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ORIGINAL RESEARCH PAPER

STUDIES ON WHITE MUST CLARIFICATION USING ENZYME PREPARATIONS

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Abstract

The use of pectolytic enzymes to clarify the white grape juice is a simple and economical solution for positively influencing the subsequent technological steps. The positive effects refer to improving the filterability of the final wine, the behavior of the wine during the stabilization treatments, and, most important, wine quality. The experimental study consisted of treating the grape juice with Zymoclaire Pro Ice for the maceration and extraction steps, and Zymoclaire CC Plus for the clarifying step. The evolution of grape juice clarification parameters such as turbidity, oxidation stability, and the dynamics of the alcoholic fermentation were monitored. The study revealed that after 24 h of treatment, a higher volume of clarified grape juice was achieved. The volume of clarified grape juice was 55% in the control samples, while in case of the sulfited grape juice, clarified with maceration and extraction enzymes or with clarification enzymes, reached 72% and 84%, respectively. The optimal turbidity levels of the juice's enzymatic clarifying stage were reached after 24 hours in case of both enzyme treatments. The comparative analysis of the physicochemical parameters did not reveal significant differences between samples regarding free and total SO_2 contents, potential alcoholic concentrations, volatile acidity, and residual sugars. Also, the enzymatically treated samples were the most appreciated from a sensory point of view. In conclusion, the results revealed that the enzyme tested in these experiments showed high efficiency in grape juice clarifying.

Keywords: white grapes, clarification, enzymes, juice, fermentation

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Introduction

According to Kechagia *et al.* (2008), grapes juice clarification benefits the resulting white wines. However, negative effects have also been reported on their composition and typicity. This happens due to the clarification technique used (Ancín *et al.*, 1996; Burin *et al.*, 2016; Mierczynska-Vasilev and Smith, 2015) and as a result of the influence of some factors and technological interventions in the pre-fermentative stage (Tirelli *et al.*, 2022).

In recent literature, sedimentation, filtration, and flotation are well-established methods for clarifying grape musts from white grapes varieties (Vázquez-Pateiro *et al.*, 2022). Also, clarifying agents of inorganic, organic, vegetable, and animal origin are of interest nowadays (Albuquerque *et al.*, 2021; Marangon *et al.*, 2019; Mierczynska-Vasilev and Smith, 2015; Parish *et al.*, 2016). The treatment with pectolytic enzyme preparations is only considered a helpful tool in the sedimentation process of the suspensions from the grape musts subjected to experimentation (Armada *et al.*, 2010).

However, the literature emphasizes the role and importance of commercial enzyme preparations in white winemaking (Espejo, 2021). Khodakov *et al.* (2021) showed that pectolytic enzymes in the pre-fermentative cold maceration of grape musts induce a slight increase in the phenolic compounds and terpene alcohol contents, while reducing the extract and the optical density. Also, it ensures a more pronounced aromatic expressiveness of the resulting white wines. Other authors (Roldán *et al.*, 2021) noted the significant release of free varietal aromas from their precursors compared to other extraction techniques, while others demonstrated the need for more effective management of the enzymatic treatment in white grape musts with high pH for antioxidant protection (Botezatu *et al.*, 2021).

Many reported research papers evidenced the significant role that enzyme preparations played in clarifying the grape musts. Particularly highlighted are those grape musts from white grape varieties rich in macromolecular compounds that form a protopectin fraction with a high impact on suspension formation and precipitation (Ridge *et al.*, 2021; Samoticha *et al.*, 2017). In the literature, white wines are classified based on scientific compositional criteria: predominantly monoterpene, predominantly thiolic together with predominantly thiopyrolic white wines (Croitoru and Râpeanu, 2019; Gammacurta *et al.*, 2019). At the same time, a worldwide tendency to use enzyme preparations in the pre-fermentative stage of white winemaking is observed, to extract the free varietal aromas and their precursors from the skins of the berries. This step solves efficiently, economically, and quickly both the extraction and the clarification of the resulting white grape musts, even when the clarification operation is carried out by flotation (Croitoru, 2012; Vázquez-Pateiro *et al.*, 2022).

The main objective of the study was to investigate the importance of grape juice clarification using enzymatic preparations as a distinctive technique from static clarification of sulfite-added grape juice. At the same time, the possible implications on the kinetics of alcoholic fermentation and the analytical and sensory profile of the

resulting wine are tracked, without minimizing the influence of the selected yeast strain used in the fermentation process.

