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INFLUENCE OF ENZYMES TREATMENT ON PHISICO-CHEMICAL PARAMETERS OF FETEASCĂ REGALĂ WINES

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Abstract

Enzymes are protein substances with an important influence on winemaking industry, generating biochemical reaction essential to the quality of the wine. The study aimed to analyse the influence of enzymes on physical-chemical parameters of white wine samples obtained in Iasi vineyard. The grapes representing ''Fetească Regală'' variety were harvested in autumn 2018 at full maturity from Iasi vineyard and processed by the classic method for obtaining white wines. The wine was fermented in 50 L demijohns. Twelve variants of wine were analysed, only six were treated with bentonite. Saccharomyces yeast (Levulia esperide) was inoculated, each variant containing a different commercial enzymatic preparation based on pectolytic enzymes and β-glucosidases, thus contributing to release aroma compounds. Following the analysis of physical-chemical parameters in accordance with OIV regulations, significant influence in the composition of the analysed samples was observed, depending on the enzyme preparations used.

Key words*: enzymatic preparations, 'Fetească Regală', pectolytic enzymes, physical-chemical parameters, β-glucosidases.*

INTRODUCTION

Wine is definitely a complex mixture of different chemicals compounds that are responsible for its quality (Samoticha *et. al.*, 2017). In modern winemaking technology, the treatments applied to the must have an important role in wine quality. Several studies have been made on the influence of the oenological practices on the final wine composition (Losada *et. al.*, 2011).

Enzymes play a fundamental role in winemaking process, especially to improve clarification and filtration of must and wine, increasing their stability and improving the aromatic profile or colour of wines (Armada *et. al.*, 2010). These enzymes originate from the grape, from the yeast, fungi or bacteria related with vineyards and wine cellars. The actions of the endogenous enzymes are limited to the pH and SO_2 conditions associated with wine-making process. Since the grape enzymes are neither efficient or sufficient under winemaking condition, commercial enzymes are widely used as supplements (Rensburg & Pretorius, 2000).

The commercial enzyme preparations are obtained from microorganism cultivated on substrates under favourable development conditions. The most used method is culture in immersed medium. The application of commercial enzymes is legally controlled in Europe by the International Office of Vine and Wine (Gómez-Plaza *et. al.*, 2010; *International oenological codex*, 2013). The International Organisation of Vine and Wine established that only *Aspergillus niger* and *Trichoderma* species may be used as source organism for wine enzymes. The most commonly used enzymes available for commercial enological preparation are pectinases, glycosidases and hemicellulases (Mojsov *et. al.*, 2015).

Fungal pectinases manifest a good resistance to wine-making conditions (Mojsov *et al.*, 2015). Pectinases enzymes are the most basic commercial enzymes; they are used to improve clarification and filterability of musts and wines. Pectolytic enzymes can also be used after alcoholic fermentation, to obtain clear wines and improve the visual quality, very important in white wines (Gómez-Plaza *et al.*, 2010). Glycosidases help release aromas that are bound to sugars and are therefore odourless. Glycosides allow winemakers to obtain wined with intense aromatics in a shorter time (Mojsov *et al.*, 2015).

In the last years, enzyme preparations have been increasingly used for improving the quality of wines, by accelerating the wine-making process and obtaining more aromatic wines (Mojsov *et al.*, 2015). Commercial enzyme preparations are eco-friendly and have great economic benefits (Mojsov, 2013).

In the technology of white and *rosé* wine production, there is a tendency to remove excess protein, the most efficient treatment being bentonite, an oenological product widely to reduce the concentration of undesirable constituents, thus reducing the risk of protein haze (Cotea., 1985; Moroșanu *et. al.,* 2016).

''Fetească Regală'' is an authentic Romanian grape variety and the wines resulted are characterized by wild flowers notes, almond and dried apricots, depending on the wine-making process (Moroșanu, 2018).

The study aimed to analyze the influence of enzymatic oenological preparation on physical-chemical parameters of *'Fetească Regală'* wine samples obtained in Iași vineyard. Physical-chemicals parameters (color, pH, acidity, ethanol content, density, malic acid, lactic acid, total sugars, free SO_2 and total SO_2, total dry matter and non-reducing dry extract), were analysed. The study results are useful in improving wine-making process and its sensorial quality.

MATERIALS AND METHODS

Grapes samples and winemaking

'Fetească Regală' grapes were harvested in autumn 2018 at full maturity from Iasi vineyard, they were destemmed and crushed. The must was transferred in 50 L demijohns. *Saccharomyces* yeast (*Levulia esperide,* AEB) at dose of 20 g/hL and 30 g/hL yeast nutrient (FERMO PLUS CH, AEB), both were dissolved in warm must, was inoculated in each variant.

