The Effects of Macerating Enzyme Treatments and Aging on Phenolic Content and Chromatic Characteristics in Vranec Wines

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Abstract - **This study evaluates the effect of using of pectolytic enzyme preparations on the phenolic content and chromatic characteristics of young red wines produced from Vranec (*Vitis vinifera* L.), the important grape variety in Macedonia. Phenolic compounds and chromatic characteristics of young red wines were investigated by means of enzyme treatments with diverse enzyme preparations (Vinozym Vintage FCE, Rohapect), with four doses and time of aging (6 months). Enzyme treatments and maceration time influenced the phenolics extraction from grapes into wine. In the wines produced with 6 days of enzyme maceration, highest concentrations of phenolic components were observed, and aging for 6 months lead to increasing the phenolic content to 32 %, total anthocyanins to 66 %. The color intensity rose with aging in enzyme treated grapes. Results from the analysis showed that all enzyme preparations increased the extraction of polyphenols and elicited an improvement in the visual aspect of the wine.**

Keywords - enzyme preparations; red wine; phenolic compounds; color; spectrophotometry

I. INTRODUCTION

Wines and grapes contain a number of polyphenolic constituents classified as flavonoids (anthocyanins, flavan-3-olmonomers and polymers, flavonols and dihydroflavonols) and non-flavonoids (hydroxybenzoic acid and hydroxycinnamic acid and their derivatives, stilbenes and phenolic alcohols) that play a major role in enology [1].

The polyphenolic compounds are responsible for the characteristics, color and quality of wines. In red wine, proanthocyanidins (condensed tannins) and anthocyanins are the most important phenolic classes. Proanthocyanidins are the compounds responsible for bitterness, and astringency and anthocyanins, for the color stability of red wines. Tannins contribute to the mouth feel of wines, but they also form pigmented polymers in association with the anthocyanins to provide the stable pigments which give the long-term stable color of the red wine.

Grape anthocyanins are red pigments, located in the first external layers of the hypodermal tissue and mainly in the vacuole, as well as in special structures called anthocyanoplasts [2, 3]. Anthocyanins are the first components that are extracted from the grape skins together with the skin tannins at the beginning of fermentation. During winemaking and wine aging, anthocyanins may be modified to create stable substituted pigments and wine making and colored and non-colored phenolics have an important role on the color, taste and the mouth feel of wines.

Wine-making 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 anthocyanins and copigmentation cofactors. Over the past few decades there is an increased interest in application of enzymes in the wine-making process. This is due to the fact that enzymatic treatments of grape, musts and wine have multiple purposes, because they influence wine clarification and filtration, juice yield, color and aroma extraction as well as wine stability [4–8].

Pectolytic enzyme preparations commonly used in fruit and wine processing industry, and in general, are the most effective at degrading polysaccharides, to increase extraction of colored compounds and releasing phenols. Meyer *et al.* [9] compared the phenol release from grape pomace by using conventional solid-liquid extraction and extraction assisted by pectinases. They reported an increase of ~ 32 % of the phenol release, confirming the use of enzymes as a tool to improve conventional extraction. Pardo *et al.* [10] obtained an increase of ~ 40 % in the amount of anthocyanins, during vinification and conservation of wines treated with pectinases. Bautista *et al.* [11] also showed significant differences in the amount of total phenols when some wine samples were subjected to pectinase maceration. Numerous recent studies have reported on the use of enzymes in wineries to facilitate the extraction of grape phenolics and to aid color stability [12–16].

Since there are not published results for the effects concentrations and aging of pectolytic enzyme preparations on the phenolic content and chromatic characteristics of Vranec wines from Macedonia the main

purpose of this study was to conduct laboratory trials to evaluate the efficacy of two commercial macerating pectolytic enzyme preparations (Vinozym Vintage FCE and Rohapect VR-C) on phenolics extraction and color intensity of Vranec wines.

The total phenolic content, total anthocyanins, total flavonoids, total catechins, color intensity and Hue of those wines and control wines with no added enzyme have been determined in two phases after maceration and 6 months of storage.

