

## THE INFLUENCE OF ENZYME PREPARATIONS ON THE STABILITY OF WHITE DRY WINES TO PROTEIN AND COLLOIDAL CASES

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**Abstract:** This article includes the results regarding the influence of enzymes on the stability to protein and colloidal cases in technological treatment schemes in white dry wine materials of the season 2015.

The use of highly active pectinase concentrated enzyme preparation Zymoclair CG resulted in smaller doses of fining auxiliary materials. Thereby, the test on the presence of pectins was negative in white dry wine materials treated by means of the optimal technological scheme.

**Keywords:** enzymes, bentonite, protein content, turbidity, white wine.

### Introduction

One of the relevant tasks of the wine industry of the Republic of Moldova is to improve the quality of products. The need for increased wine stability warranty periods in today's economy and Moldova's entry to the international market requires development and improvement of more reliable instrumental methods of determining the bottling stability [1].

Cases of colloidal nature play an important role among the wine cases of physico-chemical nature. According to some scientists (G.G. Valuiko; E.N. Datunashvili; V.I. Zinchenko, N.M. Pavlenko, V.N. Yezhov, V.A. Zagoruiko; E.G. Manrikyan; G.I. Dyaur; T. Somers; L. Usseglio-Tomasset; J. Glories; H. Oli), both individual macromolecular compounds and their complexes are of crucial importance for the formation of aggregates of colloidal nature [3, 4]. However, nowadays wine producers often can't ensure the stability of the wine materials that are difficult to clear, which cannot be treated by conventional methods. According to the research, carried out by the scientists G.G. Valuiko, V.I. Zinchenko, N.A. Mekhuzla, it is recommended to use enzyme preparation, which facilitate efficient cleavage of compound biopolymer complexes [5].

At present foreign manufacturers of auxiliary materials for the wine industry, such as “Enartis” (Italy), “Erbslöh” (Germany), “Lallemand” (Canada), “Sodinal” (France), etc. offer a wide range of enzyme preparations to process and stabilize the wines, whose action is poorly studied in terms of our winemaking industry.

In order to study the effect of individual enzyme preparations on the technological efficiency of treatments in the Republic of Moldova we conducted a research at the factory “CRICOVA” JSC in 2015-2016, dry white wines Chardonnay and Sauvignon, worked out during the wine-making season of 2015, were used as test samples of wine materials.

The main objective of our research was to investigate the influence of enzyme preparations for the stabilization of dry white wines on the colloidal cases, as well as their impact on the physical and chemical characteristics of the treated wines.

### **Methods and materials of the research**

All technological operations aimed to prepare white dry wine materials (crushing, draining of must, sulfitation, clarification, fermentation of must) have been carried out, using modern technological equipment made of stainless steel and produced by “PADOVAN” company (Italy), as well as the roll crusher DP-25 and the pneumatic press ECP -150.

The following additional materials were used for technological treatments: bentonite Pluxbenton, tannin TanBlanc, fish glue Finecoll and gelatine of “Enartis” company (Italy), as well as a complex enzyme preparation Zymoclaire CG produced by “Sodinal” company (France). Zymoclaire CG is a highly concentrated pectinase enzyme preparation, which has a balanced set of essential substances for additional activities: pektinhydrolase, pektinalakturonase and pektinesterase. Zymoclaire CG is a universal enzyme preparation to treat mash, must and young wine.

The evaluation of various technological schemes aimed to process dry white wines Chardonnay and Sauvignon is carried out according to the following indices:

- the degree of wine filterability;
- characteristic features of the resulting sediment;
- the stability to protein case;
- the stability to colloidal case;
- minimal doses of fining agents;
- changes in basic physical and chemical characteristics of wine.

The methods used to analyze musts and wines in our research, were chosen in accordance with OIV recommendations. We used the technique developed by the Oenological Institute Champagne (France) to test wine materials for the presence of pectins. This test is based on the ability of pectins to denaturate in the presence of alcohol. The technique developed in NIViV "Magarach" was used to determine the content of phenolic compounds in wine, and the concentration of protein content in wine materials was determined by the Lowry method [6].

The turbidity of the must and wine materials was determined by the turbidity tester Hanna. The method aimed to determine the turbidity is based on measuring the light intensity of a specific wave length, which passed through the cuvette that contained the studied solution.

### **Results and discussions**

Initial physical and chemical indices and degustation evaluations of dry white wine Chardonnay and Sauvignon, worked out at “CRICOVA” factory during the winemaking season of 2015, are presented in Table 1.

The results of the physico-chemical analyses, reported in Table 1, show that the studied white dry wines are characterized by a high alcohol content, moderate content of titrable acids and a high content of the total dry extract.

Next, the untreated white dry Chardonnay and Sauvignon wine materials were tested for stability to the protein, colloid, crystal and microbiological cases, and there

were also carried out some tests to identify the presence of pectins in their structure. The results are shown in Table 2.

