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**ORIGINAL RESEARCH PAPER**

# EFFECT OF MACERATION ENZYMES ADDITION ON THE AROMATIC WHITE WINEMAKING

NICOLAE LAURENȚIU ITU
*S.C. Vinex Murfatlar S.R.L., 1 Cochirleni Str., Cernavodă, Romania, niculaur_itu@yahoo.com*

GABRIELA RÂPEANU, TRAIAN HOPULELE
*Dunarea de Jos University, 111 Domneasca Street, 800201Galati, Romania*
*gabriela.rapeanu@ugal.ro, traian.hopulele@ugal.ro*

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White grapes of Muscat Ottonel variety were treated enzymatically with maceration enzyme preparation and then fermented with selected yeast. These treatments resulted in an increase of the extraction of the compounds involved in wine aroma and of the must yield. Also an improvement of filterability and a decreasing of the sedimentation and clarification time were noticed. The enzymatic treatment presented a benefic effect on the dynamics of the alcoholic fermentation and the final wine composition. By using maceration enzymes, the wine aromatic intensity is emphasized, because the enzymatic preparation contains high concentrations of both glycosidase acting on the first stage of the enzymatic mechanism and the β-glucosidase acting on the second phase of enzyme mechanism able to release the aromatic constituents. These results are also closely related to the sensory analysis, which indicated that the glycosidase enzymatic treatment seemed to be effective for the improvement of the aroma of Muscat Ottonel wines.

**Keywords:** aromatic wines, enzymes, terpenes

## Introduction

Wine aroma is due to a lot of volatile compounds with different chemical natures and origins, found at a wide range of concentrations.

Some grape varieties have the ability to synthesize and accumulate in the skins aromatic substances such as terpenols (linalool, limonene, terpineol etc.), which confers a specific final wine aroma. The typical flavour of Muscat wines is mainly due to aromatic compounds coming from grapes. Grape aroma composition and its influence on the aroma of wines have been reviewed by many authors (Flanzy, 2000).

In general, aroma precursors are located mostly in the grape skins (Sanchez Palomo *et al.,* 2006), so the must skin contact technique has been proposed to increase the aroma of white wines and enhance the varietal character (Cabaroglu *et al.,* 2002, Romero-Cascales *et al.,* 2008).

Volatile compounds from glycosides can be released by acid or enzyme hydrolysis, thus enhancing the aromatic profile of wines. Acid hydrolysis occurs very slowly during wine storage or can be accelerated by heat induction (Gunata *et al.*, 1990a,b), but both processes can induce a deterioration in wine quality.

The effect of skin maceration on wines depends on the grape variety and the skin contact conditions, such as time, temperature and equipment used (Falque and Fernandez, 1996).

Enzymatic hydrolysis, due to grape or yeast glycosidases, is very limited, since these enzymes present low activity under fermentation conditions (Fernández-González *et al.,* 2003). Enzymes from *Aspergillus niger* are widely used in wine-making, largely because their pectinolytic activity is useful for must clarification and colour extraction. However, some of these enzymes possess considerable glycosidase activity (Gunata *et al.,* 1995).

Moreover, changes taking place due to enzyme treatment may differ from one wine to another depending on the chemical composition of the grapes and on the wine-making techniques used.

The aim of this study done under microvinification conditions, was to evaluate the main effects of the use of maceration enzymes to improve the degree of extraction of aromas precursors, to increase the must yield and also to increase the must filtrability and clarification, effect on the dynamics of alcoholic fermentation and final wine composition.

## Materials and methods

Experiments were done on Muscat Ottonel grapes obtained in Murfatlar vineyard in climate conditions of 2009, by using pectolytic enzymes (pectinases category, Lallzyme Cuvee Blanc, Lallemand) and selected *Saccharomyces cerevisiae* yeast sp. (Lallvin QA-23, Lallemand). Lallzyme Cuvee Blanc was applied on grapes before pellicular maceration (2 g/100 kg of grapes).

The following variants were studied:

- V1 free run must clarified by static sedimentation and fermented spontaneously by epiphytic microflora;
- V2 pressing must clarified by static sedimentation and fermented spontaneously by epiphytic microflora;
- V3 free run must treated with macerating enzymes and fermented spontaneously by epiphytic microflora;
- V4 pressing must treated with macerating enzymes and fermented spontaneously by epiphytic microflora;

- V5 free run must treated with macerating enzymes and fermented with selected yeasts;
- V6 pressing must treated with macerating enzymes and fermented with selected yeasts maceration.