Materials and methods

Grapes

White grapes (*Vitis vinifera* var. Șarbă) were harvested (2020 year) at full maturity and were provided by a local producer from Odobești vineyard, Vrancea County, Romania.

Technological variants

Grapes were destemmed and crushed, and SO_2 was added to reach a final concentration of 50 mg/kg grape. Enzyme preparations were added to the press, and the grape juice was collected after pressing. Two different enzymatic preparations were used as recommended by the producers: for maceration and extraction - Zymoclaire Pro Ice (450 PLU/g and 1000 β-d-GLU units/g) at a 2.5 g/hl dose, while for clarification - Zymoclaire CC Plus (450 PLU/g) at a dose of 2.5 g/hl. Both enzymatic preparations presented no defined specific activities but usually had a high level of pectinase activity (450 PLU/g). They are generally used in wine cellars for white grape maceration, extraction of grape juice, and clarification.

The technological variants developed to study the influence of maceration - extraction and clarification enzymes addition in the vinification of white grapes are V1 - sulfited grape juice clarified by static sedimentation as a control sample; V2 - sulfited grape juice clarified with maceration and extraction enzymes Zymoclaire Pro Ice; V3 - sulfited grape juice clarified with clarification enzymes Zymoclaire CC.

Influence of the grape juice clarification parameters

The dynamics of the grape juice clarification process were evaluated by the variation of the unclarified grape juice volume (%), variation of turbidity, and variation of OD_{420nm} over time. In brief, 500 mL volumetric cylinders were filled with grape juice of each variant. After vigorous homogenization, the samples were kept at 20 °C in static conditions. After 4, 8, 12, 18, 24, and 36 h of incubation, samples of each variant were analyzed. The volume of unclarified grape juice was expressed as %. Turbidity was measured using a turbidimeter, expressed in Nephelometric Turbidity Units (NTU), and the absorbance was measured using a UV-VIS spectrophotometer with data analysis software (Libra S22, Biochrom, UK).

Dynamics of grapes juice during alcoholic fermentation

The evolution of the grape juice density during the alcoholic fermentation was monitored every 5 days, over a total period of 25 days.

The alcoholic fermentation of the grape musts from the three experimental variants was conducted with the same selected strain of *Saccharomyces cerevisiae* (Fermactive® Rose) yeast (dose of 15 g/hl) at a controlled temperature of 18 °C. This strain has the metabolic particularity of ensuring the formation of significant glycerol concentrations. Also, it presents an interesting production of organic acids

associated with an appreciable content of esters expressed by intense aromas of flowers and fruits. In addition, the selected strain shows a valuable fermentative capacity, since it can metabolize a high content of fermentable sugars in a wide range of temperatures, producing a low content of volatile acids and not presenting particular nutritional requirements.

Analytical characterization of young white wines

Characterization of white wines from Şarbă grapes was performed according to the analytical methods recommended by the *Organisation Internationale de la Vigne et du Vin* (OIV): SO_2 (OIV-MA-F1-07), Alcohol (OIV-MA-AS312-01), Total acidity (OIV-MA-AS313-01), Volatile acidity (OIV-MA-AS313-02), Reduced extract (OIV-MA-AS2-03B), Residual sugar (OIV-MA-AS311-01A), Glycerol (OIV-MA-AS312-05) (OIV, 2006).

Sensory characterization of young white wines

The sensory evaluation of wine samples was conducted with a trained panel of 10 certified wine tasters. They used a hedonic scale of 9 points with scores from 1 (very dislike) to 9 (very like), as described by Iosip (Dragomir) *et al.* (2022). The wines were cooled to 9 °C and served at ambient temperature. During a preliminary sensory evaluation, the repetitions of each treatment were compared to verify variations. Repetitions of each treatment indicated no differences and were combined and used for the main sensory evaluation. Thereby, panelists described and evaluated each sample in two repetitions (Fauster *et al.*, 2020). The sensory evaluation descriptors were used: olfactory intensity, olfactory quality, flower character, gustatory intensity, gustatory quality, freshness, fruitiness, roundness, and global appreciation.

Statistical analysis

Statistical analysis was conducted using MINITAB Version 17 (Minitab, USA). The experimental measurements were performed in duplicates, and the data are expressed as mean value ± of the standard deviation. The significance of the difference between samples obtained through different enzyme treatments was analyzed using one-way ANOVA and Tukey's test ($p < 0.05$).

