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ORIGINAL ARTICLE

THE DYNAMICS OF OXIDATIVE ENZYMES DURING THE WHITE WINEMAKING*

CARMEN POPESCU

Elena Doamna Food Industry College Galati, 169 Domnească St, 800189, Galați, România
popescucarmen_18@yahoo.com

ELENA POSTOLACHE

Research and Development Institute in Viticulture and Vinification Bujoru, 65 G-ral E. Grigorescu St, 805200, Galați, România
postolache_elena2000@yahoo.com

GABRIELA RAPEANU, MIRCEA BULANCEA, TRAIAN HOPULELE

Dunărea de Jos University of Galați, Faculty of Food Science and Engineering, 111 Domnească St, 800201, Galați, România
Gabriela.Rapeanu@ugal.ro

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The aim of this paper was to monitorise the evolution of the oxidative enzymes activity during the process of winemaking from white grapes. The second objective was to evaluate the browning capacity of the grape must during the alcoholic fermentation stage and in the resulting wine. During the alcoholic fermentation, the activity of the oxidative enzymes was reduced, thus the enzymatic activity of the peroxydase decreased by approximately 60% of the initial activity, the enzymatic activity of the tyrosinase decreased by approximately 40% of the initial enzymatic activity and the enzymatic activity of the laccase decreased by approximately 40% of the initial enzymatic activity.
The activity of the oxidative enzymes in wines diminished progressively after three months of wine aging. The enzymatic activity of the tyrosinase decreased by 25-60% of the initial enzymatic activity. The enzymatic activity of the peroxydase was reduced appreciatively by 10% of the initial enzymatic activity. The enzymatic activity of the laccase reached the value 0 at three months after the wine had been obtained.
During the alcoholic fermentation stage the values of the PFO and IB indices increased and during the evolution of the wine (after 3 months) the values of the PFO and IB indices decreased.

Keywords: polyphenols, polyphenoloxidase index (IPFO), browning index (IB), tyrosinase activity, laccase activity, peroxidase activity

1. Introduction

Due to the biochemical processes of oxidation and condensation, catalyzed by the tyrosinase, the phenolic compounds, the colour and the browning capacity of the must and wine undergo significant changes.

The activity of the laccase and the browning capacity of the must and wine depend on the degree of mould contamination of the harvest. Kovac and Vrbasni, (1979), demonstrated that the oxidation capacity of the must and wine is influenced by the presence of *Botryotinia fuckeliana* as well as by the presence of other types of mould *(Penicilium*, *Aspergilus* etc*)*. Valeria Ioniță, (1996), observed the role of the substrate in the oxidation phenomena of the laccase. She demonstrated the existence of a correlation between the activity of the laccase in the must and the index of polyphenoloxydase, during

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the alcoholic fermentation. The most important factors that influence the variation of the polyphenoloxydase index during the period of fermentation are the degree of contamination of the grape harvested, the oxygen level in the must, the conditions in which the fermentation takes place (the parameters of fermentation), the grape variety etc. Even at three months after the wine has been obtained, the chemical composition of the brut wines is influenced by the activity of the laccase from the must and by the biochemical processes that occur during the alcoholic fermentation.

In other researches, Postolache *et al.*, (1995), Bulancea *et al.* (2003), identified values of the tyrosinase activity for the must (*Fetească albă* variety) situated within the limits of 3.66-5.4 OD/min. At the same time, the content of total polyphenols and the colour intensity of the musts had normal values for the different varieties of white grapes.

The content of tyrosinase in white musts undergoes processes of deactivation and degradation during the alcoholic fermentation (Ioniță *et al*., 1994).

2. Materials and methods

The researches were carried out at the Research and Development Institute in Viticulture and Vinification Valea Călugărească, the "*Dealu Mare*" vineyard during the period 2007-2008. The varieties of white grapes used for the analysis were: *Italian Riesling, Fetească regală, Sauvignon, Chardonnay* (10% contamination with mould) and *Muscat Ottonel* (10% contamination with mould).

