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CHANGE OF THE PHYSICO-CHEMICAL INDICES AND THE OXIDATIVE ENZYMATIC ACTIVITIES DURING THE WHITE GRAPES RIPENING

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The aim of this study was to follow up the evolution of both the physico-chemical indices and the dynamics of the oxidative enzymes during the maturation of white grapes, more particularly until the grapes reach technological maturity. Studying the dynamics of the physico-chemical indices during the maturation of the grapes an increase of the content of glucides, polyphenols, antiocyanins, simultaneously with the decrease of the acidity was noticed. The enzymatic activity of tyrosinase is high at the moment when the grapes are in the beginning of the ripening period and afterwards it presents a decline during the ripening of the grapes, having a relatively low level before harvesting. The enzymatic activity of the laccase increases up till the full maturity of the grapes, then it decreses a little before the harvesting of the fruit, having lower values than at the start of ripening. The enzymatic activity of the peroxidase increases up till the full maturity of the grapes, then it decreses a little before the harvesting of the fruit, having lower values than when it begins to ripen.

Keywords: tyrosinase, laccase, peroxidase, mass of 100 grapes berries, sugar, acidity

1. Introduction

The activity of the polyphenoloxidase in grapes was studied by many researchers due to its implications in the wine technology. Dubernet and Ribereau-Gayon P., (1973), Dubernet, (1974), highlighted two enzymes: tyrosinase, present in all grapes, and laccase, secreted by *Botryotinia fuckeliana,* specific to the grapes prone to mould attack. Sapis et al., (1983), diversified the substrate of the action of polyphenoloxidase on the hydroxicynamic esthers. Nakamura et al., (1983), studied some of the properties of these enzymes in Romania, the activity of the oxidative enzymes was studied by Valeria Ioniță et al., (1994), Valeria Ioniță, (1996), and Maria Avramescu et al.,(1996), when the dynamics of the enzymes during the grape maturation period as well as during the alcoholic fermentation was quantified.

The study of the laccase activity in grapes has concerned many researchers. Cordonnier et al., (1979), studied the relationship between the degree of contamination with mould at harvest, the activity of the laccase and the ability of wine and must oxidative brownness. It was concluded that, besides the laccase, an important part is played by the nature of the substrate and its concentration.

Fregoni et al., (1993), showed that the ELISA test offers variable information depending on the variety, the nature and percentage of the contamination, assessed visually on the grapes. The contamination at the lever of grapes berry is done by the presence of the extracellular enzymes secreted by *Botryotinia fuckeliana*. This enzyme causes the hydrolysis of the cellular membrane and the oxydation of some chemical compounds. The laccase secreted by *Botryotinia fuckeliana* catalyses

the biochemical oxydations of the phenols located in the skin of the grapes and which, most often than not, lead to the oxydative brownness of the wine.

The oxidative enzymes (polyphenoloyidase and the peroxydase) within the grapes are genetic characters of plants and depend on the climatic and agrotechnical factors (Sapis et al., 1983).

2. Materials and methods

2.1. Materials

The research was carried out at the Research and Development Institute in Viticulture and Vinification Valea Călugărească, the "Dealu Mare" vineyard in 2007-2008. The varieties of white grapes used for the analysis were: *Italian Riesling, Fetească regală, Sauvignon, Chardonnay and Muscat Ottonel.*

2.2. Sampling

At the beginning of the maturation stage, the grape samples were drawn every 5 days starting with the beginning period of the ripening time specific to each variety, and as the maturation process advanced, the drawing was performed more often, every 3 days, up till the moment of the harvesting. The samples consisted of approximately 2-3 kg of grapes from each variety so as to present the average maturity of the variety.

Samples were picked up from different farms of land from the Dealu Mare vineyard, which came from at least 10-20 vines from the lot situated on more than just one row.

2.3. Methods

The physico-chemical analysis of the grape samples consisted in dynamics of the grape berries mass, the sugar and acidity evolution for each variety. The resulting grape berries were numbered separating the healthy grape berries from the damaged ones (crushed, mouldy).

The average samples were submitted to the following determinations:

- the mass of 100 grape berries (g) by picking 300 grapes berries, weighting them with the lab technical scales and ralate them to 100 grapes berries. After weighting, the grapes berries were crushed by using gauze and hand-press. The must was separated and kept in the refrigerator for 2-3 hours for clarification;
- the sugar concentration (g/l) was estimated by the refractometric method;
- the titrimetric determination of the total acidity (g H_2SO_4/L).