Five commercial enzymes based on pectolytic and β-glucosidases were added to musts before alcoholic fermentation, thus contributing at increasing release of aroma compounds (Endozym Thiol, AEB – V1; Endozym β-Split, AEB – V2; Zymovarietal aroma G, SODINAL – V3; Endozym Ice, AEB – V4; Zimarome, BSG WINE –V5 and no enzyme– V6), at dose of 3 g/hL (all enzyme preparations were diluted with must 1:10) and 3 mL/hL respectively. 12 variants were obtained. The fermentation was carried out at 16-18 °C for about three weeks. When the alcoholic fermentation was finished, a part of each variant was filtered through sterile membrane filter followed by sulphur dioxide addition (to preserve wine from microbiological damage) and bottled, while the rest were conditioned with bentonite, filtered and bottled after a week (V1', V2', V3', V4', V6'). The samples were kept under controlled condition and analysed after about 3 months.

Color determination was made according the Commission Internationale d'Eclairage (CIE, 1976), using characteristics of specific qualities of visual sensation: clarity, tonality, chromatic parameters, saturation, luminosity, hue (OIV-MA-AS2-11). Evaluation of chromatic characteristics was made using a Specord UV-VIS spectrophotometer. CIELab system characterizes colour variations as perceived by the human eye, representing a uniform 3-dimensional space defined by colorimetric coordinates L*, a*, and b*. The vertical axis noted with L* measures from 0 – completely opaque, to 100 - completely transparent, and parameters "+a*" red, "-a*" green, "+b*" yellow, "-b" blue were registered. (Main *et. al.*, 2007).

Standard chemical analyses according to International Organization of Vine and Wine methods. Each variant was analysed for: total and volatile acidity, ethanol, pH, malic acid, lactic acid, density, total sugar, free and total sulphur dioxide, total dry extract and non-reductive extract.

Sensory characteristics are important for the quality of wines. The wine samples were assessed for sensory characteristics by 15 tasters according to the evaluation method proposed by International Union of Oenologists. The parameters were evaluated with ratings from 0 to 10 and the mean of all results was calculated.

RESULTS AND DISCUSSIONS

Effects of enzymatic pre-treatment on basic parameters of wine

The samples analysed were dry wines with over 12.3 % vol. Ethanol content in the final products was not significantly affected by the type of enzymes, except V1. However, alcohol content ranged from 12.3 to 14.7 % vol. Total acidity varied from 3.6 to 4.2 g of tartaric acid/L on samples treated with enzymes and under 3.5 and 4.1 g of tartaric acid/L on samples treated with bentonite. Acid content is relevant for conservation and is liable for sensory characteristics of final wine. Its content in must and wine may depends on grape variety, maturity, climatic conditions, wine-making technology and wine storage conditions (Samoticha *et. al.*, 2017).

The total dry extract refers to all non-volatile compounds under specified physical conditions (OIV-MA-AS2-03B). The values registered vary between 18.4 and 27.1 g/L. The content of non-reductive extract of Romanian wines varies between 13 and 35 g/L, according to variety, grape health conditions, grape processing technology and wine treatments (Cotea, 1985). The analysed samples recorded values of non-reductive dry extract between 17.1 and 23.7 g/L, the lowest value was noted at V6' value, followed by V5'. The highest value was registered by V1 and V1'variants.

Table 1. Physical-chemical parameters of analysed wines

Samples	Total Acid. (g tartaric acid/L)	pH	Ethanol (% vol.)	Malic Acid	Vol. Acid. (g acetic acid/L)	Density	Total Sugars (g/L)	Lactic Acid	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Total dry extract g/L	Non-reductive extract g/L
Samples treated with enzyme preparations												
V1	3.6	3.27	14.7	2.2	0.13	0.9916	3.4	0	23.03	56.2	27.1	23.7
V2	4.1	3.35	12.6	2.8	0.1	0.9908	1.6	0.1	23.03	56.2	19	17.4
V3	4.1	3.2	12.7	2.8	0.07	0.9906	1.5	0.2	25.5	58.7	18.8	17.3
V4	4.1	3.27	12.6	2.7	0.13	0.9906	1.3	0.2	25.5	58.7	18.5	17.2
V5	4.2	3.26	12.6	2.7	0.09	0.9906	1.2	0.2	23.03	58.7	18.5	17.3
V6	4.1	3.27	12.7	2.8	0.11	0.9906	1.3	0.2	25.5	56.2	18.8	17.5
Samples treated with bentonite												
V1'	3.5	3.30	14.6	2.5	0.03	0.9908	1.4	0	20.47	56.2	24.8	23.4
V2'	4.1	3.30	12.6	2.7	0.07	0.9907	1	0.1	23.03	58.7	18.8	17.8
V3'	4.1	3.28	12.5	2.7	0.07	0.9907	0.9	0.1	23.03	58.7	18.5	17.6
V4'	4.1	3.26	12.5	2.6	0.13	0.9907	1.1	0.1	23.03	58.7	18.5	17.4
V5'	4.1	3.27	12.3	2.6	0.13	0.9908	1.2	0.1	25.5	58.7	18.4	17.1
V6'	4.1	3.27	12.4	2.6	0.13	0.9906	1.4	0.2	25.5	56.2	17.7	16.3

