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Research Paper

A Comparison of Effects of Three Commercial Pectolytic Enzyme Preparations in Red Winemaking

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Abstract: *Red grape mashes of Vranec were treated with different pectolytic enzyme preparations. These treatments resulted in increases on the organoleptic (colour) and rheologic characteristics (filterability, amounts of solids that settled). The results of our experiments gives a comparison of the efficiency of preparations applicable in winemaking. Preparations Vinozym Vintage FCE and Trenolin Rot DF showed a more intensive extraction of red grape pigments (anthocyanins) and increased colour intensity. The time of filtration was three times shorter, by using the enzyme preparations Vinozym Vintage FCE and Trenolin Rot DF compared to the control sample. By using the enzyme preparations Vinozym Vintage FCE, Rohapect VR-C and Trenolin Rot DF, the speed of desliming was twofold faster, compared to the control sample.*

Keywords: Pectolytic enzymes, Red grape *Vranec*, Anthocyanins, Filterability, Settling rate.

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1. Introduction

Vranec is a variety of red grape cultivated in Republic of Macedonia. It is capable of producing high quality red table wines in this country. Although the composition of the grape

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depends on its variety, the soil and the climatic conditions, there is little variation in the actual cell structure of the plant. Grape skin cell walls are a physical barrier on red grape pigments and aroma compounds. As pectic polysaccharides play a major role in cell walls rigidity they are the main limiting factor [9]. To the main polysaccharide chains other shorther or longer, straight or branched, saccharide chains are attached.

The main enzymes used during winemaking are pectinases. Pectinases occur naturally in all fruit (including grapes) and are partly responsible for the ripening process. Grape pectinases are however inactive under the pH and SO_2 conditions associated with winemaking. Fungal pectinases are resistant to these winemaking conditions. The method used to produce wine enzymes for use in the European Union is regulated by the OIV. The OIV has established that only *Aspergillus niger* and *Trichoderma* can be used for enzyme production. The most widely used commercial enzymes are: pectinases, hemicellulases, glucanases and glycosidases. The latter three types are generally sold as blends with pectinases. The red skin contact enzymes have very low concentration of anthocyanase activity. Anthocyanases are able to break off sugar units from more complex molecules. Grape anthocyanins are stabilized by covalent linkage with one glucose unit. They become unstable and become colourless when these linkages are broken. It is important that commercial red wine enzymes do not contain this activity.

The pectic enzymes play an important role in braking down grape pulp and skin cells and are able to split those chains and saccharide bonds between the chains [29]. Enzymes cannot act on grapes if they are whole. Therefore, grapes should always be crushed before enzymes are added to enhance extraction. In red wine, tannins and anthocyanins are the most important phenolic classes. Tannins contribute to the mouthfeel of wines but they also form pigmented polymers in association with the anthocyanins to provide the stable pigments required to give red wine its longterm colour stability. Grape anthocyanins are red pigments, located in the first external layers of the hypodermal tissue and mainly in the vacuoles [25], as well as in special structures called anthocyanoplasts [20].

Winemaking is a biotechnological process in which enzymes play a fundamental role. The use of enzyme preparations break down the cellular structure of the grape skin and aids in the release of anthocyanin and copigmentation cofactors.
Pectic enzyme treatments on grape mashes resulted in increased filtrerability and clarity, amounts of solids that settled [3-6, 9, 10, 12-14, 18, 21, 23] and a more efficient extraction of desirable red grape pigments [1, 2, 8, 16, 19, 24, 30]. The aim and importance of the research were a comparison of the efficiency of enzyme preparations applicable in winemaking.

2. Materials and Methods

2.1 Commercial pectolytic enzyme preparations

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- Vinozym Vintage FCE, Novozymes A/S, Bagsvaerd, Denmark; 2, 3, 4, and 5 g/100 kg grapes
- Rohapect VR-C, AB Enzymes GmbH, Darmstadt, Germany; 2, 3, 4, and 5 g/100 kg grapes
- Trenolin Rot DF, Erbslöh Geisenheim AG, Geisenheim, Germany); 10, 15, 20, and 25 ml/100 kg grapes

These enzyme preparations are derived from cultures of Aspergillus niger which is a species accepted as G.R.A.S. (Generally Recognized As Safe) [9].

