREATMENTS ON DYNAMIC OF OXIDATIVE ENZYMES FROM WHITE GRAPES

INFLUENȚA UNOR PROCEDURI OENOLOGICE ASUPRA DINAMICII ACTIVITĂȚII ENZIMELOR OXIDATIVE DIN STRUGURI ALBI

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Abstract. *Use of combined treatments like sulphite dioxide and bentonite addition is able to ensure the biological stability of white grape must. After the combined treatment the activity of oxidative enzymes (tirosinase, laccase and peroxidase) decreased gradually and the polyphenoloxidase and browning indexes have the same trend. The favourable effect of this combined treatment is based on the presence of protein fractions involved with the first deposit collected just after must alcoholic fermentation. The aim of this study was to analyse the activity oxidase enzymes from white grapes and fresh must. In the same time the inhibition of oxidative enzymes that have been achieved by combining the experimental variates of different doses of sulphur dioxide and bentonite was quantified. In fresh musts oxidative enzyme activity is increased immediately after the grapes crushing, as a result of oxygen penetration and oxidation of phenolic compounds. Tyrosinase activity during alcoholic fermentation have a decrease of 33-42% in the case of sulphite dioxide addition and coupled sulphite dioxide and bentonite addition, while for the bentonite addition only the tyrosinase activity was total inhibited. During the alcoholic fermentation, enzymatic activity of peroxidase and laccase were reduced by 27-73% correlated with the dose of sulphur dioxide and bentonite used. After the alcoholic fermentation of grape must in the case of variants where used similar doses of sulphur dioxide and bentonite were, a reduction of oxidative enzyme activity of approximately 27-90% was observed.*

Key words: enzymes, must, phenolic compounds, alcoholic fermentation, bentonite.

Rezumat. *Utilizarea tratamentelor combinate de sulfitare şi bentonizare, pentru tratarea mustului din struguri albi, asigură o stabilitate sporită inhibând activitatea enzimelor oxidative (tirozinaza, lacaza, peroxidaza), fapt evidențiat prin cuantificarea şi evaluarea indicilor de polifenoloxidază şi de brunificare. Efectul favorabil al acestui tratament combinat se bazează pe faptul că enzimele oxidative sunt reținute de burbă, deoarece enzimele sunt susceptibile la dioxidul de sulf care denaturează enzima, iar bentonita o separară din sistem. Scopul acestui studiu a fost de a analiza activitatea oxidazică inițială din struguri şi mustul proaspăt după care*

s-au realizat variantele experimentale prin combinarea diferitelor doze de dioxid de sulf şi bentonită. In mustul proaspăt activitatea enzimelor oxidative este in creştere imediat după zdrobirea strugurilor, ca urmare a pătrunderii oxigenului şi oxidarea substanţelor polifenolice. Activitatea tirozinazei în timpul fermentaţiei alcoolice scade cu 33-42% la variantele sulfitate şi cele cuplate (bentonizare şi sulfitare) în timp ce la variantele bentonizate activitatea enzimatică a tirozinazei este inhibată total. În timpul fermentaţiei alcoolice, activitatea enzimatică a lacazei şi a peroxidazei se reduce cu 27-73% la variantele studiate, proporţional cu doza de dioxid de sulf şi cantitatea de bentonită folosită. După finalizarea fermentaţiei alcoolice la variantele la care s-au folosit doze similare de dioxid de sulf şi bentonită se observă o reducere a activităţii enzimelor oxidative de aproximativ 27-90%.

Cuvinte cheie: enzime, must, compusi fenolici, fermentatie alcoolica, bentonita

INDRODUCTION

Browning is an oxidative process involving sugars, lipids, amino acids or phenols in food. It is one of the main problems encountered during the vinification of wine as it on one hand, adversely affects the sensory properties of wine (loss of colour, flavour and aroma, and increase of astringency) (Escudero, Asensio, Cacho, & Ferreira, 2002) and on the other, results in the loss of nutritional value of wine (Sioumis, Kallithraka, Tsoutsouras, Makris, & Kefalas, 2005).

The enzymatic oxidation of phenols, particularly in the presence of atmospheric oxygen and oxidoreductases, takes place in the early stages of processing and is well known to be a cause of browning in foodstuffs. In the intact cells of fresh fruit or vegetable tissues, phenols located predominantly in the vacuole and oxidoreductases located in cytoplasm cannot meet due to different cell membrane systems, whereas enzymic browning will arise once the cells are bruised or wounded in air (Wang, 1990).

The main oxidoreductases responsible for browning during grape processing are PPO and peroxidase (POD) (Li et al., 2005). PPO is a copper-containing enzyme and able to oxidize substrates characteristic for tyrosinase and laccase.

Tyrosinase, also called catecholase (E.C. 1.10.3.1), is naturally produced in grape berry and can catalyze the oxidation of monophenols and o-diphenols (Singleton, 1987).

However, laccase (E.C. 1.10.3.2) is produced by moulds and able to oxidize lots of substrates, especially 1,2- and 1,4-dihydroxyphenene (Toit et al.,2006).