Results and discussion

Evolution of unclarified grape juice volume during grape juice clarification

For all three experimental variants, the unclarified grape juice t volume followed a decreasing trend as a function of time. The unclarified grape juice volume differences according to the grape juice clarification duration for each variant are shown in Figure 1.

The difference in the percentage volumes of unclarified grape juice between samples is less evident after the first 5 h of treatment, being 96% for the V1, versus 89% for the V2 and V3 samples. After 10 h of treatment, the control sample (V1) presented significantly higher value of 84% compared to V2 and V3 (48% and 60%, respectively). This difference is maintained after the end of the investigated period (Figure 1). It was found that after 24 h of treatment, the volume of clarified grape

juice was 55% in the control samples, while in V2 and V3, this parameter reached 72% and 84%, respectively. The difference registered between V1, V2 and V3 samples are due to the high pectinase activity (PLU) of 450 units/g in both enzyme preparations, Zymoclaire Pro Ice and Zymoclaire CC Plus. The results agree with those reported by Dal Magro *et al.* (2016), which tested 8 enzyme preparations on the Concord grapes variety, revealing a 9% increase in grape juice volume compared to the untreated control sample in the enzyme preparation based on pectinolytic and cellulolytic activities. To increase the volume of white grape juice, applying enzymatic treatment is crucial because it increases the release of juice through the degradation of pectin and facilitates extraction. According to Fauster *et al.* (2020) this is a result of the changes occurring in the structure and viscosity of the treated must. By accelerating pectin degradation and reducing suspension sedimentation time, other researchers (Moio *et al.*, 2004) confirmed the efficiency of applying the enzymatic treatment for grape juice clarification, which induces a faster increase in the volume of clarified grape juice.

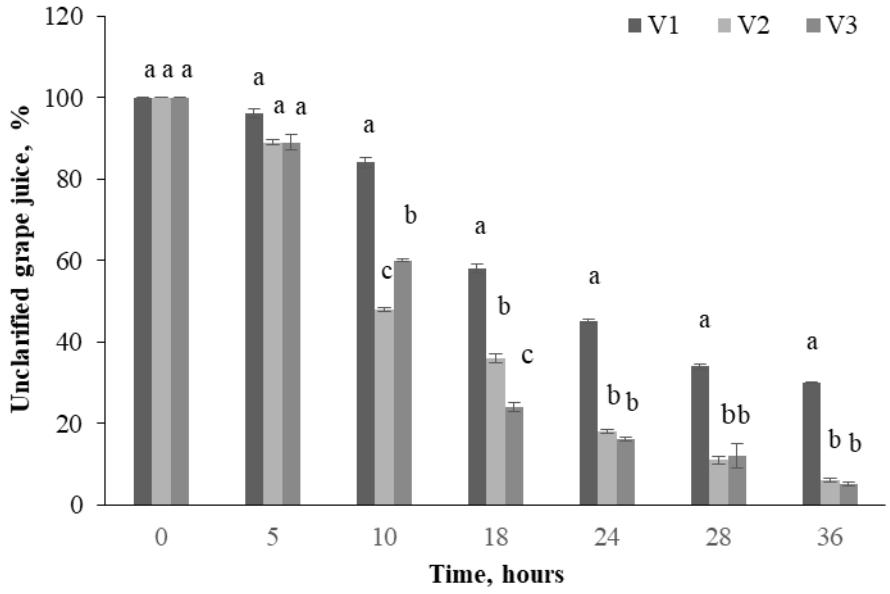

Figure 1. Influence of enzyme treatment on the evolution of unclarified grape juice volume during grape juice clarification. V1 - sulfited grape juice clarified by static sedimentation; V2 - sulfited grape juice clarified with maceration and extraction using Zymoclaire Pro Ice enzymes; V3 - sulfited grape juice clarified with Zymoclaire CC Plus enzymes. Average values, for the same clarification period, that do not share the same superscript letters are significantly different at $p < 0.05$.

Evolution of optical density ($OD_{420\ nm}$) during grape juice clarification

The stage of grape juice oxidation and browning phenomena from various technological causes can be analytically evaluated by measuring the optical density of white grape juice at 420 nm, which is considered satisfactory for the demands of

wine production (Ribéreau-Gayon *et al.*, 2017). The oxidation of polyphenols to quinones and their polymerization during white winemaking cause the formation of yellow-brown compounds (Baron *et al.*, 2000). The different $OD_{420\ nm}$ values observed in the treated grape musts may be related to a different ability to adsorb brown quinones in the colloids derived from adding clarification proteins (Pettinelli *et al.*, 2020). However, it was found that there was no strict relationship between total polyphenols and the browning potential of white grape musts, since this potential depended on the type of polyphenols, especially hydroxycinnamates and flavanols (Cosme *et al.*, 2012).

