JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2010, *16* (1), 13-18

Journal of Agroalimentary Processes and Technologies

The effect of noble mould (*Botryotinia fuckeliana*) contamination on the dynamics of the enzymatic oxidation

Carmen Popescu[a*], Elena Postolache[b], Aurel Ciubucă[b], Gabriela Râpeanu[c], Traian Hopulele[c]

[a] *Elena Doamna Food Industry College, Galaţi, Str. Domnească, nr. 169, 800189, Galaţi, România*
[b] *TheBujoru Research-Development Station for Viticulture and Wine-making, Str. G-ral E. Grigorescu 65, 805200, Galaţi, România*
[c] *Lower Danube University Galaţi, the Faculty of Food Science and Engineering, Str. Domnească nr. 111, 800201, Galaţi, România*

Received: 12 December 2009; Accepted: 15 Februar 2010

Abstract

During the winemaking it is known that the browning phenomenon has an enzymatic origin, the tyrosinase and peroxydase being the main oxidative enzymes. The laccase is mainly accumulated in the mouldy (botrytized) berries and only a small quantity is soluble in the must. It is little affected during the alcoholic fermentation and it is more stable in wine. The wines obtained from different harvests, especially from white grapes, are unstable, very susceptible to oxidative browning. The more intense cassation of the must and wine is when the mould and rot of the grapes are present. The aim of this study was to analyse the initial oxidative activity in the healthy grapes and mouldy ones with different degrees of contamination with the noble mould *Botryotinia fuckeliana*, from the fresh must, the must during the alcoholic fermentation and from the young wine after which the experimental variants were made. At the same time, it was studied the oxidative cassation of the wines obtained by processing healthy and mouldy grapes.

Keywords: Browning, cassation, laccase, noble mould *Botryotinia fuckeliana*, peroxydase, tyrosinase

1. Introduction

The capacity of enzymatic browning of a must or wine obtained from mouldy grapes contaminated with Botryotinia fuckeliana depends on: the variety of the vine, the climate conditions during the maturation period, the wine production technology, the degree of mould infestation and the type of mould (noble or grey) [6-8]. The visual estimation of the percentage of rotten berries, in rapport with the overall number of grapes, is the only method used in practice in order to appreciate the sanitary state of the vineyards. [4]. The negative effect of the laccase on the quality of the must is manifested from a certain degree of contamination, which varies according to variety and the specific spreading area of the vineyards. Under certain biotope conditions, the parasite has a beneficial effect, contributing to the increase of the quality features of the harvest. Due to the low atmospheric hygroscopicity and to the high concentration of sugars, an evaporation process occurs, followed by the shriveling of the berries. Thus, there takes place a process for which this form of pathogen agent contamination is known as "noble mould" [9-11].

The limited activity of the mould causes alterations in the grape which allows the winemakers to produce sweet white wines of high quality, which are liquorlike, oily, with a characteristic taste [2].

The predisposition to browning is more frequent in the wines obtained from damaged harvests and

Corresponding author: *e-mail:* popescucarmen_18@yahoo.com

especially in those made from grapes touched by the grey or noble mould (*Botryotinia fuckeliana*).

The aim of this study was to analyse the initial oxidative activity in the healthy grapes and mouldy ones with different degrees of contamination with the noble mould *Botryotinia fuckeliana*, from the fresh must, the must during the alcoholic fermentation and from the young wine after which the experimental variants were made. At the same time, it was studied the oxidative cassation of the wines obtained by processing healthy and mouldy grapes.

2. Materials and method

The research was done at The Bujoru Research-Development Station for Viticulture and Wine-making during the period of 2008-2009. The white grapes Riesling Italian variety were harvested at overmaturation. The grapes were sulphitated with 30 mg/kg of sulphur dioxide. The following variants of the healthy and mouldy grapes were made, with different degrees of contamination with the noble mould *Botryotinia fuckeliana*:

V1- healthy grapes - reference;
V2- grapes with 25% mould and healthy grapes 75%;
V3- grapes with 50% mould and healthy grapes 50%;
V4- grapes with 75% mould and healthy grapes 25%;
V5- mouldy grapes 100% .