*Enzymatic activities assay*s. The activity of the tyrosinase and laccase was quantified using the method described by Dubernet et al., (1974). The activity of the peroxidase was assessed by using the method described by Ciopraga et al., (1978).

One unit of enzymatic activity (UA) was the increase in optical density at 420 nm for tyrosinase and peroxidase and 520 nm for laccase per minute.

At the same time, the polyphenoloxydase index (IPFO) and the browning index (IB) were calculated according to the method described by Leglise et al., (1969), and Mantis, (1980), cited by Ioniță et al., (1996).

All the determinations were done twice and the relative standard deviations were lower than ± 1%.

3. Results and discussions

At the beginning of the ripening period in the grapes, an accumulation of sugars, polyphenols and antocyanins at the same time with the decrease of the acidity, was observed. The parameters analysed during the year 2008 were directly connected with the degree of grapes maturation and the climatic conditions of the vineyard. The dynamics of the physico-chemical indices during the white grapes maturation in the Dealu Mare vineyard 2008 are shown in Figures 1, 2 and 3.

The weather conditions in autumn 2008 led to a low contamination of grey mould favorable to high quality wines making. During the vegetation period (01.04- 30.09.2008) the rains fallen in the Dealu Mare vineyard caused the forced ripening of the grapes and full maturity was reached between 30. 08- 09. 09. 2008. In the Dealu Mare vineyard, the varieties of white wine reached full maturity at different times.

The evolution of sugar content, acidity and mass index of 100 grape berries are depicted in figures 1, 2 and 3. For the white grapes *Riesling Italian* variety (farm 4, lot 3344) full maturity was reached on September 4th 2008 with a sugar content of 271 g/l, acidity level of 5.00 g/l H_2SO_4, the mass of 100 grape berries was 136 g. The full grape maturity for *Feteasc*ă *regal*ă variety (farm 2, lot 4668) was reached on September 4th 2008 with a sugar content of 175 g/l, acidity level of 4.31 g/l H_2SO_4, and the mass of 100 grape berries of 159 g. For *Sauvignon* variety, the full maturity of grapes was reached on September 9th 2008 with a sugar content of 160 g/l, acidity level of 4.00 g/l H_2SO_4, the mass of 100 grape berries being of 160 g. The full maturity of white grapes *Chardonnay* variety was reached on September 9th 2008 with a sugar content of 186 g /l, acidity level of 4.3 g/l H_2SO_4, the mass of 100 grape berries being of 153 g. For the grapes *Muscat Ottonel* variety (farm 2, lot 4784) full maturity was reached on August 30th 2008 with a sugar content of 151 g/l, acidity level of 3.43 g/l H_2SO_4, and the mass of 100 grape berries was 144 g.

The grapes harvesting time was the same with the technological maturity which happened during the period September 20th – September 30th 2008. Table 1 shows values of the sugar, acidity and the mass of 100 grape berries at the technological maturity of grapes.

Table 1. Sugar content, acidity and the mass of 100 grape berries at the technological maturity of grapes varieties studied

Grapes variety	Sugar, g/l	Acidity, g/l H_2SO_4	Mass of 100 grape berries, g
Italian Riesling variety (farm 4, lot 3344)	234	3.0	95
*Feteasc*ă *regal*ă variety (farm 2, lot 4668)	191	3.5	138
Sauvignon variety (research lot)	182	3.0	140
Muscat Ottonel variety (farm 2, lot 4784)	191	2.6	120

Analyzing Figure 1 and 2, the presence of varieties with low sugar content (g/l) and acidity (g/l H_2SO_4) (*Muscat Ottonel*) and varieties with high sugar content and acidity (*Italian Riesling*) were noticed.

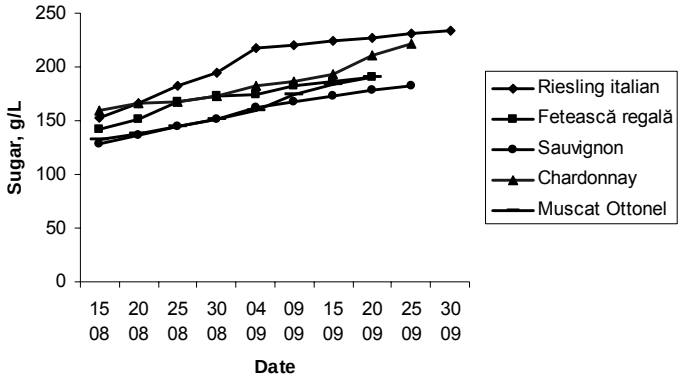

Figure 1. The dynamics of the sugar content during the maturation of white grapes in Dealu Mare vineyard (2008)

The mass of 100 grape berries presented an increasing in evolution until the moment when the grapes were fully matured, and between the full maturity and the technological level of maturity of the grapes the mass index of the 100 grapes berries decreased gradually.