The comparative evolution of $OD_{420\ nm}$ during the clarification of the grape juice samples treated with different enzyme preparations is shown in Figure 2. In case of the V1 sample, the reduction of $OD_{420\ nm}$ was very slow, becoming significant only after 24 h of clarification (15.93 %). A more pronounced reduction, following an exponential trend, was noticed after another 4 h of treatment. In case of V2 and V3 samples, the reduction of the $OD_{420\ nm}$ became significant after only 10 h of treatment. A progressive decrease of the OD420nm values was further noticed for both samples until the end of the clarification step. Comparing the evolution of the $OD_{420\ nm}$ values corresponding to the V2 and V3 samples, one can observe that the clarification treatment with Zymoclaire CC Plus enzymes (V3) became more effective compared to Zymoclaire Pro Ice (V2) after 10 h of treatment (Figure 2).

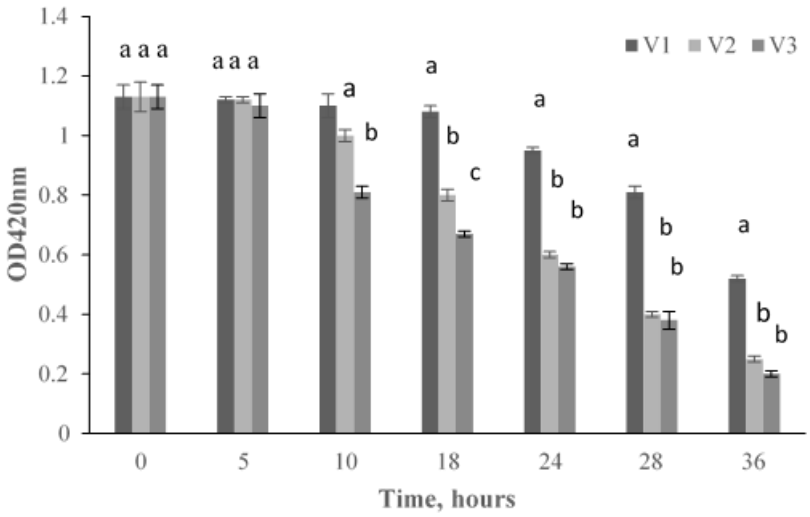

Figure 2. Influence of enzyme treatment on the evolution of OD_{420nm} during grape juice clarification. V1 - sulfited grape juice clarified by static sedimentation; V2 - sulfited grape juice clarified with maceration and extraction Zymoclaire Pro Ice enzymes; V3 - sulfited grape juice clarified with Zymoclaire CC Plus enzymes. Average values, for the same clarification period, on the same hour, that do not share the same superscript letters are significantly different at $p < 0.05$.

In a recent study regarding the clarification of the white grape musts by enzymatic treatment and/or flotation using various clarifying agents reported by Pettinelli *et al.* (2020), no significant differences were reported regarding the OD_{420nm} value after performing the mentioned treatments. OD values of 0.168 were reported after the enzyme assisted clarification step, and between 0.165 - 0.180 in the case of applying clarification by flotation, using various agents of plant and animal origin. In the same study, it was shown that the treatment with the preparation based on legume protein yeast extract (LEGYEAST) presented lower values of OD_{420nm}. Most clarifying agents tend to reduce OD_{420nm} values, as demonstrated for casein or pea protein (Meistermann and Pinsun, 2018), which, together with other vegetable proteins, can exert a clarifying efficiency comparable to gelatin (Pettinelli *et al.*, 2020). However, this is accompanied by a change in the specific sensory profile of the treated wine (Granato *et al.*, 2018).

Conversely, the ability of pea protein to reduce the OD_{420nm} values was less effective compared to the potassium caseinate (Cosme *et al.*, 2012). In another study, Ridge *et al.* (2021) analyzed the thermal denaturation of the endogenous enzymes of the white grape seeds produced by pasteurization, using the brown color difference analyzed at 420 nm as an analytical indicator. They demonstrated the presence of polyphenol oxidase in unpasteurized grape juice samples in agreement with the results of previous research (Wu, 2014). The same study also revealed the reduction of protein content in pasteurized grape juice samples that affected the effectiveness of enzymatic clarification treatment in most of them. However, it should be mentioned that the clarification of white grape musts with exogenous enzyme preparations is superior to the traditional one in which the grape juice contains endogenous enzymes (unpasteurized) or was deprived of them (pasteurized grape juice) (Ridge *et al.*, 2021). Our OD_{420nm} results registered for V2 and V3 samples after 18 and 24 h of clarification treatment, are in agreement with the results presented in the literature for the white grape juice (Pettinelli *et al.*, 2020).