The alcoholic fermentation was done at 17-21°C. During the alcoholic fermentation were collected daily samples for physico-chemical and enzymatic determinations. The dynamics of the oxidative enzymatic activity was quantified in the must, fermented must and young wine. The activity of the tyrosinase and laccase was quantified using the methods described by Dubernet et al. [3]. The activity of the peroxydase was evaluated by using the method described by Ciopraga et al. [1]. At the same time, the index of the polyphenoloxydase (IPFO) and the browning index (IB) were calculated by the method described by Ioniță et al. [5]. The musts and wines obtained were analysed for the content of total polyphenols by means of the reaction using the Folin-Ciocâlteau reagent and were expressed as g/l of gallic acid. In order to analyse the musts and wines the oficial methods were used (OIV). All the determinations were done three times and the standard relative deviations were lower than ± 1%.

The predisposition of centrifuged wines to oxidative cassation in laboratory conditions was tested as follows: the optical density of the white wines was measured when the samples were exposed to air and light, for 0, 24, 48 and 72 hours. There was observed the modification of the colour of wines, by the increase of the OD value as a result of the browning (oxidative cassation).

3. Results and Discussion

The relationship between the degree of noble mould contamination of the grapes and the activity of the oxidizing enzymes of the fresh must

In figure 1 there is presented the noble mould Botryotinia fuckeliana which contaminated the Italian Riesling berries.

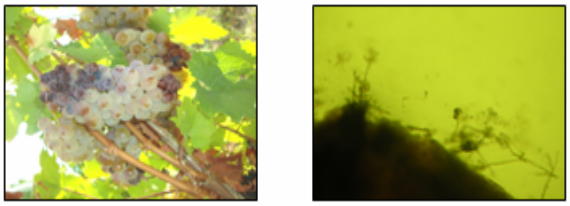

Figure 1 *Italian Riesling* mouldy with the *Botryotinia fuckeliana* noble mould

The physico-chemical characteristics of the grapes were the following: the reducing sugar content of the grapes varied between 220-270g/l, the total acidity between 3.2-2.8 g/l H_2SO_4, the total polyphenols content varied between 0.136-0.273 g/l gallic acid and the colour of the must ($DO_{420\ nm}$) varied between 0.193-0.605 (table 1, figure 2). The quantity of total polyphenols (expressed as g/l gallic acid) decreassed by 32.6-50.1% for the V_2-V_5 variants compared to the reference sample (V_1) at the same time with the increase of the degree of noble mould contamination of the Riesling Italian grapes. This happened due to the damaging of the berry skin at the same time with the increase of the degree of noble mould contamination. The colour of the must increased with the increase of the degree of noble mould contamination of the grapes.

As can be noticed from table 2 and figure 3, the increase of the degree of mould contamination of the must (V_2-V_5) led to the increase of the enzymatic activity in the must compared to the reference sample (V_1). There was noticed an increase of both indices (IPFO, IB) at the same time with the increase of the degree of mould contamination of the grapes.

Table 1. The physico-chemical characteristics of the Italian Riesling must

Variant	Sugar, g/l	Total acidity, g/l H_2SO_4	Total polyphenols, g/l acid galic	$DO_{420\ nm}$
V_1	220	2.4	0.273	0.193
V_2	238	2.3	0.200	0.315
V_3	250	2.5	0.184	0.457
V_4	261	2.6	0.152	0.528
V_5	270	2.8	0136	0.605