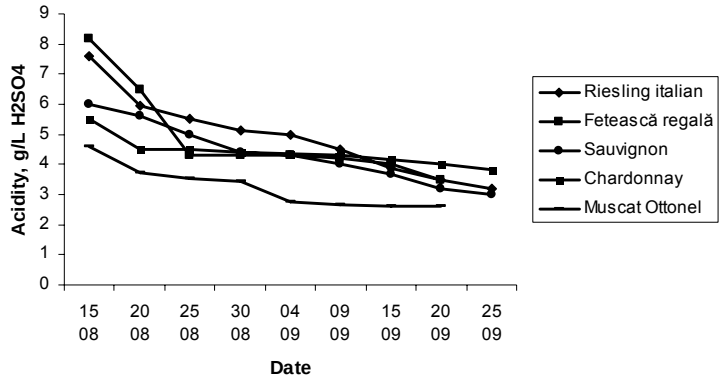

Figure 2. The dynamics of the acidity during the maturation of white grapes in Dealu Mare vineyard (2008)

The mass of 100 grapes berries had low values in the case of *Italian Riesling* and high values for *Sauvignon* (Figure 3).

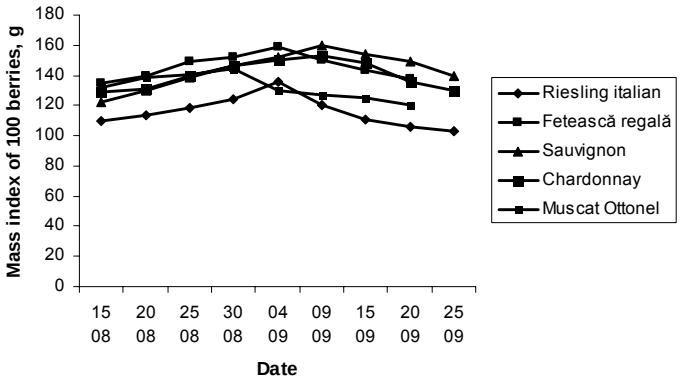

Figure 3. The dynamics of the mass index of 100 grape berries during the maturation of white grapes in Dealu Mare vineyard (2008)

The dynamics of the tyrosinase activity during the maturation of white grapes

The evolution of the tyrosinase activity according to the time to which the determination was made is presented in Figure 4 and it was noticed that they are similar for all the varieties of white grapes studied (with the exception of *Italian Riesling*). The evolution of tyrosinase activity depends on the climate conditions of the environment during the maturation period of white grapes, especially on the sum of rainfalls in the studied period of time (2008), which determined variations in the physiologic development of the grapes.

As it can be noticed in Figure 4 at the beginning of the ripening period the *Chardonnay* variety had the highest tyrosinase activity (40 UA), followed by the *Muscat Ottonel* variety (37 UA), *Fetească regală* (35 UA), *Riesling italian* (30 UA) and *Sauvignon* (23 UA). The white grapes presented a tyrosinase activity which varied within limits set quite apart depending on the variety. The *Italian Riesling* variety was remarked during the grapes maturation (September 20th, 2008) as having a maximum activity (64 UA) and the *Fetească regală* variety with minimum activity (11.4 UA).

At full maturity phase the white grapes evolve a tyrosinase activity which varied according to the variety, being remarked the *Chardonnay* variety with a maximum activity (28 UA), followed by the *Fetească regală* variety (24 UA), *Riesling italian* (20 UA), *Muscat Ottonel* (16 UA) and *Sauvignon* with a minimum activity (12 UA).

At the harvesting moment close values of the tyrosinase activity were found for the white grape varieties studied.

The activity of the tyrosinase is higher by 16.66- 58.92% at full maturity than at the harvesting moment. The tyrosinase activity for each variety varies for the entire period of the grapes ripening and differs from one variety to another according to the climate conditions of the vineyard and to the year of the harvest.