**Table 1.** Physico-chemical parameters and degustation evaluations of white dry wine materials Chardonnay and Sauvignon (harvest of 2015)

Indicator name	Unit of measurement	Name of the wine material	
		Chardonnay	Sauvignon
Volume concentration of ethanol	vol. %	13.4±0.2	13.0±0.2
<b>Mass concentration of:</b>			
sugars	g/dm <sup>3</sup>	1.8±0.03	1.6±0.02
titrable acidity	g/dm <sup>3</sup>	5.1±0.2	6.5±0.2
volatile acidity	g/dm <sup>3</sup>	0.46±0.04	0.43±0.04
sulphur dioxide (total)	mg/dm <sup>3</sup>	77±2	74±2
iron	mg/dm <sup>3</sup>	1.0±0.1	1.0±0.1
total dry extract	g/dm <sup>3</sup>	21.5±1.4	21.7±1.4
phenolic substances	mg/dm <sup>3</sup>	199±10	207±10
proteins	mg/dm <sup>3</sup>	58±4	75±4
pH	-	3.46±0.01	3.48±0.01
Degustation evaluation	point(s)	8.0±0.01	8.1±0.01

**Table 2.** The stability of untreated white dry wines Chardonnay and Sauvignon

Wine material	Stability to cases				Test on the presence of pectins
	protein	colloidal	crystal	microbiological	
1. Chardonnay	-	-	-	+	positive
2. Sauvignon	-	-	-	+	positive

**Notations:** “-“ not stable; “+” stable.

According to the results presented in Table 2 you can clearly see that untreated dry white Sauvignon and Chardonnay wine materials are unstable to protein, colloidal and crystal cases. Wine test samples are stable to microbiological cases, which proves the compliance with technological requirements of grape processing. However, tests on the presence of pectins showed positive results, indicating a high content of pectins in the white dry wine materials Chardonnay and Sauvignon.

Taken into account the instability of wine test samples to protein cases, we have further studied the state of white dry wines after the heating test and we have determined the turbidity of wines after this test. The results are presented in Table 3.

Table 3 shows that, as a result of the heating test there was observed a floccy sediment in the studied samples of wine, which amounted to 1.5% in the Chardonnay wine and to 2% in the Sauvignon wine. In the wines tested on the stability to heat, we determined the turbidity, which equalled to 598 and 402 NTU for the white dry wine Chardonnay and Sauvignon respectively. High levels of turbidity, floccy sediments as a result of the heating test, instability to colloidal case and the presence of pectins – all these characterize dry white wine materials Chardonnay and Sauvignon as wine materials that are unstable for bottling and difficult to clean.

**Table 3.** Stability evaluation of untreated wines Chardonnay and Sauvignon to protein case

Wine material	Stability of white dry wines to protein case		
	Description of sediment and its volume as a result of the heating test	NTU, as a result of the heating test	stability to protein case
Chardonnay	floccy, 1.5%	598	–
Sauvignon	floccy, 2%	402	–

Notation: “–“ not stable.

To determine the optimal technological processing schemes and to establish the minimum doses of auxiliary substances, the test samples of dry white wines Chardonnay and Sauvignon were subjected to a test fining.

To study the impact of the enzyme preparation Zymoclaire CG while fining wines on the doses of fining substances, as well as on the filtering process, test samples of dry white wines Chardonnay and Sauvignon were treated with and without adding enzymes. The results of test findings are presented in Table 4.

**Table 4.** Technological schemes of processing and bottling stableness of white dry wines Chardonnay and Sauvignon

Indicator	Chardonnay		Sauvignon	
	Scheme I	Scheme II	Scheme III	Scheme IV
<b>Stability to cases:</b>				
protein	+	+	+	+
colloidal	+	+	+	+
crystal	–	–	–	–
microbiological	+	+	+	+
<b>Turbidity during the heating test, NTU</b>	1.65	1.27	1.44	0.97
<b>Test on the presence of pectin</b>	Negative	Negative	Negative	Negative

\*Scheme I: Tannin 0.05, fish glue 0.002, bentonite 2.3; Scheme II: Enzymes 0.03, tannin 0.05, fish glue 0.002, bentonite 1.7; Scheme III: Gelatine 0.005, bentonite 2.5; Scheme IV: Enzymes 0.03, gelatine 0.005, bentonite 2.0.

Notations: “–“ not stable; “+” stable.

Having analyzed the results presented in Table 4, we conclude that enzymes Zymoclaire CG significantly reduce the dosage of bentonite at fining of white dry wines Chardonnay and Sauvignon. To ensure the stability of the wine material Chardonnay, bentonite dose was decreased by 0.6 g/dm<sup>3</sup>, while for the wine material Sauvignon it was decreased by 0.5 g/dm<sup>3</sup>, while the remaining doses of auxiliary substances remained constant (tannin 0.05 g/dm<sup>3</sup>, fish glue 0.002 g/dm<sup>3</sup>, gelatine 0.005 g/dm<sup>3</sup>). Stability to protein, microbiological and colloidal cases in the studied wine materials was achieved after treatment processes, but these wines were unstable to crystal case, which means that they should be treated with cold.