II. EXPERIMENTAL

A. Pectolytic enzyme preparations

- 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

These enzyme preparations are derived from cultures of *Aspergillus niger* which is a species accepted as G.R.A.S. (Generally Recognized As Safe) [17]. All enzyme preparations were first diluted to a 10 % solution using cool, clean water, and added to the freshly crushed grapes and an equivalent volume of deionized water as a replacement for the enzyme which was added to the control treatment.

B. Grape samples

Grapes from Vranec variety *(Vitis vinifera* L.), cultivated in Ovce Pole vineyard, Povardarie wine region, harvested into 20 kg boxes at optimal maturity (22 ^{o}Brix, 6.5–7.5 g/L total acids and pH 3.1–3.3) (2013 vintage) were used

C. Wine samples. Micro-vinification

Wines were prepared in the experimental laboratory of Imako Winery Stip using a standardised microvinification procedure. Grapes were separately processed (crushed and destemmed) in the same way, and crushed grapes were collected in 10 L plastic fermentation tanks, and 50 mg/L SO_2 was added to each portion of musts after crushing. Pre-fermentation enzyme maceration for 12 hours was carried out at a 20 °C temperature in controlled room with addition of one commercial pectolytic enzyme preparation with four different doses. Then, musts were inoculated with 200 mg/kg selected yeast *(Saccharomyces cerevisiae)* NEUTRE SC (Lallemand), and plunged twice a day until completion of fermentation. The control vinifications and the enzyme treated wines were all made in triplicate. After the period of maceration, the pomace was removed. The new wines were inoculated with selected malolactic bacteria (Lalvin) and cold stabilized at – 4 °C. Control trials were in all same with experimental trials exept for conducting pectolytic enzyme. Wines were filtered and bottled in 0.5 L glass bottles with addition of 30 mg/L SO_2 and stored 6 months.

After 6 months of storage, wines were analyzed and results were compared in order to check the influence of storage temperature on the polyphenolic concentration. Color intensity and concentration of total phenolics, total anthocyanins, total flavonoids and total catechins in all wines were analyzed after 6 days and after 6 months of storage at the 20 ^{o}C.

D. Instrumentation, chemicals and standards

Agilent 8453 UV-Vis spectrophotometer was used for analysis of polyphenolic components and chromatic characteristics in the wines. All analyses were performed in triplicate. The reagent p-(dimethylamino) cinamaldehyde (*p*-DMACA), gallic acid and (+)-catechin were from Fluka (Switzerland), and the Folin-Ciocalteu reagent was from Merck (Germany). All the other used reagents were of analytical purity grade.

E. Wine chemical characteristics

Wine analysis for pH-value, ethanol content, titrable acidity, relative density, total extract, reducing sugars, ash and total SO_2 were determined according to the OIV Official methods [18].

F. Wine chromatic characteristics

The color intensity (CI) was calculated as the sum of the absorbances of undiluted wine at 620 nm (blue component of the color wine), 520 nm (red component of the color wine) and 420 nm (yellow component of the

color wine), according to Glories [19]. The Hue of wine was calculated as the ratio of absorbance at 420 nm and absorbance at 520 nm (A_{420}/A_{520}).

G. Total phenolics determination

Total phenolics in wine samples were determined with Folin-Ciocalteu method [20, 21]. In brief, an aliquot (1 mL) of appropriate diluted wine was added to a 10 mL volumetric flask, containing 5 mL of distilled water. Then, 0.5 mL of Folin-Ciocalteu reagent was added and mixed. After 3 min, 1.5 mL 20 % solution of Na_2CO_3, followed with addition of distilled water making up the total volume to 10 mL. After 16 min storage of the samples at 50 $^{\circ}$C (water bath) in sealed flasks, samples were cooled and read their absorbencies at 765 nm against the blank prepared with distilled water. The concentration of total phenolics was expressed as gallic acid equivalents (mg/L GAE) based on a calibration curve obtained with using standard of gallic acid.

