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Activity of the oxidizing enzymes during the white winemaking

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Abstract

The biochemical processes, especially the oxireductive ones, which occur when grapes are processed, influence directly the character and direction of the transformation of the substances contained in the grapes. The way of grapes processing influences the activity of the oxidative enzymes, the quantity of marcs of grapes in the must, the colour and the content of polyphenols. In this article has been observed the influence of the technological operations which are part of the technological flow for the winemaking of white grapes Feteasca Regala variety. Its influence on the activity of the oxidative enzymes, the wine colour, polyphenols content, the index of polyphenoloxidase(IPFO) and on the index of browning(IB) were quantified as well

Keywords: lacase, peroxydase, processing, tyrosinase

1. Introduction

During the past years there has become more obvious, at world level, the wine consumers' preference for white wines with a light colour, clear fragrance, strong freshness and fruity taste, with a variety type and taste harmony. The modern technological lines for the white winemaking allow the careful processing of the grapes in order to avoid the extraction of certain undesired components by means of: destemming before crushing the grapes, the clearing of the marcs of grapes in the wine and the efficient and quick decantation of the musts, which can be improved by using the enzymatic preparations; the reduction of frictions during the pumping stage and the use of some pneumatic membrane presses in which can be introduced either whole grapes or the crushed grapes mixture coming straight from the crushing machine; the fermentation under controlled conditions (yeasts, temperature, alcoholic fermentation at low temperature) and the proper carring for the young wine (Kontek et al., 2004).

During the technological process for the grapes must obtaining the oxidative enzymes (tyrosinase and peroxydase) catalyse the oxidation of some phenolic compounds, naturally present in grapes, thus producing undesired modifications of the colour and fragrance of the wine (Sapis, 1985). The activity of the peroxydase during the wine making process has been less researched (Kochhar et al., 1979; Mujdaba et al., 1985; Avramescu et al., 1996) in comparison to the activity of the tyrosinase (Dubernet, 1974; Cordonnier et al., 1979; Lee, 1980; Sapis et al, 1984; Bayonove et al., 1984). In the presence of the air oxygen, during the processing of the grapes, the activity of the tyrosinase increases by increasing the soluble forms of the enzyme.

The first operation the crushing-dstemming of the grapes is especially favourable for the oxidative

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processes and is generally accompanied by an increase in the tyrosinase activity. These modifications have as a result the browning of the white wines processed without any SO_2 addition, only because of the presence of the oxygen. When the pressed grapes mixture is destemmed and transported through pipes, the enriching of the must with unwanted compounds takes place. At these stages there are added phenolic compounds with a flavonoid structure to the soluble polyphenols from the must, made up mostly of tartric esthers of the hydrodynamic acids, and these phenolic compounds may react with the quinones formed in the first stage of the enzymatic oxidations catalysed by the tyrosinase (Singleton et al., 1985; Cheyner et al., 1990; Kontek et al., 1994).

The result is the condensation products with a visible effect the browning. Immediately after the pressing of the grapes, the colour of the must and the polyphenols content are reduced.

The clearing of the marcs in the must reduces the enzymatic activity and increases the content of phenolic compounds (Kontek et al., 1998). The greatest edcrease of the enzymatic activity is obtained when the effect of the sulphitation is associated to that of the clearing of the marcs. The static decantation reduces the activity of the tyrosinase and the phenols content in the must and wine.

The pressing is another technological operation with direct influence on the increase of the tyrosinase activity, but on the whole, during the vinification of the grapes has been observed a decrease of the tyrosinase activity (Rapeanu, 2005). The increase of the pressing intensity generates an increase of the content of phenolic compounds, with the exception of the unflavonoid ones (Kontek et al., 2004). The colour of the wines and the susceptibility to oxidation increases at the same time with the increase of the pressing intensity (Asany, 1985). The pressed musts suffer a more browning effect than the musts obtained without the grapes pressing.

The use of SO_2 to prevent the enzymatic browning is ussualy used in vinification. At concentrations of 25 ppm, the SO_2 slowly reduces the activity of the tyrosinase (the total disappearance being done after 45-50 days from the crushing of the grapes), while in doses of 50-100 ppm it produces a total inhibiton of the enzyme in minutes after the sulphitation (Wissemann et al., 1980). The laccase activity is little affected in the presence of SO_2. The peroxydase being a stable enzyme is inactivated partially by high doses of SO_2. The reduction of the activity of the laccase and peroxydase is directly correlated with the used concentration and quantity of SO_2.