The tyrosinase activity was high at the beginning of the ripening process, followed by a decline during the full maturity and then the activity increased to a level which did not exceed the initial value but at the end it suddenly dropped, just before the moment of the harvesting (19-23.6 °Brix). The exception was observed for the grapes *Italian Riesling* variety where the tyrosinase activity exceeded initially.

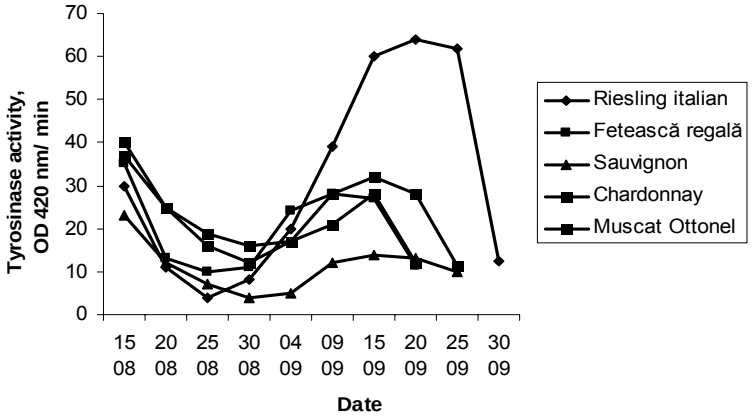

Figure 4. The dynamics of the tyrosinase activity during the maturation of white grapes in Dealu Mare vineyard (2008)

All these factors, combined with the atmospheric conditions, growth conditions and the pattern of individual development of a specific type of grape, help to explain the variation of the activity of the tyrosinase over time. The differences between the types of grapes were: the highest tyrosinase activity was recorded for the grapes of *Italian Riesling* and *Chardonnay* varieties and the lowest tyrosinase activity was quantified for the grapes of *Sauvignon* variety. Therefore, it is possible to distinguish between the varieties with high tyrosinase activity (*Italian Riesling*, *Chardonnay*) and varieties with low tyrosinase activity (*Sauvignon*). The activity of the tyrosinase is higher at full maturity than at the harvesting period, with 38% for the *Italian Riesling*, 52.50% the *Fetescă regală*, 16.66% the *Sauvignon*, 58.92% the *Chardonnay* and 25.00% the *Muscat Ottonel*.

The dynamics of the laccase activity during the maturation of the white grapes

The laccase activity depends on the contamination degree of the harvest with the *Botryotinia fuckeliana* grapes. The climate conditions of the year 2008 favored a contamination of the grapes with 10% of grey mould. From the experimental data (Figure 5) the profiles of the laccase activity evolution in the white grapes was noticed to be relatively similar. At the beginning of the ripening period, the grapes with the highest laccase activity (42 UA) were from the *Chardonnay* variety, followed by *Muscat Ottonel* (40 OD_{520} nm/min), the *Fetească regală* (36 UA), the I*talian Riesling* (31 UA) and the *Sauvignon* with the lowest laccase activity (27 UA).

At full maturity the *Muscat Ottonel* grape variety displayed the highest laccase activity (90 UA), followed by the *Chardonnay* grapes (77 UA), the *Italian Riesling* grapes (70 UA), the *Sauvignon* grapes (43 UA) and the *Fetească regală* grape variety with the lowest laccase activity (41 UA).

The values of laccase activity were determined at the moment of the white grapes harvesting and it presented the following levels: the *Chardonnay* grape variety with the highest laccase activity (20 UA), followed by the *Muscat Ottonel* grapes (19 UA), the *Sauvignon* grapes (16 UA), the *Fetească regală* grapes (15 UA) and the *Italian Riesling* grapes with the lowest laccase activity (13 UA).

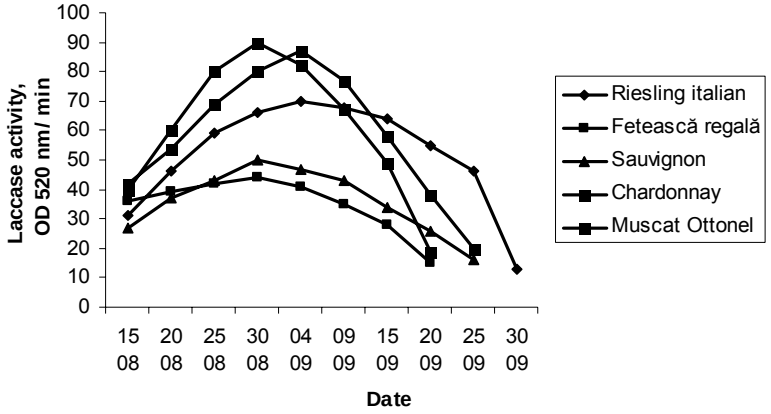

Figure 5. The dynamics of the laccase activity during the maturation of white grapes in Dealu Mare vineyard (2008)

The activity of the laccase is higher at full maturity than during the harvesting period by 81.42% for the *Italian Riesling* grape variety, 63.41% *Feteasc*ă *regal*ă variety, 62.79% *Sauvignon* variety, 74.02% *Chardonnay* variety and 78.88 % *Muscat Ottonel* variety.