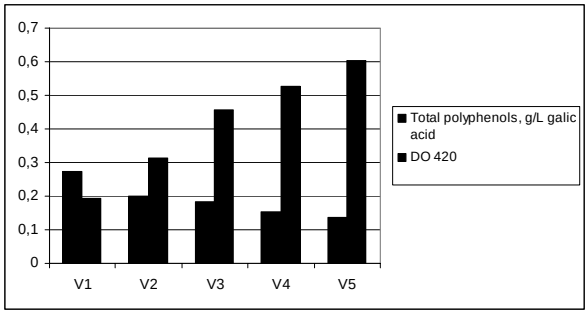

Figure 2 The physico chemical characteristics of the must

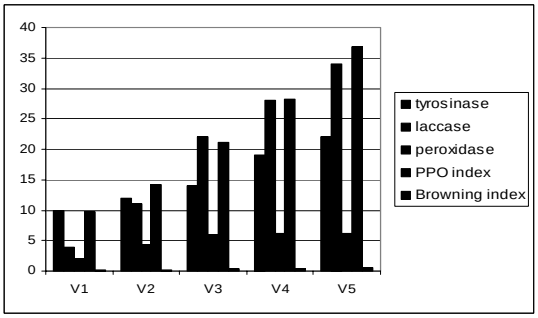

Figure 3 The enzymatic activity of the must

Table 2. The oxidizing enzymatic activity of the *Riesling italian* grapes must

Variant	The activity of the tyrosinase, DO_{420}nm/min	The activity of the laccase, DO_{520}nm/min	The activity of the peroxidase, DO_{420}nm/min	Index of polyphenoloxidase (IPFO)	Browning index (IB)
V_1	10	4	2.06	9.7	0.11
V_2	12	11	4.33	14.2	0.22
V_3	14	22	5.96	21.1	0.35
V_4	19	28	6.10	28.2	0.39
V_5	22	34	6.26	36.8	0.56

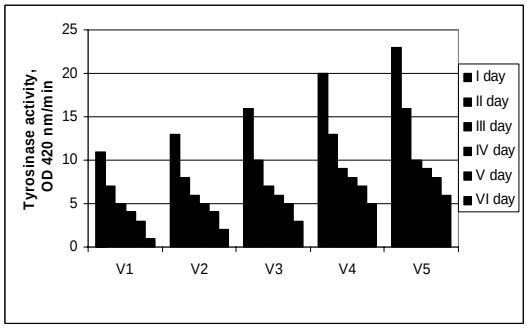

Figure 4 The dynamics of the enzymatic activity of the tyrosinase during the alcoholic fermentation

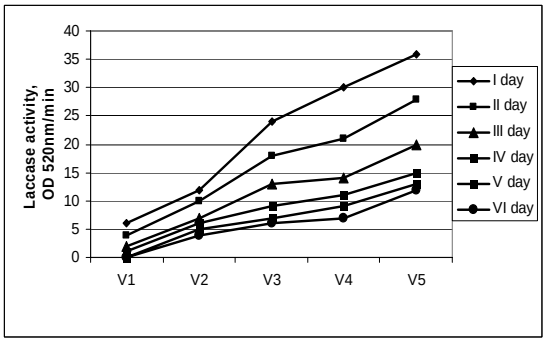

Figure 5. The dynamics of the enzymatic activity of the laccase during the alcoholic fermentation

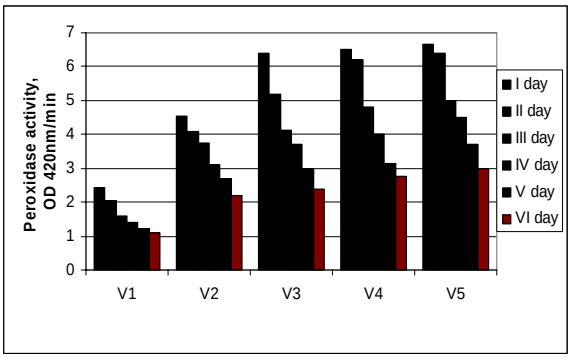

Figure 6. The dynamics of the enzymatic activity of the peroxidase during the alcoholic fermentation