During the alcoholic fermentation the activity of the tyrosinase decreases. After the alcoholic fermentation has ended, the activity of the oxydative enzymes decreases a lot due to the creation of the reductive environment in the wine (Ţârdea, 2007).

The treatment with bentonite reduces the activity of the tyrosinase and peroxydase and acts on the laccase to a very low extent. For the combined treatments of decantation and use of bentonite a significant reduction of the tyrosinase activity is obtained. The tyrosinase activity in the wine decreases when adding bentonite in the must and disappeares during the alcoholic fermentation. Because of its ineffectiveness on the lacase, the bentonite treatment cannot be considered a prevention measure against the oxidative cassation. (Răpeanu, 2005).

The aim of this study was to observe the influence of the technological operations on the oxidative enzymes activity, the wine colour, polyphenols content, the index of polyphenoloxidase(IPFO) and on the index of browning (IB when white grapes of Feteasca Regala variety are processed.

2. Material and methods

The research has been done at The Bujoru Research-Development Station for Viticulture and Wine-making in the period 2008-2009. The *Fetească regală* grapes were picked at their technological maturity (10% mould contamination). After harvesting, the grapes were processed according to the technological flow for the obtaining of white wine from microvinification and macrovinification.

The technology of the microvinification of the *Fetească regală* white wine comprises the following technological operations: the harvesting and transport of the grapes, the quality and quantity checking, the sulphitation of the grapes in the crushing-destemming bunker with 50-60 mg SO_2/kg grapes, the crushing-destemming in the crushing-destemmer device Leonida – 300 model; the crushing of the pressed grapes mixture in the hydraulic press type Ticus-40; the assembling of the must obtained without pressing with the one obtained by pressing the grapes; the

gravitational decantation of the must in demijohns of 25 litres; the next day, the pouring of the gravitational decantated must in other demijohns in which there has been done the seeding with leavens of selected yeasts *Fermactive AP* -10g/hl; the extraction of the wine from its deposit of yeast (the premature decanting); the sulphitation with a dosage of 100 mg/l SO_2 and the use of bentonite in a dose of 1g/l; the conditioning of the wine refered to the decantation, settling by using bentonite and by sulphitation folowed by filtration in a Joker type filtre, bottling, manual corking and storage.

The technology of the macrovinification of the *Feteasc*ă *regal*ă white wine comprises the following technological operations: the harvesting and transport of the grapes, the quality and quantity checking; the tipping of the grapes in a bunker with helical conveyer where there has been added 25 mg SO_2 /kg of grapes and pectolitic enzymes *Zymoclaire High CG* 0,5 g/hl; the transport of the grapes to the pessing- destemming device with egrafulopump, type Slatina where the grapes were destemmed and crushed; the transport of the crushed grapes mixture and of the must obtained without pressing by means of stainless steel pipes to the press; the pressing of the crushed grapes mixture for 5 hours in the type Enoveneta digital programming pneumatic press with various programmes, in which there has been done the sulphitation of the resulting mixture of the pressing of grapes with 40mg/kg SO_2 in order to prevent its oxidation and browning; the transport of the pressed must and of the one obtained without pressing in a pre-decantation tank (waiting); the decantation of the must by flotation using the Kiesel-Float Clear installation made up of a central equipment with digital programming of the air quantity, a gelatine feed regulator and a control system of the degree of decantation and of the gluing effect; the transport of the flotated must in a decantation tank in which there has been done the decantation and clearing of the marcs of the must for 4-6 hours by eliminating the decantated must at its bottom part; the composition corrections of the must; the transport of the decantated must in a stainless steel fermentation tank where there were added selected yeasts *Fermactive AP* - 10 g/ hl; the fermentation of the must during which there has been monitored daily the degree of the metabolization of the sugars dependant on temperature.

There have been drawn up fermentation charts (determination of the density, acidity depending on the number of fermentation days). During the alcoholic fermentation the temperature of the must has been maintained between 16-18°C, 22°C maximum. After 7 days, depending on the temperature and the sugar concentration the end of the alcoholic fermentation ended; the reducing of the fermentation void; the extraction of the wine from its yeast (decanting I), coupled with the decantation of the wine with bentonite 1g/l and an additional 100mg/l SO_2 in the rough wine; decanting II for the tartric settling of the wine; the filtration of the wine in a plate filtre; manual bottling and delivery.