After harvesting, the healthy grapes and those contaminated with grey rottenness (10%) were processed according to the technological flow of the production of white wines. The grapes sulphitation was done in the grape destemming-crusher, by spraying a solution of SO_2 6% in a quantity of 50-60 mg SO_2/kg of crushed grapes. After the crushing, pressing and the must settling, the must was poured into the fermentation tank where there have been added selected yeasts, *Fermactive AP* for the Fetească regală and Italian Riesling varieties in a quantity of 20 g/hl. For the rest of the varieties *Fermactive Sauvignon, Fermactive Chardonnay* and *Fermactive Muscat* have been used in quantities of 15 g/hl.

The must was also treated with the enzymatic preparation of *Zymoclaire G* in a quantity of 2.5 g/hl and *Endozym ICE* in a quantity of 6 ml/hl for the Italian Riesling and Fetească regală varieties. *Zymovarietal Aromă* was used for Sauvignon, Chardonnay and Muscat Ottonel in quantities of 4.4 g/hl and *Endozym ICE* as 5.1 ml/hl.

During the alcoholic fermentation for the musts, for the new wines and after the three months of maturation the physico-chemical analyses were done.

The musts and the wines obtained were analysed for the total polyphenols content by means of the reaction using the Folin-Ciocalteau reagent and were expressed as mg/l gallic acid. The tyrosinase and laccase activities were quantified using the method described by Dubernet *et al.*, (1974). The peroxidase activity was evaluated by using the method described by Ciopraga *et al.*, (1978). At the same time, the polyphenoloxydase index (IPFO) and the browning index (BI) were calculated using the method described by Leglise *et al*., (1969), and Mantis, (1980), cited by Ioniță *et al.*, (1996). The colour of the white wines was appreciated by DO 420 nm.

In order to analyze the musts and wines the official methods (OIV) were used.

All the determinations were carried out in duplicate and the relative standard deviations were less than or equal to 1%.

The technological scheme of the white wine obtained from unflavoured varieties includes the following technological operations: the quality and quantity reception, the sorting of the grapes, the crushing-destemming, the sulphitation, the enzymatic preparation addition (depending on each case), the pressing, the settling of the must, the correction of the composition (depending on each case), the controlled fermentation and the yeast separation (early decanting).

The technological scheme of the production of white wines from flavoured/semi-flavoured varieties includes the following technological operations: the quality and quantity reception, the sorting of the grapes, the crushing-destemming, the sulphitation, the enzymatic preparation addition, the pre-fermentation stage maceration, the pressing, the settling of the wine, the enzymatic preparation addition (depending on each case), the correction of the composition (depending on each case), the controlled fermentation and the yeast separation (early decanting).

The alcoholic fermentation was carried out at temperatures ranging between 17-21ºC for the white musts. During the alcoholic fermentation daily samples were taken for physico-chemical and enzymatic determinations. At the end of the alcoholic fermentation, the wines were drawn off from the yeast at a density of 1000 g/ cm^3 and they were applied a correction of free sulphur dioxide of 30 mg/l.

3. Results and discussions

3.1. The activity of the oxidative enzymes of the white must

The main physico-chemical parameters of white musts are presented in Table 1.

The total sugar content and the total acidity have close values for the varieties studied, 183-234 g/l sugar and 2.6-3.8g/l H_2SO_4 respectively.

The activity of the oxidative enzymes of the white wines is presented in Table 2.

The tyrosinase and laccase are found mainly in the grape skin and only a small part is soluble and passes on into the must during its processing. The activity of the laccase was of 13.4 (OD_{520} nm/min) for the *Italian Riesling* grape variety and of 21.0 (OD_{520} nm/min) for the *Chardonnay* grape variety. The tyrosinase activity is lower than the laccase activity with 10.6 (OD_{420}nm/min) for *Sauvignon* grape variety, 11.9-12.7 (OD_{420} nm/min) for *Feteasc*ă *regal*ă*, Chardonnay, Muscat Ottonel* grape varieties and 14 (OD_{420} nm/min) for *Italian Riesling* grape variety, respectively. The peroxidase activity was 5.1 (OD_{420} nm/min) for *Italian Riesling* grape variety and 7.1 (OD_{420} nm/min) for the *Sauvignon* grapes.