Based on the studied degree of turbidity of wine materials after technological treatments, one can conclude that the treated wine materials are stable to protein case at the turbidity value  $\text{NTU} \leq 2.00$ .

Having reached the stability of white dry wines to protein and colloidal aggregates, treated wines were subjected to physical and chemical analysis, we have also determined the content of phenol substances and proteins depending on the processing scheme. The results are presented in Table 5.

**Table 5:** Change of physico-chemical indices of dry white wines Chardonnay and Sauvignon at different technological schemes of processing

Indicator	Unit of measurement	Chardonnay			Sauvignon		
		Schemes of technological treatment, $\text{g/dm}^3$					
		Initial	Scheme I	Scheme II	Initial	Scheme III	Scheme IV
<b>Volume concentration of ethanol</b>	vol. %	13.4±0.2	13.15±0.2	13.25±0.2	13.0±0.2	12.8±0.2	12.9±0.2
<b>Mass concentration of:</b>							
<b>sugars</b>	$\text{g/dm}^3$	1.8±0.03	1.8±0.03	1.8±0.03	1.6±0.02	1.6±0.02	1.6±0.02
<b>titrable acids</b>	$\text{g/dm}^3$	5.1±0.2	5.0±0.2	4.9±0.2	6.5±0.2	6.3±0.2	6.4±0.2
<b>volatile acids</b>	$\text{g/dm}^3$	0.46±0.04	0.43±0.04	0.46±0.04	0.43±0.04	0.40±0.04	0.43±0.04
<b>sulphur dioxide (total)</b>	$\text{mg/dm}^3$	77±2	77±2	78±2	74±2	74±2	77±2
<b>iron</b>	$\text{g/dm}^3$	21.5±1.4	20.4±1.4	20.8±1.4	21.7±1.4	20.2±1.4	20.9±1.4
<b>total dry extract</b>	$\text{mg/dm}^3$	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
<b>phenolic substances</b>	$\text{mg/dm}^3$	199±10	161±10	159±10	207±10	178±10	177±10
<b>proteins</b>	$\text{mg/dm}^3$	58±4	41±4	40±4	75±4	53±4	51±4
<b>pH</b>	-	3.46±0.01	3.42±0.01	3.43±0.01	3.48±0.01	3.45±0.01	3.47±0.01
<b>Degustation evaluation</b>	point	8.0±0.01	8.0±0.01	8.1±0.01	8.1±0.01	8.1±0.01	8.2±0.01

\*Scheme I: Tannin 0.05, fish glue 0.002, bentonite 2.3; Scheme II: Enzymes 0.03, tannin 0.05, fish glue 0.002, bentonite 1.7; Scheme III: Gelatine 0.005, bentonite 2.5; Scheme IV: Enzymes 0.03, gaelatine 0.005, bentonite 2.0.

Results of the physic-chemical analysis confirmed that the technological scheme, which includes the use of the enzyme Zymoclaire CG in combination with other auxiliary agents is ideal:

- for the wine material Chardonnay: enzyme + tannine+ fishing glue + bentonite;
- for the wine material Sauvignon: enzyme + gelatine+ bentonite.

The results of the physico-chemical analyses showed an insignificant difference in the content of phenolic compounds, as well as proteins in wine samples stable to protein and colloidal cases, regardless the processing scheme.

As a result, it was found out that the use of enzyme preparations in the technological scheme of wine processing reduces the bentonite dose, which, in its turn results in a higher content of the total dry extract in processed wines (by 0.4-0.5 g/dm<sup>3</sup>).

Moreover, the use of minimal quantities of fining agents can reduce production losses of wine materials, as well as the duration of the sediment process for fining agents.

### Conclusions

The study of influence of the enzyme preparation Zymoclaire CG produced by the „Sodinal” company (France) on the efficiency of technological treatments of white dry wines Chardonnay and Sauvignon worked out during the wine season of 2015 at the „CRICOVA” factory, showed an improved clarification process and more efficient action of fining agents (reduction of the bentonite dose by 0.5-0.6 g/dm<sup>3</sup>).

In the study of the influence of enzyme preparations on the stabilization process of dry white wines that are hard to clean to protein and colloidal cases we have also used a new test on the presence of pectins, which showed an excess of pectins in untreated white dry Chardonnay and Sauvignon wine materials. The use of highly active pectinase concentrated enzyme preparation Zymoclaire CG resulted in smaller doses of fining auxiliary materials. Thereby, the test on the presence of pectins was negative in white dry wine materials treated by means of the optimal technological scheme.

We have identified higher levels of ethanol, titrable acids and the total dry extract as a result of a reduced dose of bentonite at fining of white dry wine Chardonnay and Sauvignon, as compared to the control processing scheme.

Test results confirmed that the optimal processing scheme is the scheme that involves the use of enzyme preparations, and namely:

- for the untreated wine material Chardonnay, g/dm<sup>3</sup>: 0.03 enzymes + 0.05 tannins + 0.002 fish glue + 1.7 bentonite;
- for the untreated wine material Sauvignon, g/dm<sup>3</sup>: 0.03 enzymes + 0.005 gelatine + 2.0 bentonite.

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