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The use of commercial enzymes in white grape must clarification

Nicolae Laurenţiu Itu[1*], Gabriela Râpeanu[2]

[1]*S.C. Vinex Murfatlar S.R.L., 1 Cochirleni Str., Cernavodă, Romania*
[2]*Dunarea de Jos University, 111 Domneasca Street, 800201Galati, Romania*

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Abstract

Two enzyme preparations were used in order to clarify the must obtained from white grapes, Muscat Ottonel variety. Clarification process of grape must was influenced by several factors such as must fraction (free run must and pressing must), type of enzyme preparations used (grapes maceration enzymes and clarification enzymes), the way of adding enzyme preparation and enzyme dosage used. Clarification of must can be achieved both through use of maceration enzymes and clarification those, but the dynamics of this process was different.

Keywords: white grapes, enzymes, must clarification

1. Introduction

Flavonoids The use of biotechnology in winemaking and grape processing has extended beyond the concept of yeast and malolactic fermentation technology.

Enzymes are biological catalysers very much used in the food industry and particularly in the oenological sector. Fundamentally, they are used to clarify and filter the must and wine, increasing their physical–chemical stability and strengthening the aromatic profile or colour of wines produced from certain varieties of grape [1].

The main enzymes used during winemaking are pectinases. Pectinases occur naturally in all fruit (including grapes) and are partly responsible for the ripening process. Grape pectinases are however inactive under the pH and SO_2 conditions associated with winemaking [2]. Fungal pectinases are resistant to these winemaking conditions. The method used to produce wine enzymes for use in the European Union is regulated by the OIV.

The OIV has established that only *Aspergillus niger* and *Trichoderma* can be used for enzyme production [3]. Currently, a combination of pectinases (pectin lyase, pectin methylesterase, endo and exo-polygalacturonases, pectin acetylesterase, rhamnogalacturonase, endo- and exo-arabinases), cellulases (endoglucanases, exoglucanases and cellobiases) and hemicellulases (endo- and exo-xylanases, galactanases, xyloglucanases and mannanases) - collectively called macerating enzymes - are used in the extraction and clarification of fruit and vegetable juices [4,5].

Utilisation of pectolytic enzymes for must clarification represents a simple, economic and technological method with positive effects on must filterability, stabilization treatments and quality of final wine. Naturally, pectic substances are found in any wine, and their role is to prevent wine clarification due to their colloidal character. But they can be enzymatic hydrolysated with pectolytic enzyme preparations [6].

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

The pectic enzymes play an important role in braking down grape pulp and skin cells and are able to split those chains and saccharide bonds between the chains [7].

The purpose of this study was to optimize the must clarification by enzyme preparations and to study the influence of several factors on the process of must clarification.

2. Materials and methods

Research followed the dynamics of the must clarification by using enzyme preparations. Also the influence of several factors such as must fraction (free run must and pressing must), type of enzyme preparations used (grapes maceration enzymes and clarification enzymes), the way of adding enzyme preparation and enzyme dosage used were studied.
Enzyme preparations used were:

- Maceration enzymes are pectolytic enzymes from poly-galacturonases category, cellulases and hemicelullases. Enzymes were added to the grapes in the receiving hopper of destemming-crusher.
- Clarification enzymes are pectolytic enzymes from poly-galacturonases category, pectin methyl-esterases and pectinlyases. Enzymes were added directly on grape must.

Since it was found that sediment particles in suspension, is not always directly proportional to the clearness of must, the dynamics parameters were evaluated: sedimentation rate of particles measured by the amount of sediment, % in time; and clarification rate determined by OD at 420 nm variation in time, by using 1 cm cuvette.

Enzyme preparations used for maceration was Lallzyme Cuvee Blanc (2g/100kg grapes) and for clarification was Lallzyme HC (1 g/hl).

The following variants were studied:

- V1 free run must clarified by static sedimentation;
- V2 free run must clarified by macerating enzymes Lallzyme Cuvee Blanc (2g/100kg grapes) addition;
- V3 pressing must clarified by static sedimentation;
- V4 pressing must clarified by macerating enzymes Lallzyme Cuvee Blanc (2g/100kg grapes) addition;
- V5 must (free run + pressing) clarified by static edimentation;
- V6 must (free run + pressing) clarified by clearing enzymes Lallzyme HC (1 g/hl);
- V7 must (free run + pressing) clarified by macerating enzymes Lallzyme Cuvee Blanc (2g/100kg grapes) added in mash;

3. Results and discussions

3.1. Effect of maceration enzymes on sedimentation and clarification processes - Studying the dynamics of must clarification process was observed that it is no a direct proportionality between the sedimentation rate and clarification rate of particles in suspension. In addition, this behavior differs for free run must and pressing must.