H. Total anthocyanins determination

The determination of the total anthocyanins was performed by the method proposed by Di Stefano *et al.* [22] Samples were diluted with a solution consisting of $C_2H_5OH/H_2O/HCl$=69/30/1 *(v/v/v)* and the absorbance was measured at 540 nm. The concentrations of anthocyanins was calculated using the equation:

$$TA_{\,540\ nm}\ (\text{mg/L}) = A_{540\ nm}\ 16.7\ d \qquad (1)$$

$A_{540\ nm}$ - absorbance at 540 nm
d - dilution, expressed as malvidin-3-glucoside equivalents.

I. Total catechins determination

The concentration of total catechins (procyanidins monomers) in wines was determined using the method of Di Stefano *et al.* [23] with The reagent *p*-(dimethylamino) cinamaldehyde (*p*-DMACA) and catechin as standard for construction of the calibration curve. An appropriate diluted wine sample (1 mL) was added to a 10 mL volumetric flask, followed by adding of 3 drops of glycerol and 5 mL 1 % *p*-DMACA reagent, so that the total volume was made up to 10 mL with methanol. The absorbance was read after 7 min at 640 nm against the blank-methanol. The reagent *p*-DMACA was prepared before use, and contained 1 % (w/v) DMACA in a cold mixture of methanol and HCl (4:1).

J. Total flavonoids determination

Total flavonoids were determined using the colorimetric assay with aluminum chloride [21, 24] and (+)-catechin as standard for construction of the calibration curve and the concentrations are expressed as catechin equivalents (mg/g CE). An aliquot of 1 mL of wine sample (appropriate diluted) was added to a 10 mL volumetric flask containing 4 mL of distilled water, followed with addition of 0.3 mL of 5 % $NaNO_2$ and 5 min later, 0.3 mL of 10 % $AlCl_3$ was added. After 6 min, 2 mL of NaOH (1mol/L) was added to the mixture and the total volume was made up to 10 mL with distilled water, and the absorbance was measured at 510 nm against the prepared water blank.

III. RESULTS AND DISSCUSION

The bottled Vranec wines were placed at 15 °C in a cellar, and the basic parameters of pH-value, ethanol content, total acidity, specific gravity, total extract, reducing sugars, ash and total SO_2 after six months were analyzed. There were no significant differences between enzyme treatments and control treatment in the basic chemical composition in the analyzed wines. No differences were found in percent alcohol and total acidity [12, 25].

Pectolytic enzyme preparations commonly used in fruit and wine processing industry, and in general, are the most effective at degrading polysaccharides, to increase extraction of colored compounds and releasing phenols. Meyer et al. 9 compared the phenol release from grape pomace by using conventional solid-liquid extraction and extraction assisted by pectinases. They reported an increase of ~ 32 % of the phenol release, confirming the use of enzymes as a tool to improve conventional extraction. In this investigation we found that, concentration of total phenols are from 3089.5 to 3962.7 mg L^{-1}, higher 32.3 % for sample treated with 3g $10^{-2}L^{-1}$ Vinozym Vintage FCE (VFCE) and aged 6 months (Fig.1).

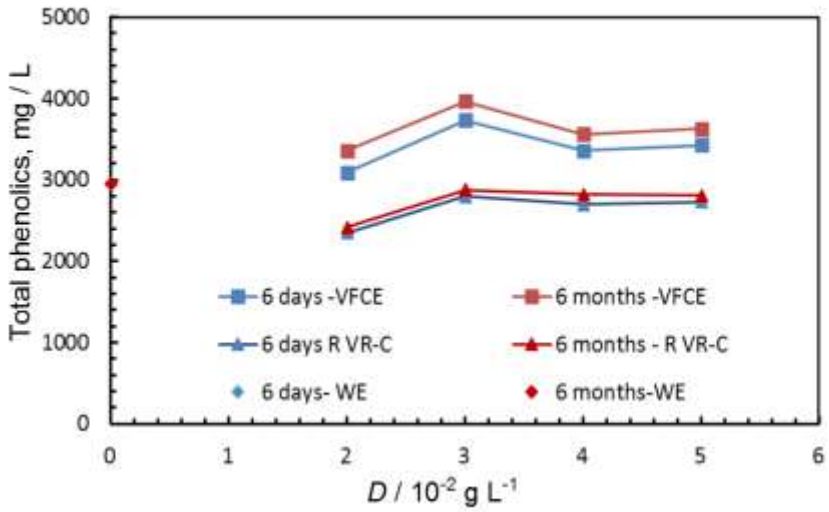

Figure 1. Effects of enzyme dose on total phenolics content in Vranec wines with different aging period, VFCE –treated with Vinozym Vintage VFCE, R VR-C –treated with Rohapect VR-C, WE untreated