During the alcoholic fermentation samples were take out daily for physico-chemical and enzymatic determinations. The musts and wines obtained were analysed for the content of total polyphenols by means of the reaction with the Folin –Ciocâlteau reagent and were expressed as g/l gallic acid. The activities of the tyrosinase and laccase were quantified using the method described by Dubernet et al., 1974.

The activity of the peroxydase was evaluated by using the method described by Ciopraga et al., 1978. at the same time, the polyphenoloxydase index (IPFO) and the browning index (IB) were calculated using the method described by Leglise et al., 1969, and Mantis, 1980, quoted by Ioniță et al., 1998. The color of the white wines was appreciated by DO 420 nm. In order to analyse the musts and wines there have been used official methods (OIV).All the determinations have been done twice and the relative standard deviations have been less than ± 1%.

3.Results and discussion

The oxidative enzymes activity of the must during the processing of the Feteasc*ă *regal*ă *grapes following the technology of microvinification and macrovinification.

The physico-chemical characteristics of the grapes must during the harvesting period are as follows: the content of sugar reductive of the *Feteasc*ă *regal*ă grapes 220g/l, total acidity 3.5g/l H_2SO_4. The influence of the pre-fermentation technological operations from the technological flow specific to the microvinification of the *Feteasc*ă *regal*ă grapes on the oxidative enzymes activity (Table 1) and on the must physico-chemical characteristics (table 2) has been studied

Table 1. The influence of the microvinification pre-fermentation operations
on the oxidative enzymes activity

Technological operation	Activity of tyrosinase, DO420nm/ min,	Activity of lacase, DO520nm/ min	Activity of peroxydase DO420nm/min
Crushing without sulphitation	15	4	3,43
Crushing-destemming with sulphitation	3	1	2,33
Pressing	14	2	7,46
Decantation	10	1	5,22

The pre-fermentation operations have an impact on the tyrosinase, peroxydase activities and less on the laccase because this acts on the level of the grape (fig. 1). The crushing-destemming is accompanied by an increase of the tyrosinase activity, due to the stress effect or the biosynthesis of the enzyme due to the release of the soluble enzyme in the environment, respectively. The activity of the tyrosinase was of 15 $DO_{420nm/min}$, the activity of the laccase was of $4DO_{520nm/min}$, and that of the peroxydase of 3.43 $DO_{420nm/min}$. The sulphitation of the grapes in the bunker of the crushing-destemming device caused a decrease of 20-68% of the oxidative enzymatic activity.

The pressing caused an increase of the enzymatic activity, because part of the cells of the core go in the must from the pressing devices, parts which carry oxireductases. The tyrosinase activity was of 14 $DO_{420nm/min}$, the laccase activity was of $2DO_{520nm/min}$, and that of the peroxydase was of 7,46 $DO_{420nm/min}$. The clearing of the marcs of the grapes in the must caused a reduction of the enzymatic activity in comparison to the pressing.

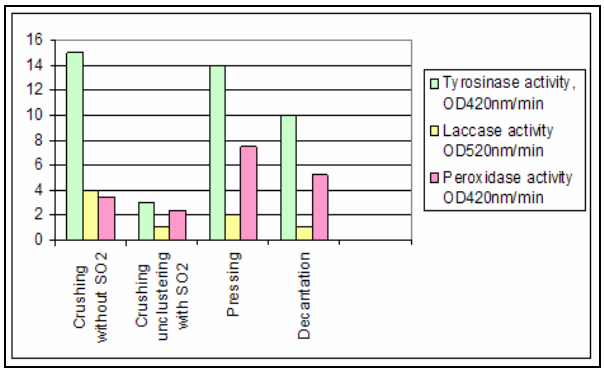

Figure 1. The influence of the pre-fermentation technological operations on the microvinification of the grapes

Table 2. The influence of the microvinification pre-fermentation technological operations on the physico-chemical characteristics of the must