***Free and bond terpenes determination***

*Sample preparation*

Fresh grapes samples were frozen immediately after being picked. Prior to analysis, the grapes were thawed. To analyse the distribution of free terpenes in the skin, pulp and juice, berries from the variety Muscat Ottonel were hand peeled and the seeds removed from the pulp. The pulp was homogenised and filtrated through cheesecloth to obtain clear juice. The weighed pulp and the skins were homogenised separately in about 200 ml phosphate buffer (*p*H 7.0) saturated with NaCl. After the skin and the pulp extracts were filtrated, the *p*H of filtrates were adjusted to *p*H 6.6–6.8 with 20 % w/v solution of NaOH.

*Isolation of monoterpenes*

The rapid distillation analytical method described by Dimitriadis and Williams, 1984 and Šapceska *et al.,* 2006 was used to determine the free volatile terpenes, as well as those released from their glycosidically bound forms by acid hydrolysis in the grape juice obtained. A sample of 100 ml grape juice was steam-distilled until 25 ml distillate was recovered. This distillate was used for determination of the content of free volatile terpenes (FVT). Without interrupting the steam flow, the juice was acidified with 5 ml of 20% (v/v) $H_3PO_4$. The distillation continued until the next 40 ml distillate was collected. This distillate contained the potentially volatile monoterpenes (PVT), derived from the polyols and glycosidically bound forms.

*Colorimetric determination of monoterpenes*

A volume of 10 ml of each distillate (FVT or PVT) was individually shaken and pipetted into Eppendorf tubes. A blank sample was prepared with 10 ml of water. A volume of 5 ml of 2 % vanilin-$H_2SO_4$ reagent was added to each precooled tube. The contents were agitated with further cooling in an ice bath. The colour was developed by heating the tubes in water bath at 60°C for 20 minutes. The tubes were than cooled at 25°C for 5 minutes and in maximum 20 minutes, the optical densities were read at 608 nm using 1 cm plastic cuvettes. The sample distillates, in reaction with the vanillin sulphuric reagents, form a complex with a blue-green colour, with intensity proportional to the content of monoterpenes. The contents of terpenes in the distillates were calculated from the standard curve prepared with linalool standard solutions containing 20 – 100 mg/l linalool. By using appropriate volumes of collected distillate, juice distilled, and aliquots taken for the colorimetric determination, the content of FVT and PVT was calculated as mg/l juice. Each experiment was repeated two times.

***Terpene separation and quantification***

Terpene separation and quantification were done by using a method described by Armada *et al.,* 2010. A volume of 100 ml must/wine was applied to a preconditioned 500 mg RP C18 SPE column. Preconditioning was performed by

purging at 3 ml $min^{-1}$ the column with 25 ml portions of methanol and water. After loading a sample onto the column it was washed with 150 ml of water. Non-polar fraction (NPF) was eluted using 25 ml of a mixture of pentane/dichloromethane (2/1, v/v). Subsequently, polar fraction (PF) was eluted using 25 ml of methanol and subjected to hydrolysis. Non-polar fraction was evaporated to approximately 500 µl firstly heating it at 30°C water bath without mixing or stirring for 30 min, then in a delicate stream of nitrogen and 1 µl of it was introduced in a splitless mode into GC system.

The terpenes from wines were separated and measured using a Hewlett–Packard GC with flame ionization detector (FID) and equipped with an HP-Innowax (60 m × 0.25 mm i.d.; film thickness 0.25 µm) capillary column. A volume of 2 µl sample of the extract was injected in splitless mode (30 s). Temperature program: 1 min hold at 45°C, ramping at 3°C/minute to 230°C, and isotherm during 25 min. Helium was used as the carrying gas (18°psi). The temperature of the injector and detector was 230°C.

***Wine analysis***

Wine characterisation was done according to the analytical methods recommended by the OIV, 2006. Sugar concentration was measured by using the clarified wine or must reaction with a specific quantity of an alkaline copper salt solution and the excess copper ions are then determined iodometrically. The titratable acidity was measured by titrimetry using NaOH 0.1 N and Bromothymol blue as indicator. Volatile acidity was measured by removing and collecting the volatile acids from the sample by steam distillation, using Parnas Wagner installation. The collected sample was titrated using NaOH 0.1 N and phenolphtalein as indicator. Alcoholic degree was measured by simple distillation and after the determination of density by pycnometer. Free sulfur dioxide is determined by direct titration with iodine in the presence of starch as indicator. The combined sulfur dioxide is subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulfur dioxide, it gives the total sulfur dioxide.