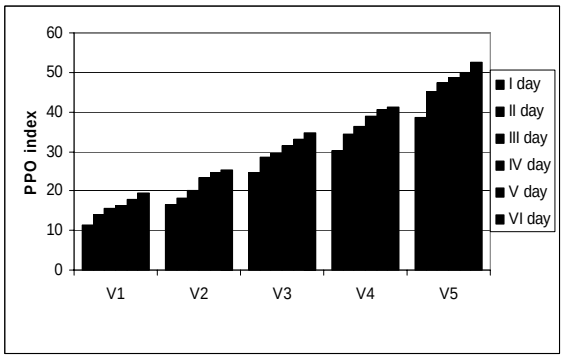

Figure 8. The evolution of the browning index during the alcoholic fermentation

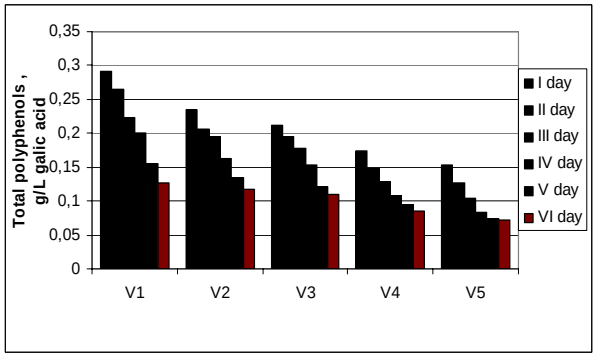

Figure 9. The evolution of the total polyphenols content of the must during the alcoholic fermentation

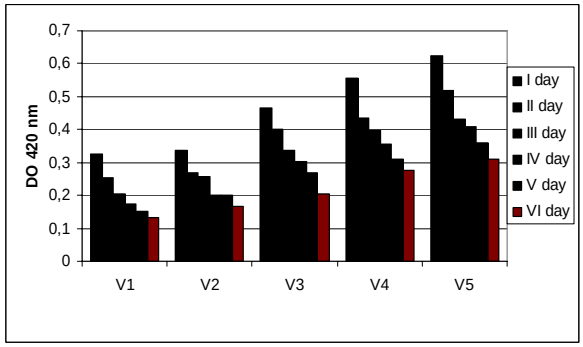

Figure 10. The evolution of the must colour during the alcoholic fermentation

The relationship between the degree of noble mould contamination of the grapes and the oxidative enzymes activity of the grapes must during the alcoholic fermentation

The oxidizing enzymatic activity in the grapes must was assessed during the alcoholic fermentation (after six days of fermentation) in the experimental variants. During the alcoholic fermentation the enzymatic activity of the laccase, tyrosinase and peroxydase gradually decreased by 66.6-76.6%, 73.9-90.9% and by 51.4-62.5% respectively (figures 4,5,6).

During the six days of alcoholic fermentation there was noticed an increase in the activity of the oxidizing enzymes at the same time with the increase of the degree of noble mould contamination of the grapes. The evolutions of the PFO (IPFO) index and of the browning index (IB) are described in figures 7 and 8. During the alcoholic fermentation the polyphenoloxydase index and the browning index increased by 35.7-70.4% and 50-100%, respectively. The browning index increased by 63.6- 79.1% on the fifth and sixth days of the alcoholic fermentation for the V_5 variant in comparison with the reference) V_1 variant. Both indices (IPFO and IB) increased during the alcoholic fermentation at the same time with the increase of the noble mould contamination of the grapes.

During the alcoholic fermentation, the total polyphenols content (g/l gallic acid) decressed by 45.9-51.9%. For the V_2, V_3, V_4, V_5 the total polyphenol content of the must decreased by 43.6-58.5%, compared to the reference (V_1) variant, at the same time with the increase of the noble mould contamination of the grapes (figure 9). During the alcoholic fermentation, the colour of the must decreased by 50.4-59.3%. On the first day of the alcoholic fermentation the intensity of the must colour increased by 90.8% for the V_2-V_5 variants compared to the V_1 reference variant, at the same time with the increase of the degree of noble mould contamination of the grapes (figure 10).