POD (E.C. 1.11.1.7) is a Fe-containing enzyme and its activity depends on the available hydrogen peroxide (H2O2) in medium. However, the browning caused by POD seems insignificant in fruits with few exceptions, such as litchi and pineapple, although some researcher found that it did enhance the degradation of phenols when coexisting with PPO (Robards et al., 1999).

In grape must, enzymatic browning is largely correlated with the content of hydroxylcinnamates such as caffeoyltartaric acid (caftaric acid) and p-

coumaroyltartaric acid (coutaric acid), and is promoted by flavanols (Oszmianski, Cheynier, & Moutounet, 1996).

It is accepted that catecholase is so sensitive to SO2 that a small amount will inactivate it, while laccase is more active, readily soluble and resistant to SO2, and it may be present in the final wine (Ribereau-Gayon, Dubourdieu, et al., 2006). However, the concentration and activity of PPO are gradually lowered during oxidation, and with the development of fermentation and such operations as fining, SO_2 treatment and ethanol production, and sometimes no PPO exists in wine (Ribereau-Gayon, Dubourdieu, et al., 2006).

The aim of this study is to evaluate the dynamic of oxidative enzymatic activity during the winemaking of white grapes by using different combinations of SO_2 and bentonite doses.

MATERIALS AND METHODS

The research has been done at the Grape and Wine Research Institute, "*Dealu Bujorului*" vineyard, in the eastern part of Romania, during 2007-2008 period. The "*Dealu Bujorului*" vineyard has a temperate-continental climate with a lot of rains at the end of summer, drought period in July and August and sunny autumns.

The grapes (***Fetească regală*** variety) were harvested at full technological maturity. After destemming and crushing, before must separation crushed grapes were treated with different combinations of SO_2 and bentonite doses: V_1 - reference sample; V_2 - addition of 50 mg/kg SO_2; V_3 - addition of 100 mg/kg SO_2; V_4 - addition of 0,5 g/kg bentonite; V_5 - addition of 0,5 g/kg bentonite; V_6 – addition of 50 mg/kg SO_2 and 0,5 g/kg bentonite.

Alcoholic fermentation was done at temperature 17-21^{o}C. During the fermentation samples have been taken daily for physico-chemical and enzymatic activities determinations. The dynamic of oxidative enzymatic activity was quantified for grapes, must, fermented must and the new wine.

Laccase and tyrosinase activities were cuantified by using the method descriebed by Dubernet et al., 1974(2). Peroxidase activity was evaluated by using the method descriebed by Ciopraga et al., 1978.

In the same time the browning index (BI) and polyphenoloxidase index (PPOI) were calculated as mentioned Mantis (1980) and Leglise et al., 1969 cited by Ionita et. al., 1998. To analyse the musts and wines official methods (OIV) have been used.

All determinations were carried out in triplicate, and the relative standard deviations are less than ± 1%.

RESULTS AND DISCUSSIONS

The oxidative enzymatic activity of grape skin and must

Physico - chemical characteristics of the grapes at harvesting period are following: the reducing sugar content of grapes 196 g/L, total acidity 3.5 g/L H_2SO_4 and total polyphenols content 0.145 g/L.

By evaluation of laccase and tyrosinase activities in both systems grape skins and must (V_1 - reference sample), some particularities were observed. Laccase activity has higher values in grape skins than musts with 12.5%. On the contrary, the tyrosinase activity was lower in grape skins and higher in must with 51.72%.

The polyphenoloxidase index in grape skins was zero and in must was 0.3.

By comparing the all variants treated with different combinations of SO_2 and bentonite doses, at variant V_3 (addition of 100 mg/kg SO_2) the oxidative enzymes activity was lower (fig.1).

The oxidative enzymatic activity of grape must during the alcoholic fermentation

The oxidative enzymatic activity of grape must during alcoholic fermentation (after 24, 48 72h and 96h) was evaluated.

The enzymatic activity of laccase, tyrosinase and peroxidase decreased gradually during the alcoholic fermentation. After 96 h of fermentation about 27-73% of oxidative enzymatic activity still remains (fig. 2,3,4). The decreasing of tyrosinase activity was directly connected with the increasing of SO_2 dose utilised. At variants V_4 and V_5 no tyrosinase activity was observed after the alcoholic fermentation.