Acetaldehyde (ethanal) in carbon decolorized wine reacts with sodium nitroferricyanide and piperidine and causes a green to violet color change whose intensity is measured at 570 nm. Esters from wine are separated by distillation of wine brought to *p*H 6.5. After saponification and suitable concentration in an alkaline environment, the distillate is acidified and the vapor condensed to separate the acetic acid liberated by saponification; the acid portion is titrated with the alkaline solution. Methanol was oxidized to formaldehyde (methanol) by potassium permanganate (acidified by phosphoric acid). The amount of formaldehyde was determined by the violet color formed by the reaction of chromotropic acid in a sulfuric medium. The intensity of the color is determined by spectrophotometry at 575 nm.

Glycerol was measured by using an enzymatic kit (Free Glycerol Determination Kit-Sigma). The total phenol content in grape skins samples was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965) using gallic acid as a standard polyphenol: 0.1 ml of

grape skin extract was mixed with 7.9 ml distilled water and 0.5 ml of Folin–Ciocalteu reagent. After 1 min, 1.5 ml of 20% $Na_2CO_3$ was added. The absorbance was measured after 120 min at 760 nm.

The concentration of the total phenolic compounds was expressed as gallic acid equivalents (g/l). The results in every assay were obtained from three parallel determinations.

***Sensorial analysis***

Sensorial analysis of wine was conducted by a panel of 10 panelists (8 men and 2 women), all persons being certified as authorized wine tasters (member of ADAR-Association of certified wine tasters in Romania).

For aromatic wines the following descriptors were chosen for sensorial analysis: olfactory intensity, purity of aroma, fruitiness, floral character, vegetable character, mineral character, bitterness, intensity bouquet, roundness, balance of taste, taste persistence. The maximum score of 5 points was awarded for excellent, 4 points for very good, 3 points for good, 2 points for less good and 1 point for poorly.

## Results and discussion

***Effect of maceration enzymes on the content of the compounds involved in wine aroma***

In choosing enzyme preparations suitable for grapes maceration, it is obvious to take into account the nature of compounds that are part of the cell wall and middle lamella, which are the main barriers to be crossed for useful compounds to must. In the case of Ottonel Muscat grapes these compounds are the free terpenes and their glycosidic precursors.

In Table 1 are listed the quantities of free and bound terpenes in free run must untreated and treated with enzyme preparation (Lallzyme Cuvee Blanc - 2 g/100 kg grapes).

**Table 1.** Free and bound terpenes content in the case of free run must treated and untreated with enzyme preparation

| Characteristics | Free run must reference (variant V1) | Free run must treated with Lallzyme Cuveé Blanc (2 g/100 kg grapes) variant V3 |
|---|---|---|
| Free terpenes, mg/l | 0.510 | 0.698 |
| Bound terpenes, mg/l | 2.330 | 3.470 |
| Bound terpenes/Free terpenes | 4.56 | 4.97 |

As can be seen in Figure 1, the content of free terpenes was lower compared to bound terpenes content. Thus, in the V1 control grape free run must, the free terpenes content was 0.510 mg/l and in the free run must treated with enzyme preparation (V3), free terpenes content was slightly higher 0.698 mg/l.

At the same time, because the enzyme preparation presents a β glucosidase activity some flavor precursors extracted are hydrolyzed during maceration, and therefore an increase of free terpenes content (26.93%) was observed for variant V3.

The ratio of free terpenes and bound terpenes in control samples had values of 4.97 to 4.56 for variant V1 and variant V3, respectively. By using the enzymatic treatment with the enzyme preparation Lallzyme Cuvee Blanc, the precursor content also increased by 32.85% for the variant V3.

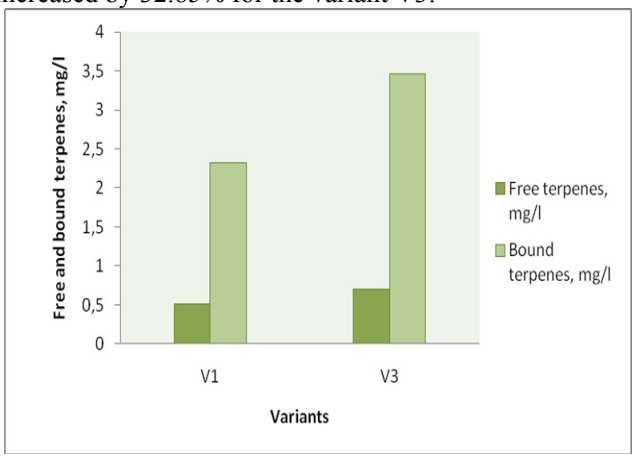



**Figure 1.** Effect of maceration enzymes addition on free and bound terpenes content in grape must

Values presented in Table 2 are terpenic compounds quantified by gas chromatography from free run must untreated and treated enzymatically with Lallzyme Cuvee Blanc (2 g/100 kg grapes).