In the case of free run must because the mechanical particles in suspension are high they are involved in must separation. The deposit occurs very quickly, but more due to mechanical than the colloidal particles, which are in small quantities (Fig. 1).

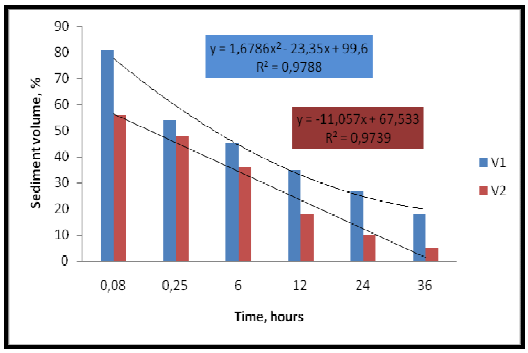

Figure 1. Dynamics of sedimentation process free run must treated with maceration enzymes

In addition, enzymatic hydrolysis of pectines found in this must fraction, under the action of endogenous pectinases, takes very long time [8, 9].

Formation of sediment in the case free run must - control variant (V1), does not significantly improve the must clearness, which continues to remain opal OD_{420nm} = 0.320 (fig. 2).

Not the same situation happens with wine obtained from grapes treated with enzyme preparation (variant V2). Exogenous pectinases begin fast their action, so that the enzymatic and electrostatic phases of sedimentation are much shortened. In this situation, the number of particles that can sediment at a given time is greater. Therefore, the changes in sediment volume for the enzymatic treated must is higher, being accompanied by a very good limpidity (DO_{420nm} = 0.124).

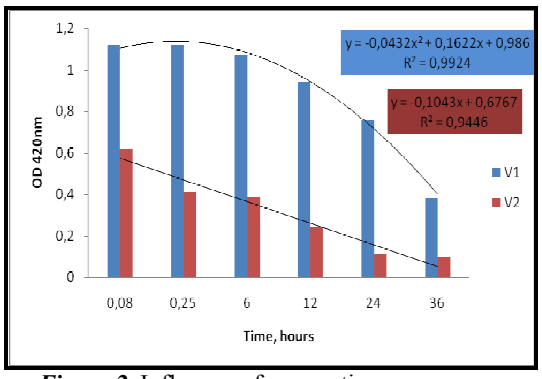

Figure 2. Influence of maceration enzymes on clarification process of free run must

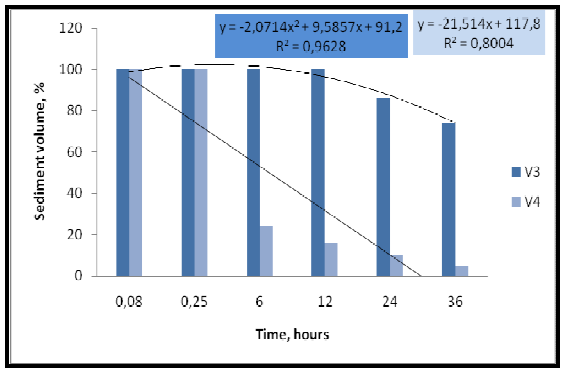

Figure 3. Influence of maceration enzymes on sedimentation process of pressing must

For the pressing must because the mechanical particles in suspension is low, they are mostly retained in the pressed pulp, which acts as a filter medium. The particles responsible for must opalescence are colloidal compounds.

The Figure 3 shows that on the first 12 hours for the control must (variant V3) it is any occurrence of sediment, no significant change of must limpidity was observed, the must remains very opal.

During the next 12 hours the presence of deposit (1.2%) was observed, due to mechanical particles which succeed to deposit without being accompanied by a must clarification. The must is being still opal. Over the next 12 hours, the sediment thickness increases due to the deposit of new particles, and the must clarity is improved. The final opalescence of this must was DO_{420nm} = 0.512, and remains constant until the start of the must fermentation.

The pressing must enzymatically treated (variant V4), has a remarkable evolution. The quantity of pectic substances is higher in pressing must then the free run must. Therefore pectolytic enzymes have the substrate on which to act in this way destabilizing the colloidal complex responsible for the opalescence and for the high suspension quantity of this must fraction.

Although no exogenous pectolytic action is noted from the first minutes (as in case of free run must), the sediment formation is visible. Its volume decrease is accompanied by the increasing of the must limpidity. Thus, after 12 hours, the volume of sediment is constant and is practically the final clearness DO_{420nm} = 0.128 (Fig. 4).