The relationship between the degree of noble mould contamination of the grapes and the oxidizing enzymatic activity of the young wine

There were studied the physico-chemical characteristics and the oxidative enzymatic activity of the young wine. The alcohol degree in young wines was specific to each variety of grapes depending on

the quantity of sugar accumulated in the grapes. It was of 12.7% (v/v) alcohol for the reference variant (V_1) and of 15,6% (v/v) alcohol for the V_5 variant. The total acidity was situated within normal limits (3,6-4,1g/l H_2SO_4). The volatile acidity was situated between 0.33-0.60 g/l acetic acid. The content in total extract was between 21.6-26.0 g/l. The SO_2 was of 1.2-2.5g/l (free SO_2) and 2.5-5.1g/l (total SO_2).The oxidative enzymatic activity was lower visibly in wines than in must and it increased at the same time with the increase of the degree of noble mould contamination of the grapes. The activity of the tyrosinase and laccase in the young wine decreassed by 56.5-92.3% and 72.2-80% respectively, compared to the must on its first day of alcoholic fermentation. The activity of the tyrosinase and laccase was not detected in the reference variant (V_1). The activity of the peroxidase in the young wine decreassed by 80.7-92% compared to the must on its first day of alcoholic fermentation. The polyphenoloxydase index decreassed by 6-13.4% and the browning index increased by 29.7-87.5% compared to the must on its first day of alcoholic fermentation. The browning index increased by 60% in the V_5 comparatively to the reference variant (V_1). The total polyphenols content (g/l gallic acid) and the colour in the young wine decreased by 47.1-55.4% and 49.5-57.1%, respectively, compared to the must in its first day of alcoholic fermentation. The total polyphenols content decreased by 42.3% in the V_5 variant comparatively with the reference variant (V_1).

The wine oxidative cassation capacity The predisposition of the young wine to oxidative cassation was tested in lab conditions by measuring the optical density by exposing the samples to air and light for 24, 48 and 72 h. There was observed the wine colour change, by the increasing DO value as a consequence of the browning (oxidative cassation). Firstly, there was noticed an oxidation and afterwards an increase of the intensity of the colours of wines (browning) for 3 days at ambient temperature at the same time with the increase of the degree of noble mould contamination of the grapes. The intensity of the colour of the wine after the 3rd storage day increased for the V_5 variant by 57.8% compared to the reference variant (V_1) (figure 11). The wines from the V_5 variant (which have the highest DO $_{420nm}$ values) were the ones most exposed to the oxidative cassation.