Figures 4 and 5 show the activity of the laccase as being higher than the tyrosinase activity. This demonstrates that *Botryotinia fuckeliana* leads both to the secretion of laccase and also to the increase of the tyrosinase activity.

The dynamics of the peroxydase activity during the white grapes maturation

The dynamics of the peroxydase activity during the white grape ripening (Figure 6) within the studied period was similar for all varieties of white grapes studied. Starting on August 15th 2008 the activity of the peroxydase recorded increases up until the beginning of September after when a drop in the activity of the peroxydase was noted.

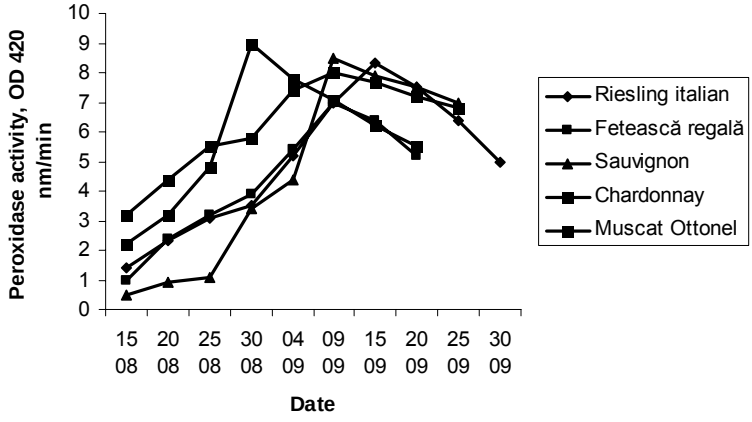

Figure 6. The dynamics of the peroxydase activity during the maturation of white grapes in Dealu Mare vineyard (2008)

Although the profiles of the enzyme activity evolution are approximately the same, the values of the peroxydase activity for the varieties of white wine differ. This can be explained by means of the climate conditions of the *Dealu Mare* vineyard, which caused variations on the physiological grapes evolution, but also by the different nature of the soil.

During the studied period the peroxydase activity was determined at the full maturity as being different from one variety to another. In case of the white grapes there were noticed: the *Muscat Ottonel* grapes variety with the highest value for the peroxydase activity (9.0 UA), followed by the

Sauvignon grapes variety (8.5 UA), the *Chardonnay* (8.0 UA) with average activity, the *Fetească regală* grapes variety (5.4 UA) and the *Italian Riesling* grapes variety with the lowest peroxydase activity (5.2 UA).

At the harvesting moment the following varieties were analyzed: the *Italian Riesling* with the lowest peroxydase activity (5.0 UA), the *Muscat Ottonel* with an average value (5.5 UA) and the *Sauvignon* with the highest value (7.0 UA).

The peroxydase activity is higher at full maturity than at the harvesting time with 3.84% for the *Italian Riesling*, 3.70% for the *Fetească regală*, 17.64% for the *Sauvignon*, 15.00% for the *Chardonnay* and 33.88% for the *Muscat Ottonel*.

4. Conclusions

During the ripening period the evolution of phisico-chemical parameters was almost the same for all varieties of grapes studied. The sugar content increased, the acidity decreased and the mass of 100 grape berries presented an increasing till the moment when the grapes were at fully maturity, and between the full maturity and the technological level of maturity of the grapes the mass index of the 100 grape berries decreased gradually.

The evolution of the oxidative enzymatic activities during the grape maturation varies depending on the variety of grapes, the harvest year and the climate conditions of the vineyard.

The activity of the laccase is higher than the tyrosinase activity. This proves that *Botryotinia fuckeliana* leads both to the secretion of laccase and also to an increased activity of the tyrosinase.

The peroxidase activity of the grapes during the maturation period is different for the varieties analysed in the present study, depending on the climate conditions of the harvest year and the physiologic state at the moment of the determination.

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