**Table 2.** Free terpenes content from free run must treated and untreated with enzyme preparation

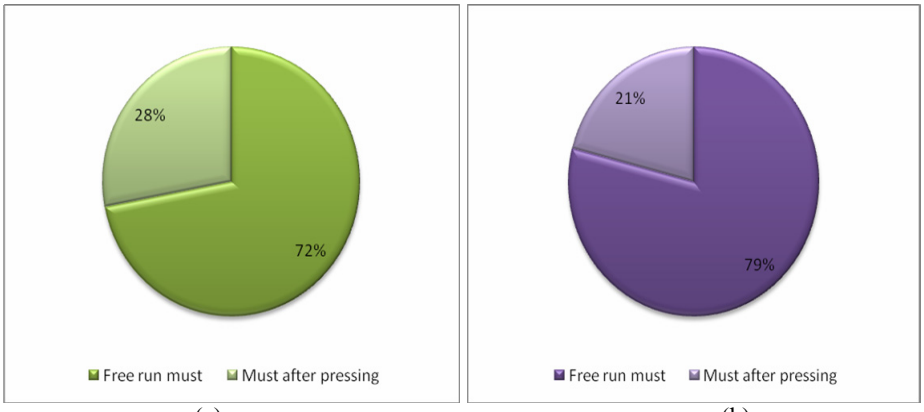
| Terpenic compounds, µg/l | Free run must reference V1 | Free run must treated with Lallzyme Cuveé Blanc (2 g/100 kg grapes) V3 |
|---|---|---|
| Linalool | 157.5 | 197.7 |
| Hotrienol | 82.6 | 98.4 |
| α Terpineol | 36.9 | 49.7 |
| Citronellol | 6.4 | 8.2 |
| Nerol | 84.1 | 106.6 |
| Geraniol | 62.3 | 132.8 |
| Geranic acid | 80.2 | 104.6 |
| **Total terpenic compounds** | **510** | **698** |

***Effect of maceration enzymes on must extraction yield***

The process of getting a big quantity of wine is influenced by different factors like: winemaking technology, the content of grapes in pectic substances and their hydrolysis rate.

Since the duration of maceration of white grapes is very short, from the beginning to the end of crushing and pressing, the effect of endogenous pectolytic is in many cases ineffective. The rate of pectins degradation by enzymatic maceration preparations depends on the duration of contact between enzyme and substrate.

By adding enzymes on grape in the destemming crusher hopper, the effect is maximal and their distribution throughout the mass of grape pulp and later in must is better.




(a) (b)

**Figure 2.** Influence of maceration enzymes on free run must yield for referance (a) and enzymatic treated sample (b)

The use of maceration enzymes whose activity is potentiated by the pectolytic cellulases, hemicellulases and sometimes protease, provides a more advanced and rapid degradation of cell walls. Also a reduction in must viscosity was observed with influence on the rate of grape must extraction like an increasing in the free run must yield (Figure 2a and b) and in the total must yield (Figure 3).

In the case of Muscat Ottonel grapes, the free run must yield increased from 49.7% to 57.4%, the pressing must yield decreased from 17.9% to 14%. Also, the total grape must yield increased from 65.8% to 71.4% (Figure 3).

The increase in the free run fraction of the must depends largely on the pectin content of grapes and is very important to pectin-rich varieties as it is the case of Muscat Ottonel grapes.

***Effect of maceration enzymes on must clarification***

From the total colloidal substances presented in wine, which cause its turbidity, pectin is about 50%. They are the main substances responsible for colloidal

stability of the grape must, which makes the spontaneous clarification take a long time.

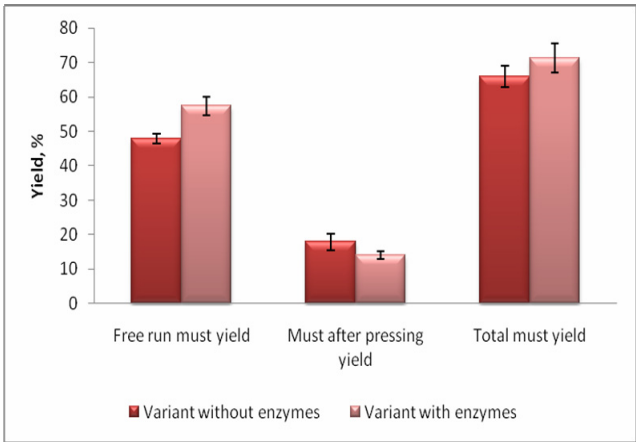



**Figure 3.** Influence of maceration enzyme on must yield

By using exogenous pectolytic enzymes, the first phase of the must clarification - enzymatic hydrolysis phase of pectin - is much shorter. Flocculation of colloidal particles and sedimentation of precipitates formed take place more rapidly.