3.2. The activity of the oxidative enzymes of the white must during the alcoholic fermentation

The activity of the oxidative enzymes was assessed in the must during the alcoholic fermentation (after 24, 48 and 72 h) (Table 3 and Figures 1, 2, 3). The enzymatic activity of the tyrosinase, laccase and peroxidase decreased gradually during the alcoholic fermentation. After 72 hours of fermentation approximately 30-60% of the oxidative enzymatic activity was still present. After the grapes crushing, the most important biochemical reactions catalized by the enzymes, especially by the tyrosinase, occur in the must. It could be noticed an increase of both indices (IPFO and IB) during the alcoholic fermentation.

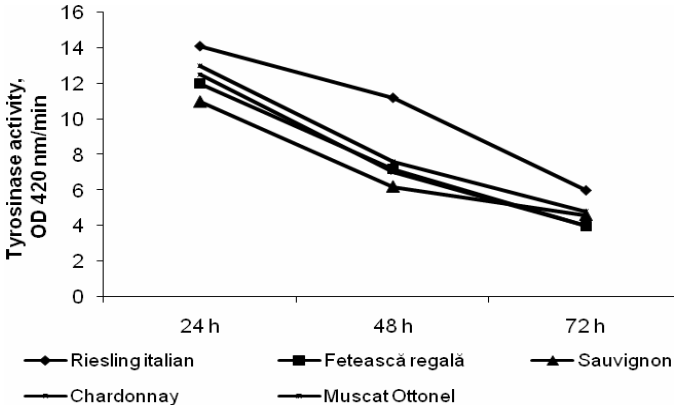

Figure 1. The tyrosinase activity evolution during the alcoholic fermentation of the white must

During the alcoholic fermentation, the total content of phenolic compounds decreases because of the precipitation and condensation reactions. The same decreasing evolution was noticed for the colour intensity (OD_{420} nm) caused by the intense oxidation phenomena which occur during the alcoholic fermentation and the macromolecules precipitation (Table 3).

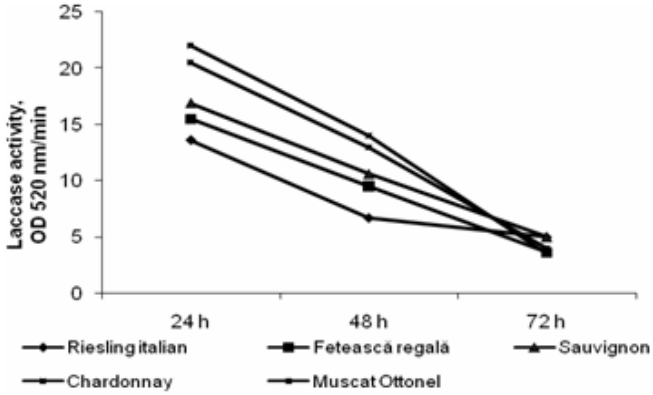

Figure 2. The laccase activity evolution during the alcoholic fermentation of the white must

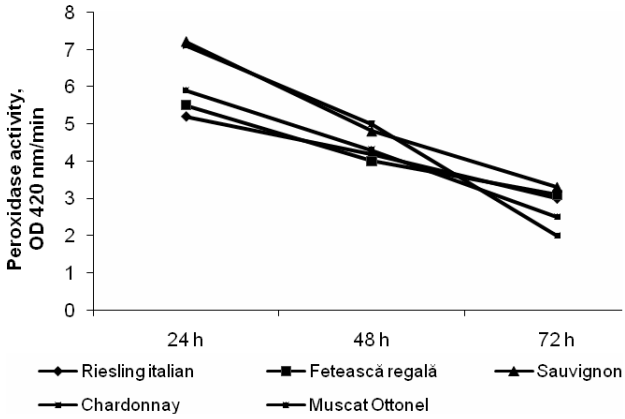

Figure 3. The peroxidase activity evolution during the alcoholic fermentation of the white must

3.3. The activity of the oxidative enzymes in the new wines

Table 4 presents the physico-chemical characteristics and the enzymatic activity in the new white wines from the *Dealu Mare* vineyard.