Evolution of turbidity during grape juice clarification

In white winemaking, obtaining clarified grape juice through an efficient extraction process is highly important. The grape juice turbidity must be very close to that desired before alcoholic fermentation, i.e., very close to the value of 200 NTU. The optimal range is between 100 and 250 NTU, depending on the variety and other factors (Ribéreau-Gayon *et al.*, 2017). In case of V2 sample, the optimum turbidity levels of 247 NTU were reached after 24 hours, whereas in case of the V3 after 18 h – 24 h interval (362 NTU after 18 h and 81 NTU after 24 h, respectively) (Figure 3). The control sample was towards the upper limit of the optimal turbidity range of the grape juice after a clarification period of 36 h (256 NTU, results not shown). It can be therefore stated that, the efficient clarification of V3 and V2samples was achieved after 20-22 h and 24 h.

The association of the enzymatic treatment on the destemming of white grape varieties with the action of a pulsed electric field (PEF) led to results that revealed the dependence of the degree of turbidity on the grape variety. The results did not demonstrate a significant difference in this clarification parameter's evolution, which

would support the aforementioned relationship (Fauster *et al.*, 2020). In other research based only on applying PEF, contradictory results on the turbidity of clarified grape juice have been reported. Thus, higher values were reported for the grape juice obtained from the Garganega grape variety (Comuzzo *et al.*, 2017) and lower for grape musts from other white varieties (Praporscic *et al.*, 2007). Anyway, other studies indicate the lack of effects of this parameter on the degree of clarification of the grape juice (Grimi *et al.*, 2009), regardless of the grape juice pressing conditions.

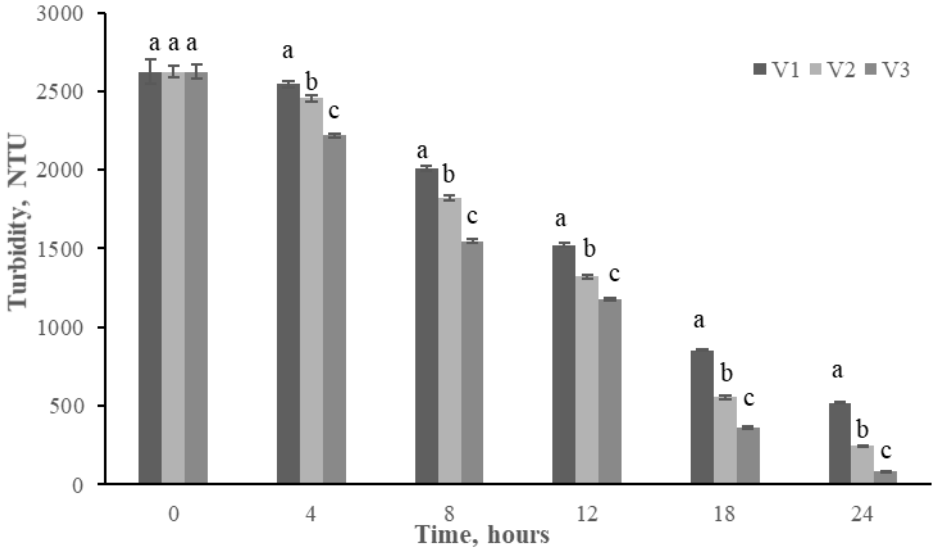

Figure 3. Influence of enzyme treatment on the grape juice turbidity during clarification. V1 - sulfited grape juice clarified by static sedimentation; V2 - sulfited grape juice clarified with maceration and extraction Zymoclaire Pro Ice enzymes; V3 - sulfited grape juice clarified with Zymoclaire CC Plus enzymes. Average values, for the same clarification period, that do not share the same superscript letters are significantly different at $p < 0.05$.

Kinetics of the grape juice fermentation

The results showing the evolution of the density of the grape juice clarified through different treatments, during the alcoholic fermentation, are presented in Figure 4.