Table 2. Chromatic characteristics of samples treated with enzyme preparations

Samples	**L Clarity**	**Chromaticity**	**Chrome C**		**Tonality H**	**Lightness**	**Hue**	**ΔE**	**ΔH**
			a*	b*					
V1	98.2	-0.11	5.6	5.6	-88.81	0.12	3.31	2.71	1.91
V2	96.9	1.43	4.93	5.14	73.6	0.14	1.94	0.59	0.37
V3	97.0	1.5	4.48	4.74	71.44	0.13	1.89	0.52	0.3
V4	96.6	1.62	5.55	5.8	73.63	0.16	1.94	0.98	0.18
V5	97.1	1.35	5.02	5.18	75.01	0.19	2.01	0.77	0.45
V6	96.7	1.7	4.58	4.93	68.61	0.16	1.75	0.11	0.1

"ΔE" represent colorimetric difference;
"ΔH" represent tonality difference.

Table 3. Chromatic characteristics of samples treated with enzyme preparations

Samples treated with bentonite									
Samples	**L Clarity**	**Chromaticity**	**Chrome C**		**Tonality H**	**Lightness**	**Hue**	**ΔE**	**ΔH**
			a*	b*					
V1'	97.6	0.52	0.53	5.54	84.47	0.14	2.57	3.23	0.28
V2'	97.7	0.94	3.68	3.77	78.77	0.12	2.05	0.29	0.14
V3'	98.9	0.23	3.76	33.76	86.34	0.08	3.05	1.03	0.57
V4'	98.0	0.82	3.84	3.95	77.74	0.12	2.1	0.12	0.02
V5'	97.8	0.88	3.66	3.76	76.32	0.13	2	0.19	0.08
V6'	98.0	0.81	3.72	3.85	77.86	0.11	2.2	0.02	0.01

"ΔE" represent colorimetric difference;
"ΔH" represent tonality difference.

All variants presented a high level of clarity, with more yellow and red shades, except V1 that presented more green and yellow shades. Parameter "a*" had the highest value at V6 (control sample) and the lowest at V1 (Endozym Thiol). The highest values of "b*" was recorded at V1 sample (Endozym Thiol), and the lowest at V3 (Zymovarietal aroma G). Some differences were measured for "L*" that corresponded to brightness. It was manifested by a less green/red and yellow color of wine. This red color is causing the "pinking" phenomenon in white wines, perceived as an undesirable phenomenon by winemakers and consumers (Cosme *et. al.*, 2018). These results may indicate the presence of low but visible amounts of anthocyanins.

Tonality has registered positive values for the majority of samples, except V1, that recorded a negative value. The lightness parameter was reduced with the addition of bentonite in most variants, excepting V1, where the value increased. The chromaticity was significantly improved by the use of bentonite. Enzymatic treatment influenced chromatic parameters of analysed samples to varying degrees. The majority of wines treated with enzymes were characterized by the decrease in a* compared to the control sample, and thus less intense green colour and more red colour. Wine making progress was accompanied by an increase in b*, that means more yellow colour. A perceptible colour difference between samples treated with enzymes and control samples can be observed, suggesting that enzyme preparation had a greater effect on colour. Delta values represents colour difference as compared with the control. Parameters shows higher values on samples treated with enzymes compared to the control. No significant differences registered between the effect of pectolitic enzymes and β-glucosidases on wine colour.

In figure 1, organoleptic charts are represented. Following the sensory analysis, significant differences can be observed due to the type of enzyme used as pre-treatment. Thus, variants V3 and V3' (Zymovarietal aroma G) were noted as having a richer aromatic profile with intense notes of ripe fruits, exotic fruits, with good persistence, texture and high minerality. V1 and V1' variants (Endozym Thiol) showed high acidity with light fruity notes. A spicy taste was noted to be more pronounced in variants V5 and V5' (Zimarome), with good floral notes and honey aroma. Wild floral notes were best noted at V4 and V4' variants (Endozym Ice). Variants treated with Endozym β-Split (V2 and V2'), showed a high level of fruity notes, with discreet notes of wild flowers. The samples treated with bentonite were more balanced in taste than unconditioned samples. The mineral flavour has been intensified by the bentonite treatment.