The alcoholic degree in new wines was specific for each variety of grapes depending on the quantity of sugar accumulated in grapes. It was 13.5% v/v for the *Italian Riesling* and 10.4% v/v for the *Sauvignon* variety.

The total acidity was situated within normal limits for all varieties (2.4- 3.5 g/l H_2SO_4). The average volatile acidity was close for the new white wines studied (0.16- 0.20 g/l acetic acid).

The values of the total extract of the white wines were situated between 20.0-22.0 g/l.

The oxidative enzymatic activity was lower in the new wines than in the musts. The tyrosinase, laccase and peroxidase activities of the new white wine had lower values compared to the must for all the grape varieties studied.

From the experimental data (Tables 3 and 4) a decrease can be noticed of the polyphenoloxydase index (IPFO) of the white wine compared to the must and an increase could also be noticed for the browning index (IB) and of the total polyphenols (g/l gallic acid) of the wine compared also with the must.

Table 1. The physico-chemical parameters of the white musts from the Dealu Mare vineyard (2008)

Grape variety	Sugar, g/L	Total acidity, g/L H_2SO_4	Total polyphenols, g/L gallic acid	$DO_{420\ nm}$
Italian Riesling	234	3.0	0.210	0,354
Fetească regală	191	3.5	0.204	0,360
Sauvignon	183	3.0	0.236	0,348
Chardonnay	223	3.8	0.215	0,338
Muscat Ottonel	191	2.6	0.216	0,367

Table 2. The oxidative enzymatic activity of the white musts from the Dealu Mare vineyard (2007-2008)

Grape variety	Tyrosinase activity, OD_{420}nm/min	Laccase activity, OD_{520}nm/min	Peroxidase activity, OD_{420}nm/min	Polyphenoloxidase index (IPFO)	Browning index (IB)
Italian Riesling	14.0	13.4	5.1	6.2	0.14
Fetească regală	11.9	15.2	5.4	7.3	0.15
Sauvignon	10.6	16.7	7.1	8.2	0.17
Chardonnay	12.2	21.0	7.0	8.7	0.18
Muscat Ottonel	12.7	20.0	5.7	9.6	0.19

Table 3. The physico-chemical characteristics and the oxidative enzymatic activity during the alcoholic fermentation of the white musts from the Dealu Mare vineyard (2007 -2008)

Grape variety	Tyrosinase activity. OD_{420}nm/min			Laccase activity OD_{520}nm/min			Peroxidase activity. OD_{420}nm/min			PFO index			Browning index			Total polyphenols. g/L gallic acid			OD 420 nm		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Italian Riesling	14.1	11.2	6	13.6	6.7	5.0	5.2	4.2	3.0	7	11	14.5	0.16	0.19	0.24	0.215	0.190	0.175	0.360	0.320	0.315
Fetească regală	12.0	7.2	4.0	15.5	9.5	3.6	5.5	4.0	3.1	7.8	10.1	12.0	0.17	0.20	0.22	0.210	0.186	0.169	0.365	0.341	0.315
Sauvignon	11.0	6.2	4.6	16.9	10.6	5.0	7.2	4.8	3.3	8.5	10.6	11.3	0.18	0.21	0.25	0.240	0.228	0.200	0.352	0.325	0.300
Chardonnay	12.5	7	4.0	22.0	14.0	3.5	7.1	5.0	2.0	9	11.5	12.4	0.20	0.24	0.26	0.218	0.196	0.175	0.340	0.315	0.290
Muscat Ottonel	13.0	7.6	4.8	20.5	13.0	4.0	5.9	4.3	2.5	10.0	12.0	13.6	0.20	0.21	0.23	0.220	0.199	0.180	0.370	0.345	0.320

Table 4. The physico-chemical characteristics and the enzymatic activity of the new wines from the Dealu Mare vineyard (2007-2008)