As seen in Figure 4, none of the tested variants led to obtaining dry wines, considering that the density values of the grape musts did not drop below 1000 g/L after the completion of their alcoholic fermentation for 25 days.

In the control sample, the evolution of grape juice density recorded constant reductions in the first 15 days of fermentation, followed by a slower fall (Figure 4). Ultimately, the resulting wine had a density of 1010 g/l, corresponding to a residual sugar content of 2.90 g/l (Table 1). In case of the V2 sample, a significant decrease of the grape juice density was observed in the first 2-time intervals (Figure 4). A smaller decrease was registered in the next 5 days (15 units after 10-15 days; 2 units after 15-20 days and 3 units after 20-25 days). The resulting wine, with a density of

1005 g/L, benefited from a residual sugar content of 2.69 g/L. A spectacular decrease in the grape juice density was measured in the V3 variant after the first five days interval (68 units, corresponding to a density reduction compared to the initial value of 69.39%), followed by an appreciable decrease of 8 units after completing the 5-10 days. A plateau was reached after 15 days of fermentation. The resulting wine with a density of 1003 g/L showed a residual sugar content of 2.90 g/L (Table 1). Comparing the evolution of the density of the control sample V1 and the V2 variant one can see a more pronounced drop on density during the fermentation of V2 compared to V1 after passing the intervals of 5 – 10 days (27 units corresponding to a decrease of 27.55 %) and respectively of 10 – 15 days (24 units corresponding to a decrease of 24.49 %). Similarly, the results revealed significant and constant reductions in the grape juice density in V3 compared to V1 after the end of the first two-time intervals (40 units corresponding to a reduction of 40.82%).

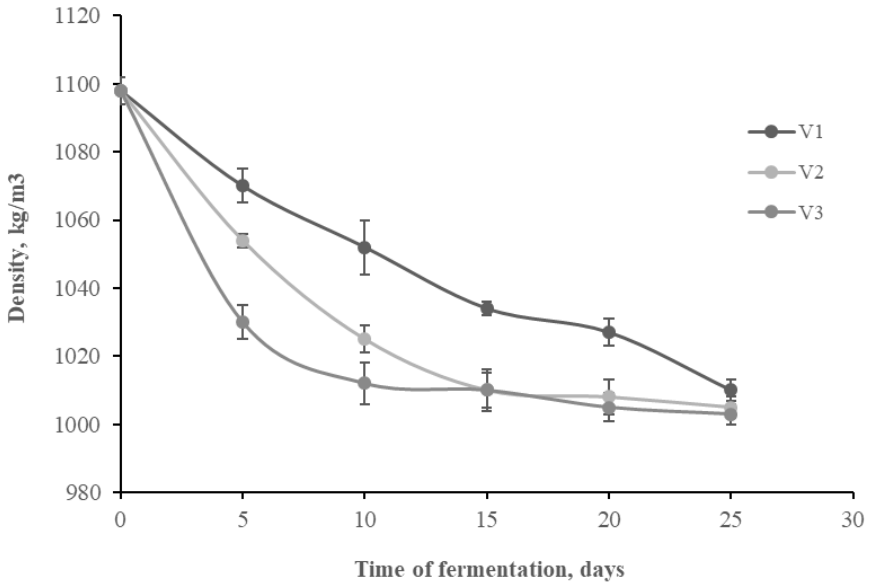

Figure 4. Influence of enzyme treatment on the evolution of grape juice density during alcoholic fermentation. V1 - sulfited grape juice clarified by static sedimentation; V2 - sulfited grape juice clarified with maceration and extraction Zymoclaire Pro Ice enzymes; V3 - sulfited grape juice clarified with Zymoclaire CC Plus enzymes. Average values that do not share the same superscript letters are significantly different at $p < 0.05$.

The above results demonstrate that the enzymatic clarification of the Şarbă grapes juice ensures the provision of the appropriate nutrients for the metabolic and fermentative functions of FERMACTIVE® ROSE yeasts in the alcoholic fermentation until the “dry white wine” stage. It should also be mentioned that for the V3 variant treated with the clarification enzyme preparation, which is probably preserved in the treated grape juice after the applied treatment, the yeast cells need a higher assimilable nitrogen content during the alcoholic fermentation (YAN).