Studying the effect of pectolytic enzymes on must clarification, their influence on the period of must sedimentation and clarification was observed.

Sedimentation rate was determined by the change in the volume of sediment over time. Clarification rate was monitored by the change of optical density measured at 420 nm by using a 1 cm cuvette.

Because the free run must has a small quantity of pectic substances, but higher in solids suspensions involved in the free run must separation, the time of sedimentation for the must control (V1) is comparable to that of enzymatically treated must (V3) (Figure 4) because the pectolytic enzymes do not have a large amount of substrate on which to act. The clarification time of the reference must is longer than the treated must, which indicates that the action of pectolytic enzymes may fill more quickly particles in suspension.

On the other hand, the pressing must is rich in pectic substances but poor in solids because they have been retained in pulp. In this case, the time of sedimentation of the enzymatically treated sample variant (V4) is lower than the control (V2). Also a decreasing in clarification time was observed for the variant V4, showing the effect of pectolytic enzymes on the must clarification (Figure 5).

The must limpidity was expressed by the optical density value at a wavelength of 420 nm (Figure 6). For the control samples it appears slightly opal with an absorbance value of $DO_{V1} = 0.320$ for variant V1 and very opal with a value $DO_{V2} = 0.509$ for the variant V2 towards great limpidity for variants treated with enzyme preparation ($DO_{V3} = 0.160$ and $DO_{V4} = 0.128$).

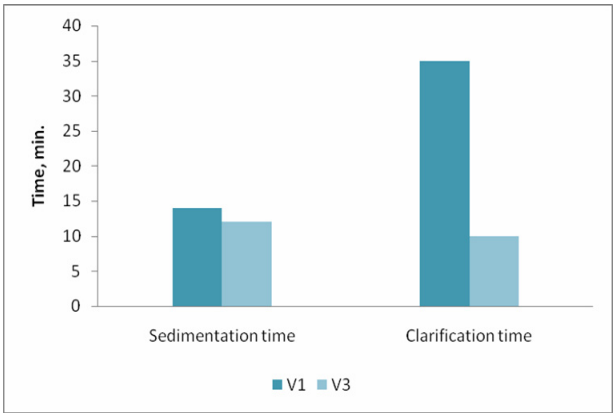



**Figure 4**. Effect of maceration enzymes on the sedimentation and clarification time

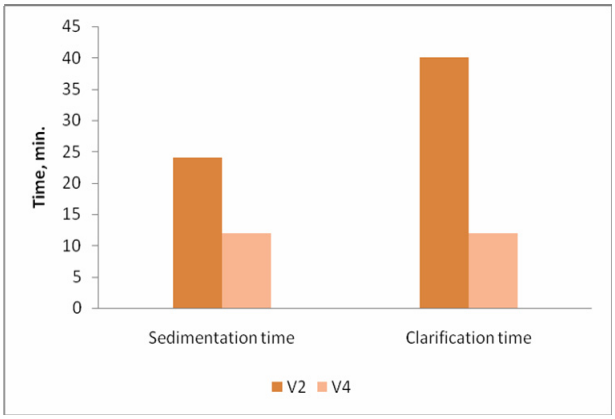



**Figure 5.** Effect of maceration enzymes on the sedimentation and clarification time of the must after pressing

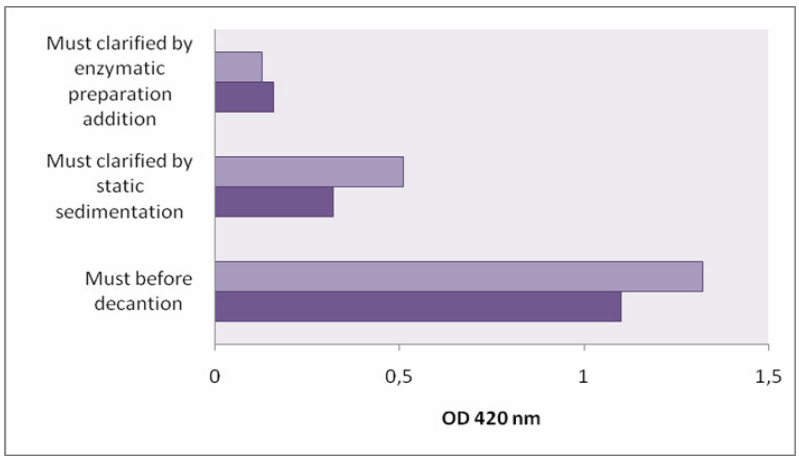



**Figure 6.** Effect of maceration enzyme on the must limpidity

***Effect of maceration enzymes on grape must filterability***

Effect of pectolytic enzyme treatment on the must clarification can be reflected also in its filterability.