Technological operation	The polyphenol oxydase index (IPFO)	The browning index (IB)	Total polyphenols, g/l galic acid	Culour of the must (DO 420nm)
Crushing without sulphitation	4,9	0,2	0,115	0,200
Crushing-destemming with sulphitation	0,2	0,1	0,275	0,105
Pressing	3,0	0	0,290	0,370
Decantation	1	0	0,253	0,312

The oxidation capacity of the wine is expressed by the polyphenoloxydase index (IPFO) and the colour of the wine. The must is strongly enriched with phenolic substances because the crushing of the grapes is the first operation in the vinification during which the must and the wine are strongly enriched with phenolic substances (0.115 g/l gallic acid). Because of the sulphitation a decrease of both indices values: of polyphenoloxydase (IPFO)(0.2) and of browning (IB)(0.1) and a decrease of the colour of the must (DO 420 nm)(0.105) was noted. The musts obtained by pressing are more availabe to oxidation. In the discontinuous pressing stage the content of polyphenolic substances increases during the pressing (0.290 g/l gallic acid). In the decantation stage the browning index had a null value.

During the macrovinification of the grapes the influence of the pre-fermentation operations on the activity of the oxidizing enzymes (table 3, figure 2) and on the physico-chemical characterisitics of the must (table 4) is presented.

Table 3. The influence of the macrovinification pre-fermentation operations
on the activity of the oxidizing enzymes

Technological operation	Activity of tyrosinase, DO420nm/min	Activity of lacase, DO520nm/min	Activity of peroxydase, DO420nm/min
Tipping of the grapes	3	4	3,03
Crushing-unclustering	6	2	4,23
Pneumatic pressing	4	1	2,73
Flotation	6	1	2,23

During the unfolding of the technological flow specific to the macrovinification a decrease of the

activity of the laccase and peroxydase and a variation of the activity of the tyrosinase has been observed. In the crushing-destemming and flotation stages the tyrosinase activity had the highest value (6 DO $_{420nm/min}$). The laccase activity was lower in the pneumatic pressing and flotation (1DO $_{520nm/min}$). The peroxydase being an oxyreductive enzyme recorded a high value in the crushing-unclustering (4,23 DO $_{420nm/min}$) and lower values for the pneumatic pressing and flotation(2,73 DO $_{420nm/min,}$ respectively 2,23 DO $_{420nm/min}$).

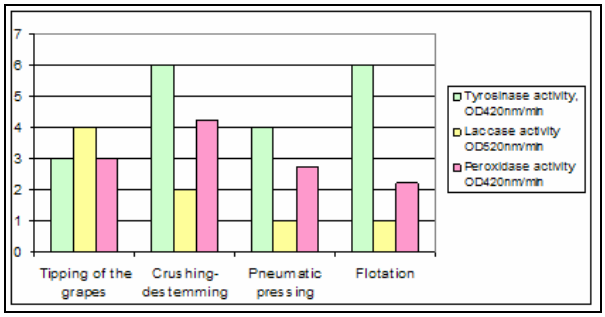

Figure 2. The influence of technological pre-fermentation operations on the grapes macrovinification

Table 4. The influence of the macrovinification pre-fermentation operations on the physico-chemical characteristics of the must

Technological operation	Polyphenoloxydase index (IPFO)	Browning index (IB)	Total polyphenols, g/l gallic acid	OD 420 nm
Tipping of the grapes	3,2	0,1	0,160	0,250
Crushing-destemming	4,7	0,2	0,210	0,394
Pneumatic pressing	5,4	0,3	0,260	0,434
Flotation	4,1	0,4	0,300	0,400

During the grapes macrovinification an increase of the browning index (IB) and of the content of total polyphenols has been noted. The polyphenoloxydase index (IPFO) varied between 3.2 -5.4 and the colour of the must (DO 420nm) between 0.250 – 0.400.

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

The oxidative enzymes activity in the microvinified *Fetească regală* grapes must, which was sulphitated, during the alcoholic fermentation (three days after fermentation) (figure 3) has been determined. During the alcoholic fermentation the activity of the tyrosinase decreases with 66.6% due to the alcohol production and its precipitation. A decrease of the peroxydase activity with 57%, of the content of polyphenols with 42% and of the must colour with 46% has been noticed. The activity of the laccase was null on the third day of the alcoholic fermentation.