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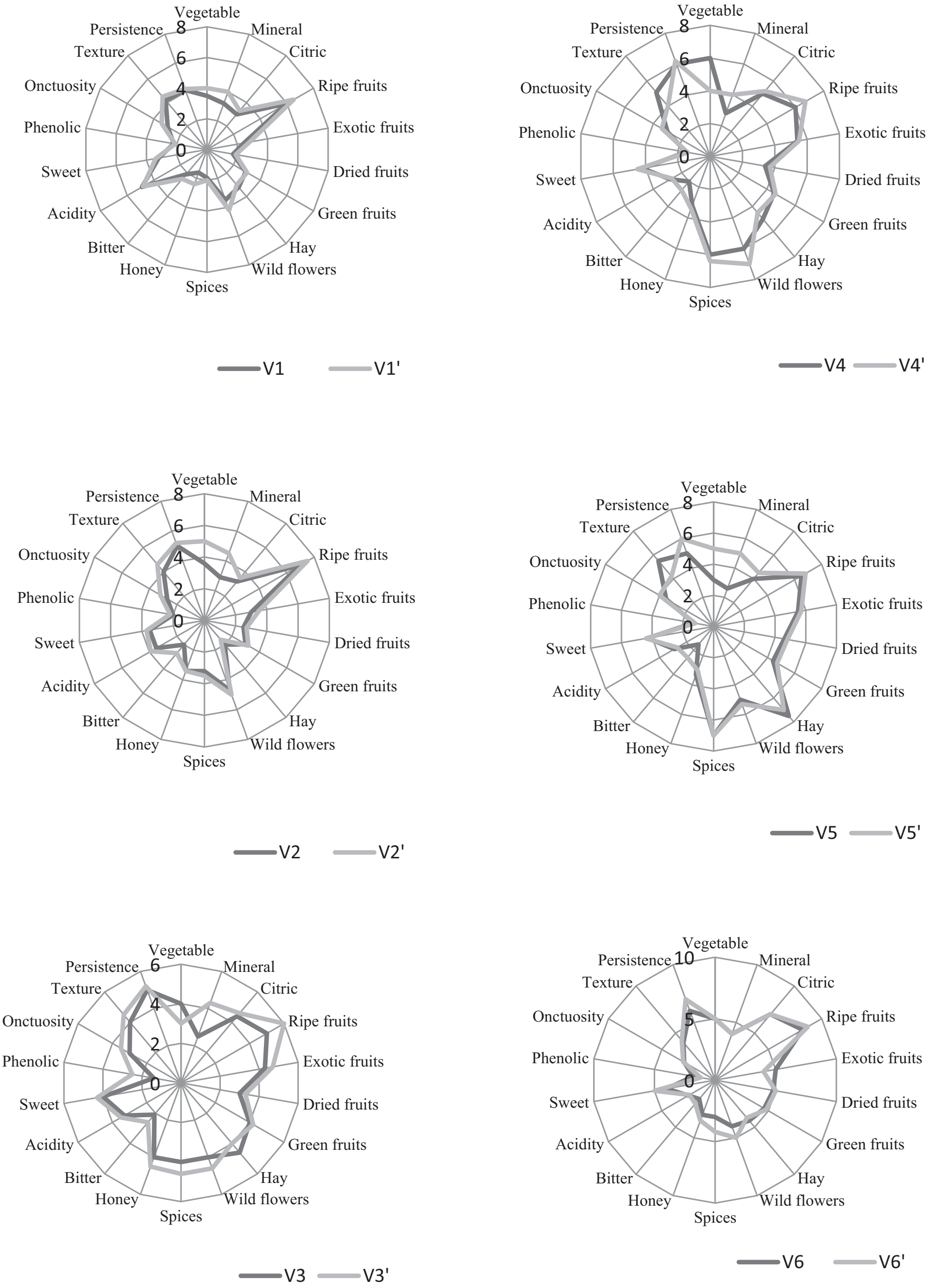

Figure 1. Comparative organoleptic graphics of the analysed sampled
(treated with different enzyme preparations vs enzyme+bentonite treatment)

CONCLUSIONS

Wine is a sensitive and extremely complex combination of chemical components that influence its quality. The process of winemaking depends on the activity of numerous enzymes. This study discusses the effects of enzymatic treatment on the improvement of chemical composition of wine. In this study, the pre-fermentative treatments didn't have a significant influence on the basic physical-chemical parameters. Enzymatic treatment influenced chromatic parameters of analysed samples to varying degrees.

The chromatic parameters were significantly improved by the use of bentonite. Following the sensory analysis, significant differences can be observed due to the type of enzyme preparation used as pre-treatment.

The samples treated with bentonite were more balanced in taste than unconditioned samples. Also, the treatments with bentonite can influence the final product perception.

The treatments applied in winemaking have an important role in deciding the wine's quality.

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