Considering filterability as the time measurement for a specified volume of must (50 ml) revealed that in the case of free run must, filtration time decreases from 30 minutes to 14 minutes and for the must after pressing the filtration time decreases from 18 minutes to 12 minutes (Figure 7).

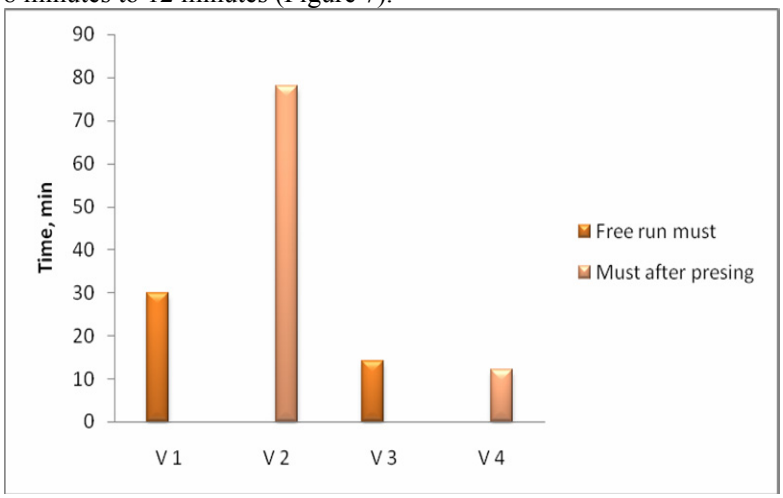



**Figure 7.** Influence of maceration enzymes on must filterability

The beneficial effect of maceration enzymes on filterability of the pressing must was also noted. Improvement of must filterability is found in improving of wines filterability, thus, increasing the amount of wine filtered per unit time and in the possibility of using lower pressure during filtration with economical benefits.

***Effect of maceration enzymes on the dynamics of alcoholic fermentation***

The doses of enzyme preparations have to be judiciously correlated with the effect of clarifying, not to make a clarification too advanced.

The main problems raised by a very advanced must clarification refer to: late start of the alcoholic fermentation, effect on fermentation dynamics and on the yeasts metabolism, knowing the role of dietary fatty acid intake from sediment required for the multiplication and development of yeasts. Also, a deep clarification of the must induces a microbial population decrease, requiring the starting of alcoholic fermentation with selected yeasts, or using activators for fermentation.

By studying the dynamics of alcoholic fermentation of free run must and pressing must it was intended to establish how the enzyme treatment must influence this process.

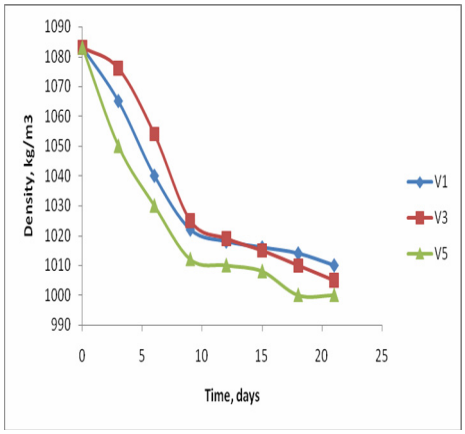



**Figure 8.** Effect of enzymatic treatment on alcoholic fermentation dynamics of the free run must

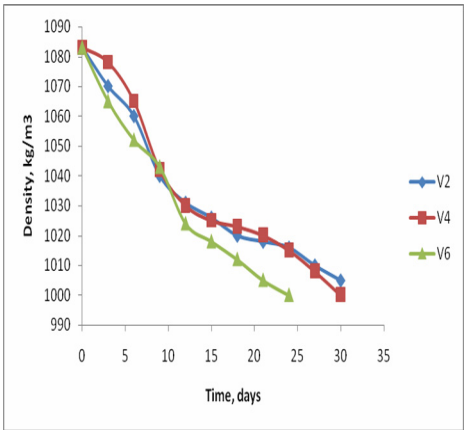



**Figure 9.** Effect of enzymatic treatment on alcoholic fermentation dynamics of the pressing must

Figure 8 shows that the fermentation of free run must fermented by epiphytic microflora treated with enzyme preparation is initiated later, but the fermentation takes less time than the control variant V1. Also, the fermentation is quieter and more uniform.