Our results agree with the study conducted by Pettinelli *et al.* (2020) that reported higher values of YAN content in grape juice clarified using enzyme treatment (184 ± 11 mg/L) compared to the clarification by flotation assisted by the use of legume proteins associated with LEGYEAST yeast extract (167 ± 11 mg/L) or associated with GEL gelatin treatment (164 ± 11 mg/L). The YAN values in the grape juice clarified through enzymatic treatment (184 ± 11 mg/L) was almost identical to the variants obtained by flotation corroborated with LEGCHIT chitin-associated legume protein clarification agent (184 ± 10 mg/L). This might be the results of the increase in nitrogen content because of the presence of chitin (Tshinyangu and Hennebert, 1996), as well as the confirmation that the mycorrhizal fungi involved used chitin as a source of nitrogen (Leake and Read, 1990).

Chemical characterization of young white wines

The main physicochemical parameters that characterize the investigated wines obtained from the Şarbă grape variety are presented in Table 1.

Table 1. Analytical parameters of young white wines, depending on the clarification variant applied to the grape juice.

Parameter		**Samples**		
		V1	**V2**	**V3**
SO_2, mg/l	**free**	19.5±2.1^{a}	12.6±1.1^{b}	18.4±1.7^{a}
	total	60.8±1.2^{a}	45.50±3^{b}	55.2±2.1^{ab}
Alcohol, %	**acquired**	12.9±1.1^{a}	12.8±0.7^{a}	12.9±0.2^{a}
vol.	**potential**	13.1±0.5^{a}	12.9±0.5^{a}	13.0±0.3^{a}
Total acidity, g H_2SO_4/l		5.0±0.5^{a}	4.8±0.5^{a}	5.1±0.1^{a}
Volatile acidity, g acid acetic/l		0.43±0.01^{a}	0.46±0.02^{a}	0.42±0.01^{a}
Reduced extract, g/l		19.2±0.7^{a}	20.3±1.1^{a}	21.8±0.8^{a}
Residual sugar, g/l		2.9±0.2^{a}	2.7±0.2^{a}	2.1±0.1^{b}
Glycerol, g/l		8.1±0.3^{a}	9.7±0.3^{a}	9.1±0.3^{a}
Esters, g/l		0.35±0.01^{b}	0.40±0.01^{ab}	0.47±0.02^{a}

Within a column, means values with different superscript letters are significantly different ($p<0.05$)
V1 - sulfited grape juice clarified by static sedimentation; V2 - sulfited grape juice clarified with maceration and extraction Zymoclaire Pro Ice enzymes; V3 - sulfited grape juice clarified with Zymoclaire CC Plus enzymes.

The results of the chemical analysis presented in Table 1 do not reveal significant differences between V1 and V3 regarding free and total SO_2 contents, potential alcoholic concentrations, volatile acidity, and residual sugars. These results agree with the results of other researchers (Pettinelli *et al.*, 2020; Scutarașu *et al.*, 2021), which confirmed that the enzymatic treatments of the grape musts did not significantly affect the physicochemical composition of the obtained white wines.

No significant differences were observed between the total acidity values of the investigated samples. In addition, Sindou *et al.* (2008) found that Debina white wines variety obtained from grape musts clarified by nitrogen flotation, with and without prior treatment using a pectolytic enzyme preparation, presented lower total acidity than wines from the same grape juice clarified by sedimentation. In turn,

Moio *et al.* (2004) showed that clarification by spontaneous decantation or filtration, with and without treatment with a pectolytic enzyme preparation, did not affect the concentration of free terpenols in the grape musts and wines from the Falanghina white grapes variety. Though, they led to a reduction in the concentration of glycosylated precursors.

Also, in Table 1, no statistically difference of reduced extract contents are reported for enzymatically treated sample compared to V1. No study reported the relationship between the value of the reduced extract in the wines produced and the enzymatic treatments applied during the pre-fermentative stage, nor compared to the total content of esters found in the wines resulting from clarified grape musts with exogenous enzymes. In addition, no studies highlighted the intensification of the fermentation abilities in the mentioned directions of a particular selected strain of yeasts. Moreover, no significant differences were found among the glycerol content of the investigated samples (Table 5), suggesting that the enzymatic extraction treatment of grape juice had no influence on the production of glycerol during the fermentation process. A higher content of soluble nutrients (amino acids, peptides, vitamin B1, and certain mineral salts) is probably provided under enzymatic action at the V2 variant. This issue should be addressed in other research to correlate the implications of the grape juice enzymatic clarification treatment on the selection of soluble nutrients necessary to express the biological ability of the selected yeast strain form glycerol in the first stage of its alcoholic fermentation.