Concentrations on phenolic content and in the samples treated 6 days with VFCE are increased, compared with samples without enzyme and treated Rohapect VR-C (R VR-C). Concentrations of phenols in samples treated with R VR-C are lower (2350.7- 2807.5 mg L^{-1}) for all doses of enzyme and aging. Concentration in samples without enzyme (WE) is 2995.5 mg L^{-1} and is not changed with aging.

Pardo et al. 10 obtained an increase of ~ 40 % in the amount of anthocyanins from 220 to 305 mg/L during vinification and conservation of wines treated with pecinases. Bautista et al. /11/ also showed significant differences in the amount of total phenols when some wine samples were subjected to pectinase maceration. Numerous recent studies have reported on the use of enzymes in wineries to facilitate the extraction of grape phenolics and to aid color stability [12–16].

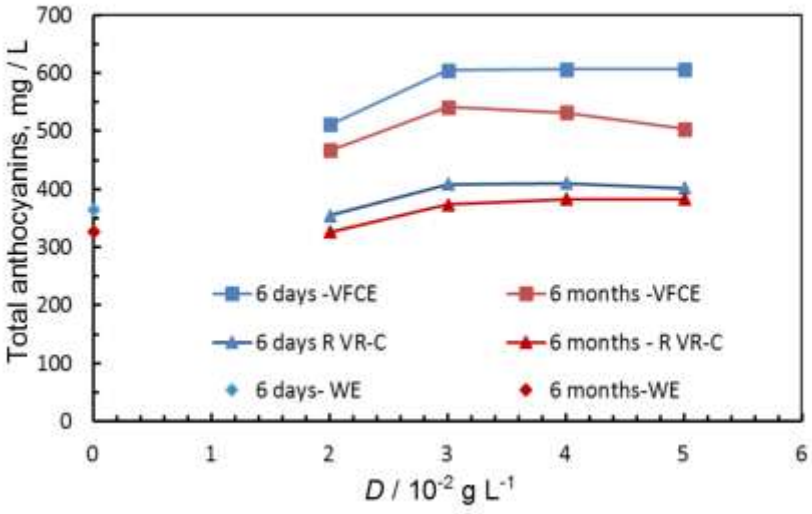

Figure 2. Effects of enzyme dose on total anthocianins content in Vranec wines with different aging period , VFCE –treated with Vinozym Vintage FCE, R VR-C –treated with Rohapect VR-C, WE untreated

The concentrations of anthocyanins in WE samples were 364 and 327 mg L^{-1} for samples and enzyme treated are close with referred /10/. In the enzyme treated samples content of anthocyanins is from 511-606 mg L^{-1}, for VFCE, and 354-410 for R VR-C (Figure 2), values higher up to 66 % for VFCE samples and up to 10 % for R VR-C. The concentration of anthocyanins decreased with aging.