In the case of the pressing must, the fermentation of free run must under the action of epiphytic microflora treated and untreated with enzyme preparation, began practically at the same time but lasts longer (Figure 9).

When selected yeasts are used, fermentation is much faster and the fermentation time is shorter.

The fermentation with selected yeasts can bring different advantages like: lower fermentation temperature, producing smaller quantities of volatile acids, different spectrum of secondary products of alcoholic fermentation responsible for the "aroma of fermentation", etc.

***Effect of maceration enzymes on wine quality***

In order to study how the treatment of maceration enzymes influences the physico-chemical and sensorial composition of wines, their main parameters were determined (Tables 3 and 4).

Analyzing data from the tables, it is observed that the combination ratio of sulfur dioxide is better in the case of variants fermented with selected yeasts.

Volatile acids content and acetaldehyde values are better to variants fermented with selected yeasts, due to the particularities of the yeast metabolism.

Residual sugar content is slightly higher for variants clarified with enzymes. On the samples fermented with selected yeasts, the content varied from 0.96 to 1.60 g/l.

The extract of the wines made from grapes macerated with enzymes is superior to reference variants, due to the better extraction of soluble compounds of grapes

skins.

The selected yeasts used in experiment are able to produce glycerol, the increase in glycerol content was from 45.5 to 50.0% compared to the control. The wines clarified by enzymes addition compared to the reference variant had an increased concentration of glycerol, thus the forming secondary compounds during alcoholic fermentation under these conditions is influenced beneficially.

**Table 3.** Physico-chemical composition of Muscat Ottonel wines, cultivar 2009, Murfatlar vineyard

| Variants | $SO_2$, mg/l | | Alcohol, % vol | | Total acidity, g $H_2SO_4$/l | Volatil acidity g acid acetic/l |
|---|---|---|---|---|---|---|
| | free | total | efective | potential | | |
| V 1 | 17.5 | 90.00 | 11.9 | 12.1 | 4.90 | 0.47 |
| V 2 | 12.5 | 82.50 | 11.7 | 11.9 | 4.74 | 0.48 |
| V 3 | 22.5 | 85.00 | 12.0 | 12.1 | 5.10 | 0.38 |
| V 4 | 17.5 | 85.00 | 11.8 | 11.9 | 5.00 | 0.41 |
| V 5 | 25.0 | 77.50 | 12.45 | 12.1 | 5.16 | 0.26 |
| V 6 | 27.5 | 82.50 | 12.25 | 11.9 | 4.95 | 0.28 |

**Table 4.** Physico-chemical composition of Muscat Ottonel wines, cultivar 2009, Murfatlar vineyard

| Variants | Reducing extract | Sugar, g/l | Glicerol, g/l | Esters, g/l | Acet-aldehyd, mg/l | Metilic alcohol, mg/l | Total poly-phenols, mg/l |
|---|---|---|---|---|---|---|---|
| V 1 | 17.70 | 2.20 | 6.14 | 0.334 | 42.7 | 47 | 250 |
| V 2 | 18.00 | 2.45 | 6.52 | 0.390 | 47.1 | 59 | 295 |
| V 3 | 19.20 | 2.58 | 8.02 | 0.440 | 30.1 | 58 | 220 |
| V 4 | 19.40 | 2.70 | 7.98 | 0.395 | 36.4 | 65 | 247 |
| V 5 | 20.50 | 0.96 | 9.50 | 0.475 | 22.7 | 76 | 185 |
| V 6 | 20.90 | 1.60 | 10.28 | 0.447 | 25.4 | 84 | 215 |

If the clarification is more advanced (V2, V5), the ester content is higher in the final wines. Ester content of wines fermented with selected yeasts is higher due to metabolic products of yeasts.

As for free run wines and for the wines after pressing, there is an increase in methanol content, when the enzymatic clarification is done. This is the result of pectin-esterases activity in the preparations used. But the increase is not significant in order to influence the wine quality. Wine from must after pressing contains more methanol than free run wine because its content was higher on pectic substances.

The rapid sedimentation of must induces a decreasing in the extraction of phenolic compounds in wine. The polyphenoloxidase activity is removed with the solid parts of grape, where the enzymes are located. Wines fermented with selected yeasts present lower polyphenol content than fermented spontaneously and clarified by enzymes, probably due to the different absorption of these compounds by yeast

cells.