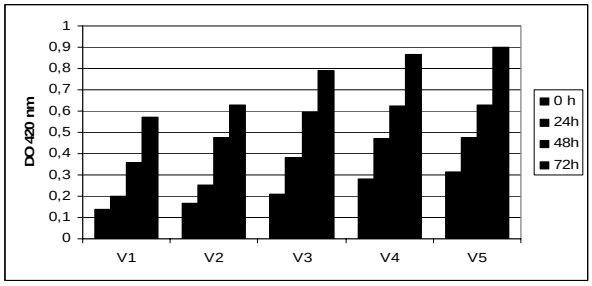

Figure 11. The wine colour depending on the storage time

4. Conclusions

1. The activity of the laccase responds to three conditions: specificity (the absence in the healthy grapes and the presence in the mouldy grapes); solubility (the easy passage into the must during extraction) and stability (it maintains its activity long enough causing the wine cassation).
2. The activity of the oxidative enzymes and of the physico-chemical characteristics of the must during the alcoholic fermentation and those of the young wine increased at the same time with the degree of noble mould contamination of the grapes.
3. The activity of the oxidative enzymes decreased on the sixth day of fermentation compared to the first day of the alcoholic fermentation by 73.9-90.9% (tyrosinase), 66.6-76.6% (laccase) and by 51.4-62.5% (peroxidase). The activity of the laccase could not be detected in the reference sample (V_1) after the fifth day of the alcoholic fermentation.
4. The activity of the oxidative enzymes decreased in the young wine by 56.5-92.3% (tyrosinase), 72.2-80% (laccase) and by 80.7-92% (peroxidase) comparatively to the must in its first day of fermentation. In the reference sample (V_1) of young wine there was not detected the activity of the tyrosinase and laccase.
5. There was noticed the oxidation and the increase of the intensity of the colour of the wines (browning) for three days of storage at ambient temperature at the same time with the increase of the degree of noble mould contamination of the grapes.
6. The biologic factors (noble mould, the oxidative cassation capacity of wines, nature and the concentration of the substrates) influenced the oxidazic activity of the grapes, musts and wines.

5. References

1. Ciopraga J; Niculescu S., Marinescu M., New method for the determination of peroxidase activity, *Rev. Roumaine de Biochimie*, **1978**, 15(4), 259-283;
2. Dharmadhikari, M., Botrytis cinerea in Winemaking, Iowa State University, Accessed **2007** Extension, http://www.extension.iastate.edu /NR/donlyres/173729E4-C734-486A-AD1678678 B3E 1CF /56372/botrytiscinerea1.pdf;
3. Dubernet, M., *Thése Doctorat* 3 cycle, Bourdeaux, **1974**.
4. Dubourdieu D., Grassin Catherine, Deruche Catherine, Mise au point d'une mesure rapide de l'activite laccase dans les mouts et dans les vins par la methode a la syringaldazine. Application a l'appreciation de l'etat sanitaire des vendanges, *Connassance Vigne Vin*, **1984**, 18(4), 237-252;
5. Ioniță, V., The study of the activity of the laccase in the grapes infested by *Botrytis cinerea* and the oxidasic cassation aptitude of the must and of the wine in theproduction technologyof the white wines. *Analele ICVV Valea Calugareasca*, **1998**, 15, 407-417.
6. Răpeanu, G. The biochemical characterization and the kinetic study of the inactivation at high temperature and/or pressure of the polyphenoloxydase from white grapes, **2005**, Thesis Doctorat, Universitatea „Dunărea de Jos din Galați".
7. Franc Čuš, Helena Baša Česnik, Špela Velikonja Bolta, Ana Gregorčič, Pesticide residues and microbiological quality of bottled wines, *Food Control*, **2010**, 21(2), 150-154, DOI:10.1016 /j.foodcont.2009.04.010;
8. Philip A. G. Elmer, Themis J. Michailides, *Epidemiology of Botrytis cinerea in Orchard and Vine Crops* In: *Botrytis: Biology, Pathology and Control,* Springer Netherlands, 2004, 243-272, ISBN: 978-1-4020-2624-9 (Print) 978-1-4020-2626-3 (Online); DOI:10.1007/978-1-4020-2626-3;
9. Jacometti, Marco Alexander Azon, Enhancing ecosystem services in vineyards to improve the management of *Botrytis cinerea*, Thesis Doctorat, Lincoln University, 2007, http://researcharchive. lincoln.ac.nz/dspace/handle/10182/27;
10. Luca Cocolin, Ashley Heisey, David A. Mills, Direct Identification of the Indigenous Yeasts in Commercial Wine Fermentations, *Am. J. Enol. Vitic*. **2001**, 52(1), 49-53;
11. Hans-Juergen Rosslenbroich, Dietrich Stuebler, Botrytis cinerea — history of chemical control and novel fungicides for its management, *Crop Protection*, **2000**, 19(8-10), 557-561, DOI:10.1016/ S0261-2194(00)00072-7.