2.2 Grape samples for laboratory trials

The grape cultivar Vranec (*Vitis vinifera*), cultivated in the Ovce pole vineyard, the Povardarie region, was harvested at optimal maturity (2009 vintage), at 200-220 g l^{-1} sugar, 6.5-7.5 g l^{-1} total acids, and pH from 3.1 to 3.3, and transported to the private winery "Imako Vino" Stip, Republic of Macedonia.

2.3 Wine samples. Microvinification

Wines were prepared in the laboratory of winery "Imako Vino" Stip. Grapes for was weighed, crushed/destemmed and divided in 5 liters plastic fermentation tanks. Red grape mashes were macerated for 6 hours (18-20 ^{o}C), with addition on one commercial pectolytic enzyme preparation. After addition of SO_2 (50 ppm) and yeast (*Saccharomyces cerevisiae*) NEUTRE SC (Lallemand) (200 mg kg^{-1} grape), maceration time of 5 days (~25 ^{o}C) was applied in order to study the effect of macerating enzymes on colour intensity and concentration of total anthocyanins in the obtained 12 different variations. After the maceration, the pomace was removed. Control trial was in all same with experimental trials only no added pectolytic enzyme preparation. All treatments were performed in duplicate.

The bottled wines (0.5 l) were stored at 4 – 6 ^{o}C. Colour intensity and concentration of total anthocyanins were measurmed after 6 months of the wine maturation.

2.4 Instrumentation and reagents

Aglient 8453 UV-Vis spectrophotometer was used for analysis of colour intensity and total anthocianins in the wines. Reagents used for analysis were of analytical grade purity. All analyses were performed in duplicate.

2.5 Total anthocyanins assay

Concentration of anthocyanins was performed by dilution of the wine samples with a solution of ethanol/water/HCL = 70/30/1 [7, 15]. The concentrations of anthocyanins was calculated using the equation: $TA_{540\ nm}\ (mg\ L^{-1}) = A_{540\ nm} \cdot 16.7 \cdot d$

A – absorbance at 540 nm, d – dilution expressed as malvidin-3-glucoside equivalents.

2.6 Colour intensity, hue and colour composition of the wines

The colour intensity in wines is determined as the sum of the absorbances at 420, 520 and 620 nm [11]. The absorbance of a wine was directly measured at 420, 520 and 620 nm using a

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2 mm optical path and the colour intensity (CI), hue (H), colour composition (%Ye, % Rd, % Bl) of the wine were calculated [11]. The hue of wine is defined as the ratio A_{420}/A_{520}.

2.7 Filtration speed

The filtration speed was measured as the time needed for filtration of a defined amount of the mash sample (10 ml must) that passed through the filtration paper.

2.8 Speed of sedimentation

The speed of sedimentation was expressed as the thickness of sediment that formed within 30 min after the mixing up of experimental bottles with the samples (10 ml must).

3. Results and Discussion

The major red grape colorants are anthocyanins-belong to the group of the phenol compounds. These are released from the tissues of berries by the action of enhanced amount of ethanol. By the action of pectic enzymes on the grape skin, this process was accelerated. Maximum release of the red grape pigments take place within 5 days after the application of pectic enzyme preparations. With enzymes, winemakers can enhance aroma, improve colour, clarify of the wine, filterability and amounts of solids that settled. Enzymes are very popular in red wine making since extraction and clarification of the must is difficult due to the presence of pectins extracted during winemaking. High viscosity and the cloud particles are kept in suspension. Enzymes also help with reducing viscosity, releasing free-run juice easily, and a more intensive extraction of desirable red grape pigments and other phenol compounds which are bound in plant cells.