In Table 5 are presented values for terpenic compound assayed by gas chromatography in wine made from untreated and treated grapes with enzyme preparation Lallzyme Cuvee Blanc (2 g/100 kg grapes).

The wine produced from free run must treated with enzyme preparation presented a higher content of free terpenes, this increasing coming from their eliberation from the bound forms during alcoholic fermentation, due to residual grape enzyme activity or enzymatic activity of yeast (Delcroix *et al.,* 1994, Delfini *et al.,* 2001).

On the other hand, the increasing of citronelol concentration is due to yeast metabolism able to synthesize it from nerol and geraniol (Dugelay *et al.,* 1992). Cyclization of nerol, linalool and geraniol in an acid medium results in the α terpineol, and cyclization in acid medium by eliminating a water molecule of the 2,6 dimethyl 3,7 octadien 2,6 diol leads to the hotrienol.

**Table 5.** Free terpenes in free run must treated and untreated with enzyme preparation

| Terpenic compounds, µg/l | Free run wine reference V1 | Free run wine treated with Lallzyme Cuveé Blanc (2 g/100 kg grapes) V5 |
|---|---|---|
| Linalool | 190.8 | 214.6 |
| Hotrienol | 134.9 | 154.2 |
| α Terpineol | 82.3 | 69.2 |
| Citronelol | 82.2 | 101.4 |
| Nerol | traces | traces |
| Geraniol | 55.9 | 81.6 |
| Acid geranic | 54.2 | 119.4 |
| **Total** | **600,3** | **740,4** |

The aromatic profile of wines from variant V1 reference and variant V5 treated Lallzyme Cuvee Blanc (2 g/100 kg grapes) is represented in the Figure 10.

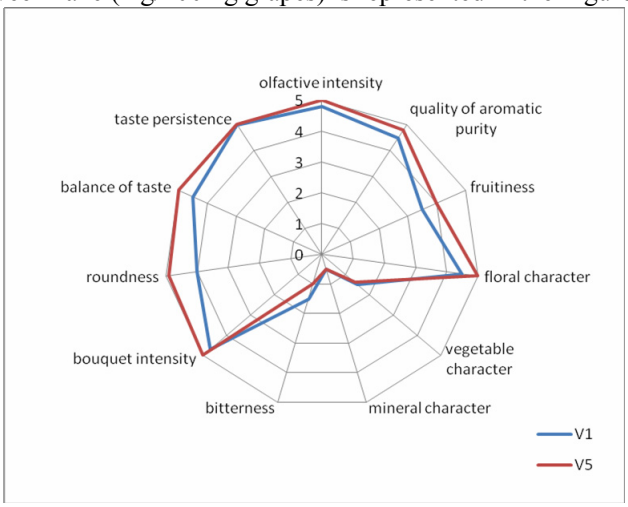



**Figure 10.** Aromatic profile of V1 and V5 wines

The wine made from must treated with enzyme preparation Lallzyme Cuvee Blanc presents superior sensory characteristics when it is compared to the reference variant.

The aromatic intensity is emphasized by using enzyme preparation addition, which contains high concentrations of both glycosidase acting on the first stage of the enzymatic mechanism and in β-glucosidase acting on the second phase of enzyme mechanism to release the aromatic constituents.

From the sensorial point of view, the wines made by using the maceration enzymes are more harmonious, more expressive, and show the best balance of flavour intensity and typicity.

Wines made from musts fermented with selected yeasts present a more attenuated flavour and the aroma of fermentation is more advanced and more evident.

## Conclusions

By adding maceration enzymes to grapes, an increase in free run must yield by 9.5% and a decrease in must after pressing yield of 3.0% were observed.

The pectolytic enzymes action, clarification time of free run must and pressing must are practically the same and equal to the sedimentation time. Reported to the classical sedimentation process, the time of enzymatic clarification is less with 70% and the clarification effect is higher with 50% for free run must and with 75% in the case of the must obtained after pressing. Depending on the presence of the suspensions in must - the same wine technology depends on the grapes composition by using pectolytic enzymes, the must filterability increased by 50% for the free run must and 85% for the must obtained after pressing.

By using the yeast selected for fermentation process on the musts clarified by enzymes, the time of fermentation decreased by 37% for the free run must and by 69% for the must obtained after pressing.

Wines obtained from musts clarified by enzymatic treatment, are distinguished by higher contents of non reducing extract, glycerol, esters, methanol and small amounts of acetaldehyde content, volatile acids and phenolic compounds. Wines produced from musts clarified with enzymes and fermented with selected yeast recorded the highest values of glycerol content, are rich in esters and present very low levels of acetaldehyde content and phenolic compounds.

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