Grape variety	Alcohol % vol.	Total acidity. g/L H_2SO_4	Volitile acidity. g/L CH_3COOH	Total extract. g/L	Free SO_2 mg/L	Total SO_2 mg/L	Tyrosinase activity. OD_{420}nm/min	Laccase activity. OD_{520}nm/min	Peroxidase activity. OD_{420}nm/min	PFO index	Browning index	Total polyphenols. g/L gallic acid	OD 420 nm
Italian Riesling	13.5	2.8	0.20	21.5	12	23	5.0	3.5	2.7	7.0	0.27	0.190	0.325
Fetească regală	11.0	3.3	0.18	21.0	14	20	3.2	3.0	2.9	5.5	0.24	0.178	0.320
Sauvignon	10.4	2.9	0.16	20.0	10	24	4.3	4.2	3.1	6.0	0.27	0.215	0.306
Chardonnay	12.9	3.5	0.19	22.0	9	22	3.5	3.0	2.8	5.8	0.29	0.192	0.298
Muscat Ottonel	10.9	2.4	0.17	21.2	11	23	4.0	3.3	2.3	7.2	0.25	0.198	0.327

Table 5. The physico-chemical characteristics and the enzymatic activity of the wines after three months of evolution from the Dealu Mare vineyard (2007-2008)

Grape variety	Alcohol % vol.	Total acidity. g/L H_2SO_4	Volitile acidity g/L CH_3COOH	Total extract g/L	Free SO_2 mg/L	Total SO_2 mg/L	Tyrosinase activity. OD_{420}nm/min	Laccase activity. OD_{520}nm/min	Peroxidase activity. OD_{420}nm/min	PFO index	Browning index	Total polyphenols g/L gallic acid	OD 420 nm
Riesling italian	13.3	2.7	0.23	21.3	22	98	2	0	2.6	5.5	0.10	0.195	0.329
Fetească regală	10.9	3.1	0.20	20.7	26	102	1	0	2.7	4.2	0.15	0.180	0.324
Sauvignon	10.2	2.7	0.19	19.9	24	110	3	0	3.0	3.6	0.20	0.218	0.310
Chardonnay	12.7	3.3	0.22	21.8	27	96	2	0	2.5	4.0	0.22	0.198	0.302
Muscat Ottonel	10.8	2.2	0.19	21.0	25	112	3	0	2.2	5.1	0.20	0.202	0.330

The colour of the wine and must highlights the biochemical reactions that characterise the alcoholic fermentation process, being influenced by both the phenolic richness of the grapes and the technological winemaking process.

As it can be noticed in Tables 3 and 4, the oxidation phenomena are in favour of the decrease of the colour of the white wines compared to the white musts.

3.4. The activity of the oxidative enzymes for the wines after 3 months of evolution

The experimental data that were obtained underlined a decrease in the two indices in the wines after 3 months of evolution compared to the new wines. The variation of the browning index (IB) depends on the degree of contamination with *Botryotinia fuckeliana* of the grape harvested, as well as on the harvest year. As it can be noticed (Tables 4 and 5) the total amount of total polyphenols content increases insignificantly for the wines after three months of evolution compared to the new wines.

At the same time differences between the tyrosinase activity, the total phenol content and the browning tendencies of the different varieties of wines were observed. Problems appear when attempts are made to correlate the total amount of phenols content and/or the activity of the tyrosinase with the tendency of the wine to brown. The scientific literature has reported that the different answers offered by wines with high browning capacity are connected to the relative quantities of the specific fractions of the phenols and less to the total amount of total phenol content.

4. Conclusions

After the crushing of the grapes the enzymatic activity is maximum due to the contact of the enzymes with the substrate (the must) when the oxygen is present.

The tyrosinase and the laccase are localized in the grapes skin and only a small part is soluble and passes on into the must during the processing.

The laccase activity is maximum for the grapes contaminated with *Botryotinia fuckeliana.*

The phenolic compounds, the colour and the browning capacity of the must and of the wine undergo significant modifications because of the biochemical processes of oxidation and condensation catalyzed by the tyrosinase. The physico-chemical composition of the white wines is strongly influenced by the activity of the oxidative enzymes from the must and by the biochemical processes which take place during the alcoholic fermentation.

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