3.1 Effects of enzyme treatments on the chromatic characteristics of red wines

In Table 1 are given results for the effect of the use of pectolytic enzymes on the chromatic characteristics of red *Vranec* wines and contol trials “no-enzyme addition”. In Table 1 it can be seen increased colour intensity (CI) by 3.976 (control, 3.341), the hue (H) values by 0.468 (control, 0.537). Percentage of red colour contribution (Rd%) by 61.7 (control, 57.8), and total anthocianins (TA, mg L^{-1}) by 513.83 (control, 57.8). Pectolytic enzyme preparation Vinozym Vintage FCE showed increased of CI for 19%, Trenolin Rot DF for 13.8%, and Rohapect VR-C for 2.5% compared to the control sample from 3.341, depend of used doses. Vinozym Vintage FCE and Trenolin Rot DF showed a more efficient extraction of total anthocianins compared to Rohapect VR-C and control sample. The obtained results for the analysed wines were in agreement with previously published data [8, 17, 19, 24, 26, 28].

3.2 Effects of enzyme treatments on the filtration speed of red wines

The enzyme preparations used are able to split pectin chains to produce short chains of saccharides. The split pectin loses the protective activity to juice colloids which makes problems during filtration. In Table 2 are given results for the effect of the use of pectolytic enzymes on the filtration speed of red *Vranec* wines and contol trials “no-enzyme addition”.

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In Table 2 it can be seen decreased time of filtration by 6.5 min (control 20.555 min). By using the enzyme preparation Vinozym Vintage FCE, was three times shorter. The time of filtration was in the case of Trenolin Rot DF and Rohapect VR-C 2.5 and 1.7 times shorter, respectively, compared to the control sample. The obtained results for the analysed wines were in agreement with previously published [4, 9, 21, 23].

Table 1. Effects of enzyme treatments on the chromatic characteristics of red wines made of red grape *Vranec*

Enzyme preparations	Dose	[a]CI	H	Ye/%	Rd/%	Bl/%	[a]TA, (mg l^{-1})
Vinozym Vintage FCE							
I-1=2g/100kg grape	I-1	3.375±0.003	0.46	28.9	61.7	9.4	425.53±0.30
I-2=3g/100kg grape	I-2	3.856±0.003	0.49	29.6	59.6	10.8	477.54±0.13
I-3=4g/100kg grape	I-3	3.765±0.001	0.47	29.1	60.7	10.2	474.03±0.12
I-4=5g/100kg grape	I-4	3.976±0.005	0.46	28.7	61.2	10.1	496.37±0.06
Rohapect VR-C							
II-1=2g/100kg grape	II-1	3.047±0.001	0.543	30.4	56.2	13.4	349.42±0.01
II-2=3g/100kg grape	II-2	2.692±0.001	0.53	30.4	57.2	12.3	324.07±3.50
II-3=4g/100kg grape	II-3	3.348±0.002	0.52	30.1	57.1	12.7	350.68±1.37
II-4=5g/100kg grape	II-4	3.425±0.001	0.53	30.4	56.7	12.8	377.42±0.89
Trenolin Rot DF							
III-1=10ml/100kg grape	III-1	3.537±0.003	0.47	28.9	61.1	9.9	438.18±0.11
III-2=15ml/100kg grape	III-2	3.803±0.001	0.48	29.1	60.2	10.6	513.83±0.61
III-3=20ml/100kg grape	III-3	3.215±0.001	0.51	30.4	59.2	10.3	323.63±0.58
III-4=25ml/100kg grape	III-4	3.245±0.001	0.50	30.2	59.3	10.5	324.34±1.63
Control-no added enzyme	0	3.341±0.001	0.53	31.0	57.8	11.2	314.57±0.23

Note: [a]The values are average from 2 replicates ±SD

CI: colour intensity, H: hue or tint, Ye%: percentage of yellow colour contribution, Rd%: percentage of red colour contribution, Bl%: percentage of blue colour contribution in the overall colour, total anthocianins, TA (mg l^{-1})

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Table 2. Effects of enzyme treatments on the time of filtration of the wine samples at the fifth day of enzyme maceration