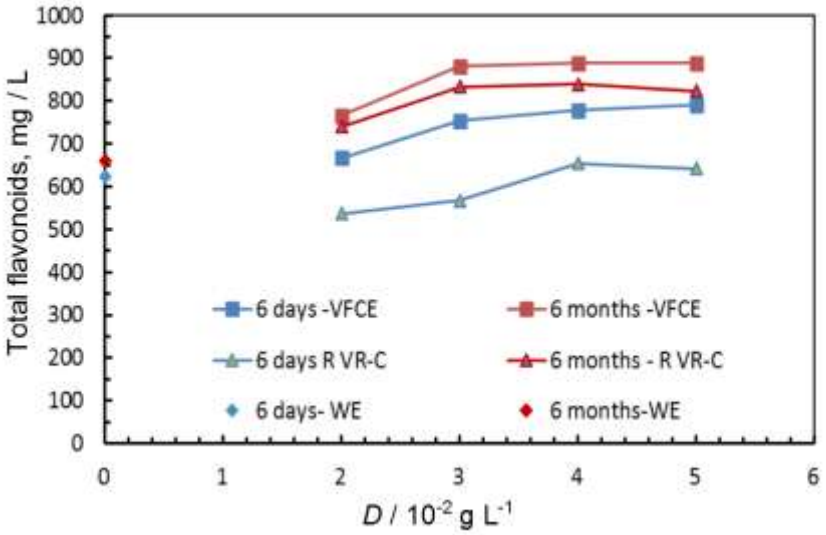

Figure 3. Effects of enzyme dose on total flavonoids content in Vranec wines with different aging period, VFCE –treated with Vinozym Vintage FCE, R VR-C –treated with Rohapect VR-C, WE-untreated

Dependence at enzyme and aging at flavonoids content is given at Figure 3. The content is higher in treated samples with VFCE 7- 27 % and rise with aging for 11 %. Aged samples treated with R VR-C have higher content at flavonoids than WE samples. The content rises with aging for all doses especially for R VRC samples.

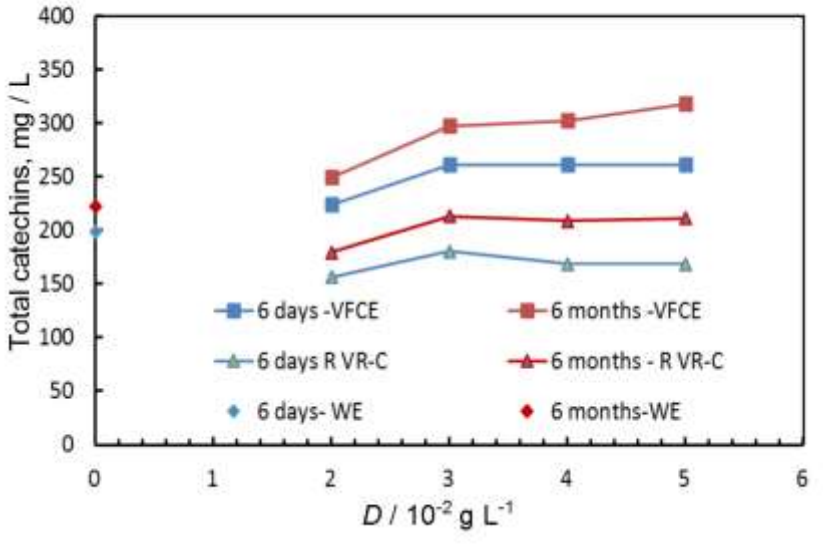

Figure 4. Effects of enzyme dose on total cathecines content in Vranec wines with different aging period, VFCE –treated with Vinozym Vintage FCE, R VR-C –treated with Rohapect VR-C, WE untreated

The concentrations at cathecines are 224 - 261 mg L^{-1} for VFCE treated samples, and 156 – 168 mg L^{-1} for samples treated with R VR-C, compared with 199 mg L^{-1} for WE samples. The concentrations of cathecines and rise for 10-20 % with aging /27, 28/.

Spectrophotometric determinations of color intensity (CI) and Hue (H) were performed for wines samples and differences were observed in wines with enzyme treatments after fermentation and after 6 months of storage.