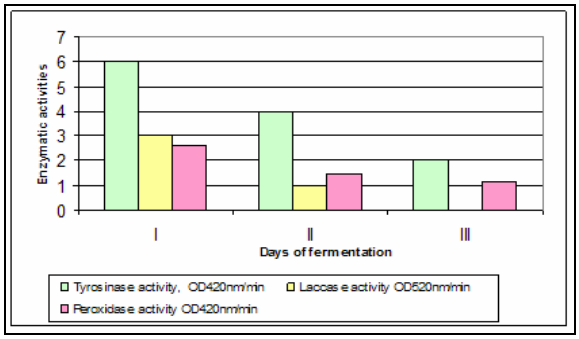

Figure 3. The activity of the oxidizing enzymes during the alcoholic fermentation of the microvinified grapes must

During the alcoholic fermentation the polyphenoloxydase index and the browning index increased. the The activity of the oxidative enzymes in the macrovinified *Feteasca regală* grapes must, sulphitated during the alcoholic fermentation (after 7 days of fermentation) (figure 4) has been determined.

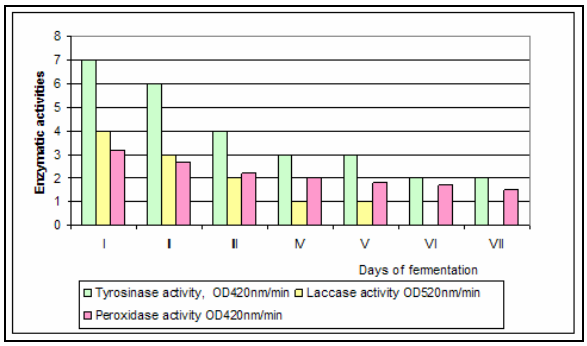

Figure 4. The activity of the oxidative enzymes during the alcoholic fermentation of the macrovinified grapes must

During the 7 days of fermentation the activity of the oxidative enzymes decreased with approximately 53-71.42%. The activity of the laccase was null in the seventh day of the alcoholic fermentation. An increase of both indices (IPFO, IB) during the alcoholic fermentation has been noticed. The total polyphenols content decrease by 26.47% due to the precipitation and condensation reactions. The same

decreasing evolution by 46.42% was noticed for the colour intensity (DO420nm).

The activity of the oxidizing enzymes in the young wines. There have been studied the physico-chemical characteristics and the enzymatic activity for the young white wines after the technology of the microvinification and macrovinification.

The alcoholic degree in young wines was specific to the type of grapes depending on the sugar quantity accumulated in the grapes. It was of 12.8% alcoholic vol. for the wine obtained by microvinification technology and 12.7% alcoholic vol. for the one obtained by macrovinification.

The total acidity was situated between normal limits (3,6-3,7g/l H_2SO_4). The average volatile acidity was close to the new white wines(0,28 - 0,40g/l acetic acid). The values of the total extract of white wines was situated between 22-23.0 g/l free SO_2 and total SO_2 between 78-82g/l. The activity of the oxidative enzymes is lower in wines than in musts. The tyrosinase activity in wine was of 1 DO $_{420nm/min}$, and peroxydase activity had a value of 0,90 DO $_{420nm/min}$ in the microvinification and in the macrovinification of 1,40 DO $_{420nm/min.}$the activity of the laccase was null in both situations. From the experimental data there has been noticed a decrease of the polyphenoloxydase index (IPFO) of the white wine compared to the must. The colour of the must and of the wine highlights the biochemical reactions which characterize the alcoholic fermentation process, being influenced both by the phenolic richness of the grapes and by the vinification technological process. As it could be noticed from the experimental data the oxidation phenomena favour the fading of the colour of the white wines compared to that of the white musts.

4. Conclusions

1. The processing manner of the grapes and of the crushed grapes mixture influences both the quality of the musts and of the wines which are obtained.
2. During the unfolding of the pre-fermentation operations there has been noticed a variation of the oxidative enzymatic activity.
3. The activity of the oxidative enzymes (tyrosinase, lacase, peroxydase) decreased by 57-66.66% during the alcoholic fermentation of the microvinified grapes must. The activity of the oxidative enzymes (tyrosinase, lacase, peroxydase) decreased by 53-71.42% during the alcoholic fermentation of the macrovinified grapes must. The activity of the laccase in the last days of the alcoholic fermentation was null in both situations.

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