Enzyme preparations	Dose	[a]Time of filtration, (min)	Time of filtration Vs control	Speed of filtration, (ml/min)
Vinozym Vintage FCE				
I-1=2g/100kg grape	I-1	18.332 ± 0.234	0.891	0.545
I-2=3g/100kg grape	I-2	13.500 ± 0.271	0.656	0.740
I-3=4g/100kg grape	I-3	6.500 ± 0.271	0.316	1.538
I-4=5g/100kg grape	I-4	12.333 ± 0.134	0.600	0.810
Rohapect VR-C				
II-1=2g/100kg grape	II-1	19.333 ± 0.134	0.940	0.517
II-2=3g/100kg grape	II-2	16.500 ± 0.271	0.802	0.606
II-3=4g/100kg grape	II-3	12.666 ± 0.134	0.616	0.789
II-4=5g/100kg grape	II-4	11.500 ± .271	0.559	0.869
Trenolin Rot DF				
III-1=10ml/100kg grape	III-1	11.333 ± 0.134	0.551	0.882
III-2=15ml/100kg grape	III-2	10.500 ± 0.135	0.510	0.952
III-3=20ml/100kg grape	III-3	8.333 ± 0.136	0.405	1.200
III-4=25ml/100kg grape	III-4	10.166 ± 0.135	0.494	0.983
Control-no added enzyme	0	20.555 ± 0.207	1.000	0.486

Note: [a]The values are average from 3 replicates ±SD

3.3 Effects of enzyme treatments on the speed of sedimentation of red wines

Clouds may be formed of grape skins and parts of pulp which are transferred to rhe must after pressing. Under the conditions of static desliming, clouds settle on the bottom of the reservoir. In this way the wine is clarified. Chemically, clouds are formed by pectin, cellulose and other stuffs and this may result in an unpleasant taste of the red wine. In Table 3 are given results for the effect of the use of pectolytic enzymes on the speed of sedimentation of red *Vranec* wines and contol trials “no-enzyme addition”. By using the enzyme preparation

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Vinozym Vintage FCE, the thickness of the sediment was 1.10-2.33 cm, i.e. the speed of desliming was 2.3 times faster, compared to the control sample (1.0 cm). The speed of desliming was in the case of Trenolin Rot DF and Rohapect VR-C twofold faster (sediment by 2.0 cm), compared to the control sample (1.0 cm). The obtained results for the analysed wines were in agreement with previously published [4, 22, 27].

Table 3. Effects of enzyme treatments on the speed of sedimentation of the wine samples after the first day of enzyme maceration

Enzyme preparations	Dose	The thickness of the sediment, (cm)
Vinozym Vintage FCE		
I-1=2g/100kg grape	I-1	1.10 ± 0.081
I-2=3g/100kg grape	I-2	1.46 ± 0.047
I-3=4g/100kg grape	I-3	2.10 ± 0.081
I-4=5g/100kg grape	I-4	2.33 ± 0.047
Rohapect VR-C		
II-1=2g/100kg grape	II-1	1.53 ± 0.047
II-2=3g/100kg grape	II-2	1.76 ± 0.047
II-3=4g/100kg grape	II-3	2.03 ± 0.047
II-4=5g/100kg grape	II-4	2.06 ± 0.047
Trenolin Rot DF		
III-1=10ml/100kg grape	III-1	1.46 ± 0.047
III-2=15ml/100kg grape	III-2	1.93 ± 0.047
III-3=20ml/100kg grape	III-3	1.96 ± 0.047
III-4=25ml/100kg grape	III-4	2.03 ± 0.047
Control-no added enzyme	0	1.00 ± 0.081

Note: [a]The values are average from 3 replicates ±SD
The thickness of the sediment, 30 min after the mixing up of the experimental bottles with the wine samples (10 mL)

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4. Conclusions

Commercial pectolytic enzyme preparations offer significant quantitative (increased juice yields) and qualitative processing improvements (colour extraction, filtration rates, lees settling rates, and clarity of wines) to both the winemaker and the grape processor.

The results presented in this paper shown that the use of enzyme preparations allows a more efficient extraction of red grape pigments (increased anthocianins) and reduction of the time needed for some technological steps (settling and filtration). Thus, a more amounts of grapes can be processed and can be produced wine with a higher sensory quality in a shorter time and a more economic way. Results from comparison of effects of pectolytic enzyme preparations in winemaking can contribute to a better orientation in the choice of suitable enzyme preparations in wine industry.

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