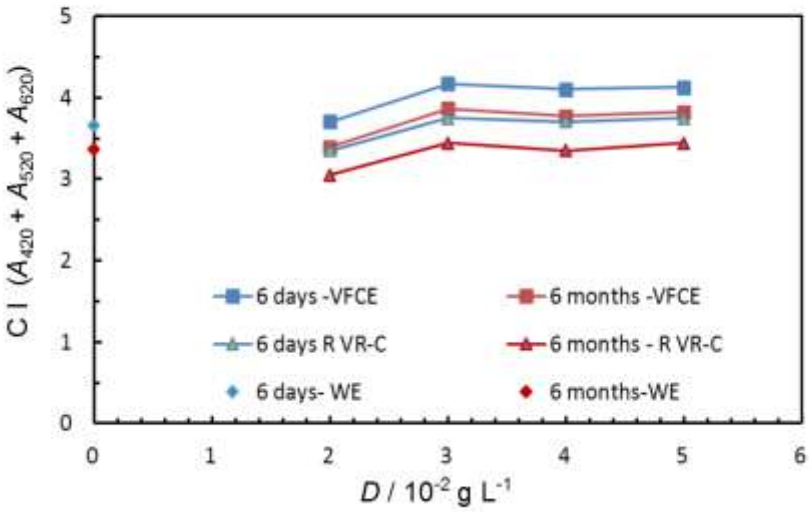

Figure 5. Effects of enzyme dose on color intensity in Vranec wines with different aging period, VFCE –treated with Vinozym Vintage FCE, R VR-C –treated with Rohapect VR-C, WE untreated

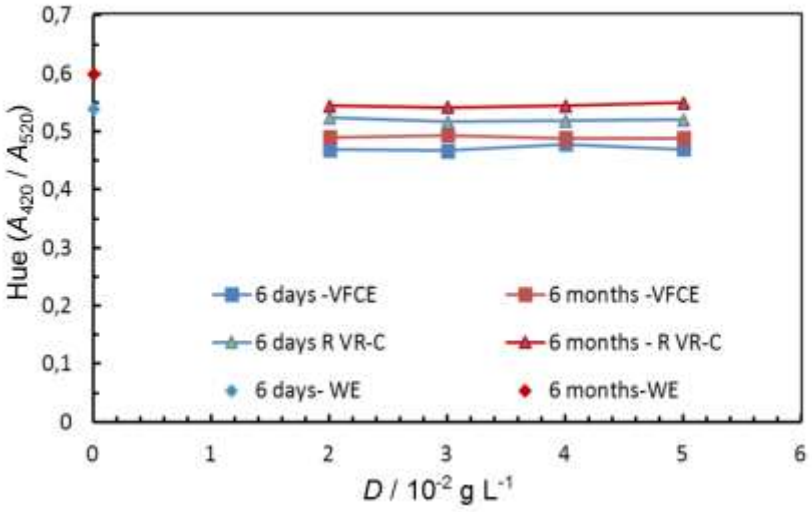

Figure 6. Effects of enzyme dose on Hue in Vranec wines with different aging period, VFCE – treated with Vinozym Vintage FCE, R VR-C – treated with Rohapect VR-C, WE untreated

Enzyme preparations Vinozym Vintage have more intensive extraction of red grape pigments except at Rohapect VR-C, who have a lower CI and higher Hue values [9-16, 29-31]. The intensity of color rises with aging for all enzyme treated samples in spite of WE samples where color intensity decreases. Effects of storage, enzyme concentration, temperature and maceration time on the Vranec wine quality, will be investigated in the feature works.

IV. CONCLUSIONS

Over the last two decades commercial enzyme preparations have gained enormous popularity in the wine industry and they are effective, specific and convenient to use, and it can be expected that the search for enzymes with improved characteristics will continue.

Apart from traditional maceration, alternative extraction technologies include enzyme methods. The application of pectolytic enzymes would facilitate breakup of the grape cell wall enabling more rapid release of anthocyanins from the anthocyanoplasts, and would also aid juice and wine clarification by breaking-down the released grape pectins. Enzyme treatment also resulted in wines with increased levels of total phenolics. From Vranec grapes, commercial pectolytic enzyme preparations Vinozym Vintage FCE produce wines with significantly higher percentage red color and phenolic compounds. Rohapect VR-C had minor effects the color and total phenolics content.

By using appropriate enzyme, wines can be produced in a shorter period of time, with less cost, tank space, refrigeration and labor. There are tremendous potential benefits for the wine producer and the consumer alike in the application of these exciting new technologies and developments.

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