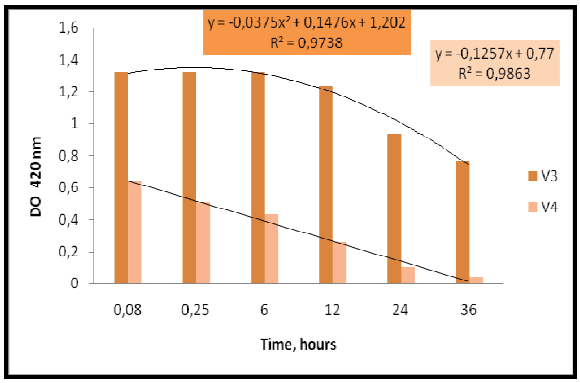

Figure 4. Influence of maceration enzymes on clarification process of pressing must

It is noted that the utilization of enzymatic preparation lead to the same period of clearing for the free run must and pressing must. The effect of clearing is very high and is always higher to reference variant. The resulted sediment is always compact and well differentiated from the liquid fraction. Deposit volume depends on the dosage of enzymes used and winemaking technology.

3.2. Effect of clearing enzymes on sedimentation and clarification processes - To study the development of sedimentation and clarification by the action of clearing enzyme preparations, the research was done on the mixture of free run must and pressing must. A dosage of 1 g/hl of the enzyme preparation Lallyzym HC was used. The reference variant (V5) present a non significant sedimentation after 2 hours, with a clearing effect total unsatisfactory (Fig. 5).

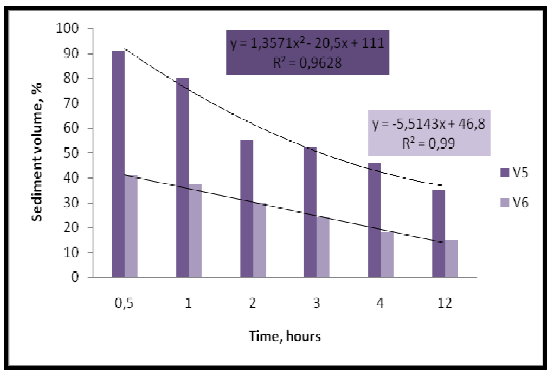

Figure 5. Influence of clarification enzymes on must sedimentation

Considering the end of the settling when the volume of sediment is constant, in this case after 12 hours (Fig. 6), the must clearness (DO_{420nm} = 0.665), is significantly lower to the variant treated with enzyme preparation (DO_{420nm} = 0.229).

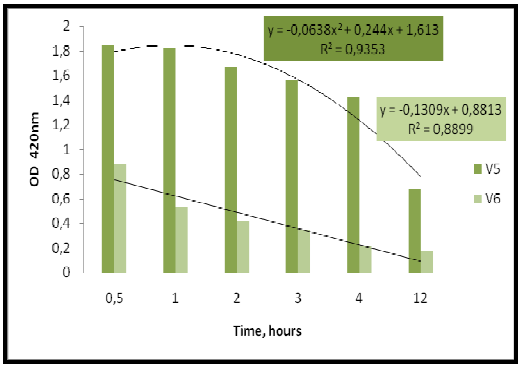

Figure 6. Effect of clarification enzymes on must clarification

For the variant enzymatically treated a direct correlation between sedimentation rate and clearing rate was observed. After 4 hours the clarification process of must treated by exogenous pectolytic preparation can be finished.

3.3. Influence of the moment of enzymes addition on the must clarification - Clarification of must can be achieved both through use of maceration enzymes and clarification those, but the dynamics of this process is different, because the moment when enzymes are added and the specific composition of these enzyme preparations.

Since enzymes are added to the mash maceration or even on grapes, the duration of contact between enzyme and substrate is higher than are using the clearing enzymes, because those have to be added directly on the must.

When the maceration enzymes are used, the stage of enzymatic pectin hydrolysis is conducted during grapes crushing (when enzymes are added to the grapes), and during the must separation and pressing (when enzymes are added to mash). During the settling phase occurs only flocculation and sedimentation of particles in suspension. Figure 7 shows that already after 30 minutes of settling in the case of the maceration enzymes use (variant V7) the sediment volume decreased to 40%. At the same time, by using the clearing enzymes (variant V6) the sediment volume decreased to 80%, which suggests that enzymatic hydrolysis of pectin phase was not completed.

By using the maceration enzymes after a period of 3 hours, the must sedimentation and clarification processes can be ended. In the situation of using treatment with clearing enzymes, that happens only after a period of 12 hours.