Sensory characterization of young white wines

The results of the sensory analysis provided in Figure 5 indicate that V2 and V3 samples were the most appreciated.

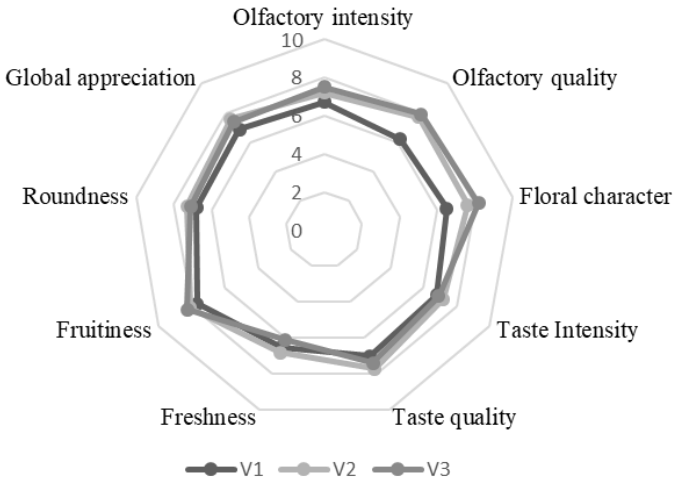

Figure 5. Sensory attributes of the young white wines. V1 - sulfited grape juice clarified by static sedimentation; V2 - sulfited grape juice clarified with maceration and extraction Zymoclaire Pro Ice enzymes; V3 - sulfited grape juice clarified with Zymoclaire CC Plus enzymes.

The results regarding the olfactory intensity and olfactory quality of the white wines revealed the favorable influence of the yeasts on the V3 grape juice sample compared to the V2. The aromas produced under the fermentation action of the yeast presented a more intense influence on the olfactory profile than the free primary varietal aromas extracted from the berry's skins in the pre-fermentative stage under the action of the enzymatic treatment. These aromas are quantified as the total content of esters with flower and fruit aromas in the wine resulted from alcoholic fermentation of the grape juice. The average values given to the assessment of floral character and fruitiness highlighted, as well, the superiority of the V3 sample over the V2. On the contrary, the comparative evaluation of taste intensity, taste quality, roundness, and freshness indicated that the V2 variant was slightly superior to the V3 variant.

The results gathered in the present study agree with those of previous research investigating the influence of higher degrees of turbidity of some grape musts during alcoholic fermentation on the sensory characteristics of the resulting white wines (Fauster *et al.*, 2020; Koenitz *et al.*, 2003; Singleton *et al.*, 1975). They indicated that wine from clarified grape musts showed a higher aromatic intensity and superior taste characteristics compared to the wine from fermented grape musts with higher amounts of solid particles in suspension. Moio *et al.* (2004) reported that higher turbidity in the grape juice induces an accentuation of astringency and bitterness in the resulting white wines. Therefore, the taste becomes harsher, sometimes accompanied by an unpleasant smell of hydrogen sulfide. Other authors reported the accentuation of green/vegetal fruits flavors in the samples of white wines from the Fetească regală variety because of the grape juice treatment with clarifying enzymes in the pre-fermentative stage (Moroșanu *et al.*, 2018). They explained that this was due to the intimate contact between the skins, pulp, and seeds of the grape juice subjected to enzymatic treatment.

Conclusions

The usage of exogenous enzyme allowed a more efficient clarification of the white grape musts compared to the traditional treatment. Enzymatic clarification of the grape juice from the Șarbă variety ensured a significant reduction of the unclarified grape juice volume and a level of turbidity that allowed the rapid initiation of alcoholic fermentation. The sediment volume followed a decreasing trend as a function of time, and it was found that after 24 h of treatment, the volume of clarified grape juice was lower in the control samples compared to the enzymatically treated sample. A higher reduction in grape juice density was observed in case of the sulfited grape juice treated with clarification enzymes compared to sample treated with maceration and extraction enzymes in the 5-10 days interval, with no density differences after 25 h of treatment, between all the samples. The analysis of the physicochemical parameters does not reveal significant differences between enzymatically treated samples regarding potential alcohol concentration, volatile acidity, total acidity values, and glycerol contents. In turn, small differences were

observed in terms of ester and free and total SO_2 contents. The sensory analysis revealed that the enzymatically treated samples were the most appreciated. All the results indicated that the investigate enzymes showed high efficiency in clarifying the evaluated grape juices.

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