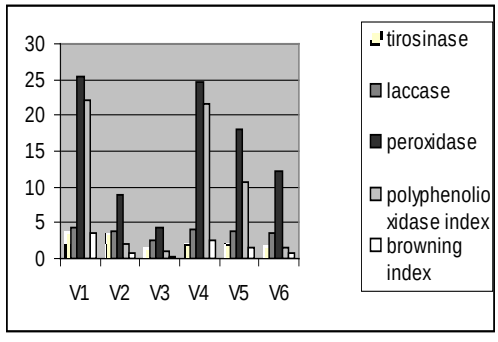

Fig.1. Enzymatic activities of musts

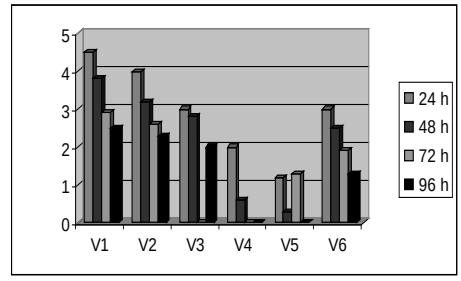

Fig.2. Dynamic of enzymatic actitivity of tyrosinase during the alcoholic fermentation

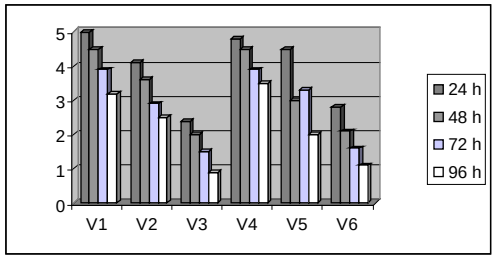

Fig.3. Dynamic of enzymatic actitivity of laccase during the alcoholic fermentation

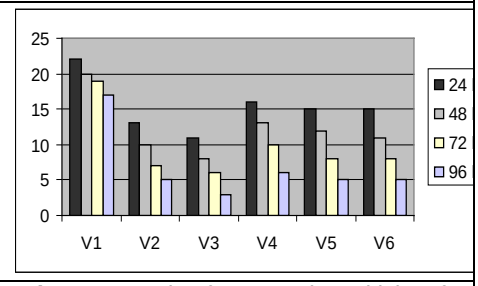

Fig.4. Dynamic of enzymatic actitivity of peroxidase during the alcoholic fermentation

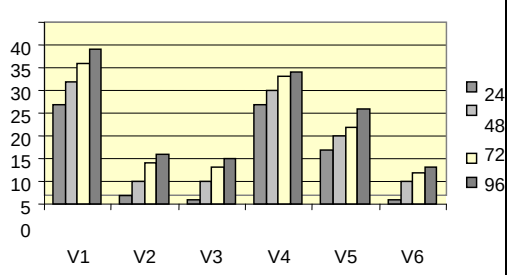

Fig. 5. Evolution of the polyphenoloxidase index during the alcoholic fermentation

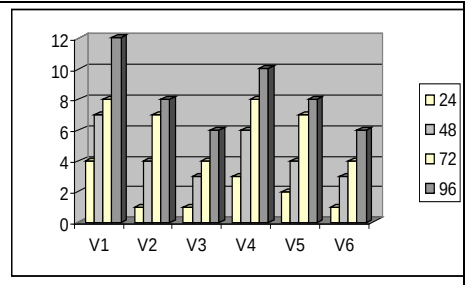

Fig.6. Evolution of browning index during the alcoholic fermentation

The evolution of PPO index is depicted in Fig. 5. An increasing about 30-75% of PPO index was observed for variants V_1, V_4 and V_5 in comparation with V_2, V_3 and V_6. The browning index was almost the same evolution (fig. 6).

The oxidative enzymatic activity of the new wine

Oxidative enzymatic activity was visibly lower at wines than must. Tyrosinase activity in the new wine was zero for all variants studied except the variant V_1 (reference sample).

Laccase activity was zero for variant V_3 where the higher concentration of SO_2 was used, followed by variant V_6 where a combined treatment was used.

Peroxidise activity was still present in the new wine, the lower value was observed for the high SO_2 doses used for variant V_3 (fig. 7).

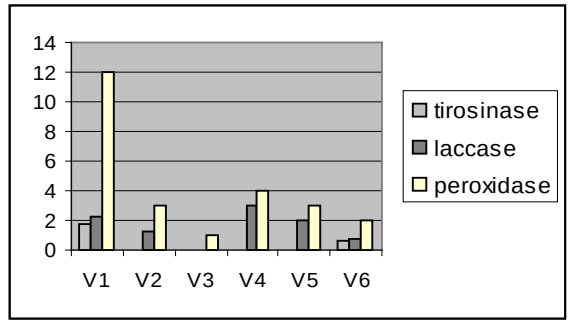

Fig.7. Total oxidative enzymatic activity of the new wine

CONCLUSIONS

The activity of PPO (tyrosinase and laccase) was gradually lowered during the alcoholic fermentation and no PPO activity was detected in the new wine, when different amounts of SO_2 and bentonite were added.

Tyrosinase activity during alcoholic fermentation has a decrease of 33-42% in the case of sulphite dioxide addition and coupled sulphite dioxide and bentonite addition, while for the bentonite addition only, the tyrosinase activity was total inhibited.

During the alcoholic fermentation, enzymatic activity of peroxidase and laccase were reduced by 27-73% correlated with the dose of sulphur dioxide and bentonite used.

After the alcoholic fermentation of grape must in the case of variants where were used similar doses of sulphur dioxide and bentonite, a reduction of oxidative enzyme activity of approximately 27-90% was observed.

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