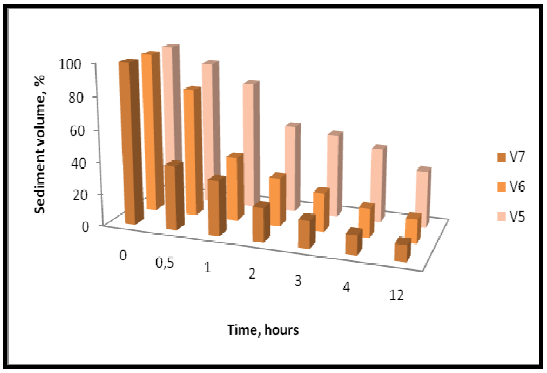

Figure 7. Influence of enzymes addition moment on the sedimentation of must

But, after this period of 12 hours the limpidity of must for the variant V6 (DO_{420nm} = 0.129), is more advanced than the variant V7 (DO_{420nm} = 0.160), which is rich in compounds that contribute to opalescence but also to the extract of this must fraction (Fig. 8).

This is possible because enzymatic preparations contain extraction enzymes (pectolytic enzymes and cellulases, hemicellulases, etc.) of the components from the berry in the must.

3.4. Influence of enzyme dosage on the dynamics of must clarification - Maceration or clearing enzymes are used in doses ranging between 1 and 4 g/100 kg hl grapes or wine, depending on the nature and characteristics of the enzyme preparation.

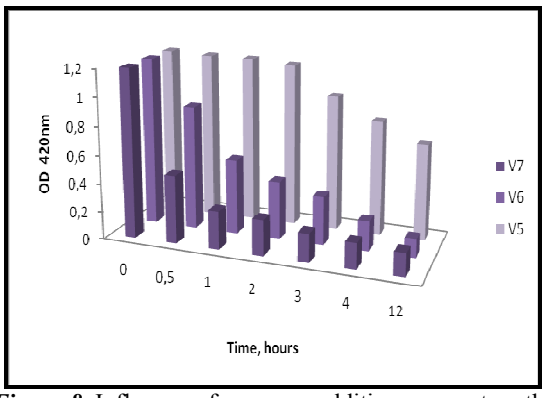

Figura 8. Influence of enzymes addition moment on the clarification of must

Thus by increasing the dose of enzymes, the sedimentation and clarification rate are increased (Fig. 9), because the same content of pectic substances (the substrate) is hydrolyzed by a large dose of enzymes.

However, by increasing the dose above the maximum limit indicated by 1g/hl, the effect remains constant because the existing substrate is covered by the equivalent amount of enzyme, and there is not present another substrate that can be hydrolyzed by the excess of enzyme added.

The enzyme dosage choosing should be judiciously determined by several factors like the type of enzyme preparation, content of grapes and must in pectic substances and the clearing effect desired.

The use of large doses of maceration enzymes will lead to maceration of the grapes too advanced and higher volume of deposits.

The use of large doses of clearing enzymes may lead to clarification of the must too advanced, with technological consequences and also with compositional consequences of the resulting wine.

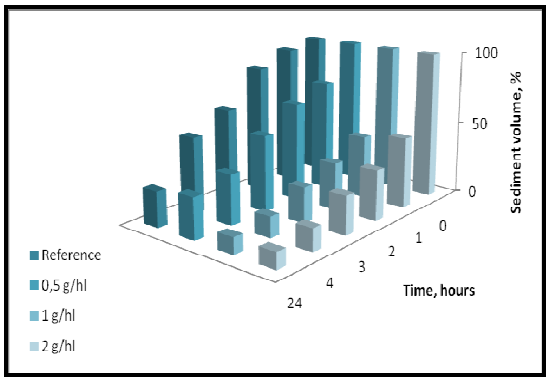

Figure 9. Influence of enzyme dose on the must clarification

4. Conclusions

Clarification process of must is influenced by several factors such as the nature and quantity of colloidal compounds of must, which depends on grape variety, degree of maturity and wine-making technology used, and the enzyme preparations and dosages used.

The must made from pectin rich grape varieties, especially those obtained from the continuous presses contain large quantities of colloidal pectic compounds, which influence the must clarification by classical methods. Preparations containing large amounts pectinlyases and polygalacturonases conduct to a faster degradation of pectic substances.

Maceration enzymes realized an advanced extraction of structural components of the berry in the must. By adding those directly to grapes allow an increased contact time between enzyme and substrate and thus an increasing in sedimentation and clarification rates.

By increasing the dose of enzymes, the sedimentation rate and the degree of clarification increase. An advanced clarification of must is not desired because of late entry into the alcoholic fermentation.

Establishing the moment when to use the enzyme preparations and doses required for must clarification will be made taking into account: the type of enzyme preparation (maceration or clarification) and the purpose (the must clarification must when is recommended clearing enzymes or improving of must clarification and the quality of resulted wine, in which case the maceration enzymes is recommended).

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