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Effects of pectolytic enzyme treatments on white grape mashs of *Smederevka* on polyphenolic content of wines

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Abstract

The aim of this work was to study the effect of the use of pectolytic enzymes on the phenolic composition of young white wines made of white grape *Smederevka (V. vinifera* L.*)*. White grape mash from *Smederevka* were macerated for 4 hours at 18 to 20 °C with addition on one commercial pectolytic enzyme preparation (Vinozym Process, Trenolin Mash DF, Rohavin LX). The obtained results of laboratory trials showed decreased concentration of total phenolics by 37.8% and results of industry trials decreased by 39.2%. These enzyme treatments resulted in clarified the white grape juice, degraded the grape mash, reduced juice viscosity and total phenols and elicited an improvement in the visual aspect of the wine.

Key words: pectolytic enzymes, wine-making, total phenolics, flavonoids, catechins

Utjecaj pektolitičkih enzima u masulju grožđa Smederevka na koncentraciju polifenola u vinu

Sažetak

Cilj ovog rada bio je odrediti utjecaj pektolitičkih enzima na fenolni sastav mladih vina Smederevka (*V. vinifera*). Masulj grožđa Smederevka maceriran je 4 sata na 18 do 20 °C sa dodatkom jednog komercijalnog pektolitičkog enzimskog preparata (Vinozym Process, Trenolin Mash DF and Rohavin LX). Dobiveni rezultati laboratorijskog pokusa pokazali su smanjenje koncentracije ukupnih fenola od 37.8%, a rezultati industrijskog pokusa smanjenje od 39.2%. Primjena pektolitičkih enzima utjecala je na bistroću mošta, smanjenje viskoziteta i ukupnih fenola te su pozitivno utjecali na vizualni izgled vina.

Ključne riječi: pektolitički enzimi, vino, polifenoli, Smederevka

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Introduction

Smederevka is a variety of white grape cultivated in Republic of Macedonia used for production of quality wines. Although the composition of the grape depends on its variety, the soil and climatic conditions, there is little variation in the actual cell structure of the plant. Wines and grapes contain a number of polyphenolic constituents classified as flavonoids (anthocyanins, flavan-3-ol-monomers and polymers, flavonols and dihydroflavonols) and non-flavonoids (hydroxybenzoic acid and hydroxycinnamic acid and their derivatives, stilbenes and phenolic alcohols) that play a mayor role in enology. Red wines contain all the above phenolics, while white wines contain mainly phenolic acids and flavanols (Ribéreau-Gayon et al., 2000). Wine phenolics are important quality components that contribute to the colour, taste, and feel of wines. Pectolytic enzyme preparations are the most widely used in fruit and wine processing industry and in general are the most efficient at degrading polysaccharide. Numerous studies have reported on the use of commercial enzymes in white grape juice clarification, enzyme treatment effects on quality of white grape musts and wines, effects of different commercial pectolytic enzyme preparations on white grape musts and wines et al. (Ough C. and Crowell E., 1979; Brown M. and Ough C., 1981; Sreenath H. and Krishnaswamy S., 1992; Lao et al., 1997). Treating crushed white grapes with pectic enzyme increases juice yield, sensory tests indicate that wine quality is either enhanced or unchanged (Ough C. and Crowell E., 1979). White grape musts of eight different varieties were treated with pectic enzyme preparations.These treatments resulted in increases in total juice yields, clarify of the wine, filterability, methanol production, wine quality, browning capacity and amounts of solids that settled (Brown M. and Ough C., 1981). A commercial pectinase from *Aspergillus niger* containing various polysaccharases clarified the white grape juice to an extent of 98-99% and also degraded the grape mash by 25-30%. After pectinolytic juice clarification, both juice viscosity and total phenols were reduced by 25% and 32% respectively (Sreenath H. and Krishnaswamy S., 1992). Grapes of three white cultivars were treated with a commercial pectic enzyme preparation on an industrial scale. Pectic enzyme treatment increased the higher alcohols, free hydroxycinnamic acids and volatile phenols, whereas amounts of most esters, herbaceous alcohols and hydroxycinnamate derivative esters were reduced. When tasted by an expert panel, differences were found in sensory characteristics, with wines from untreated judged higher quality than those from treated grapes (Lao et al., 1997). Enzyme treatments on mushs speed up settling and ensure efficient sediment compaction (Cruess et al., 1951; Höhn et al., 2005; Trepo, 2008). The clear must is then racked from the lees without problems.

Material and methods

Commercial pectolytic enzyme preparations

In this study were used for laboratory trials three commercial macerating pectolytic enzyme preparations with four doses:

Vinozym Process, Novozymes A/S, Bagsvaerd, Denmark;

Doses: 3, 4, 5, and 6 g/100 kg grapes (I-1, I-2, I-3, I-4)

Trenolin Mash DF, Erbslöh Geisenheim AG, Geisenheim, Germany);

Doses: 1, 2, 3, and 4 mL/100 kg grapes (II-1, II-2, II-3, II-4)

Rohavin LX, AB Enzymes GmbH, Darmstadt, Germany;

Doses: 2, 3, 4, and 5 mL/100 kg grapes (III-1, III-2, III-3, III-4)

and for industrial trials used one commercial pectolytic enzyme preparation (Trenolin Mash DF, 2 mL/100 kg grapes) along with controls with no added enzyme(IV). These enzyme preparations are derived from cultures of Aspergillus niger which is a species accepted as G.R.A.S. (Generally Recognized As Safe) (Canal-Llauberes, 1993).

Grape samples for laboratory trials

The white grape cultivar *Smederevka* (*Vitis vinifera*), cultivated in the Ovce pole vineyard, the Povardarie region, Republic of Macedonia, were harvested at optimal maturity (2009 vintage), at 170-190 g L^{-1} sugar, 6.0-7.0 g L^{-1} total acids, and pH from 3.0 to 3.2, and transported to the private winery "Imako Vino" Stip, Republic of Macedonia.

Grape samples for industrial trials

The white grape cultivar *Smederevka* (*Vitis vinifera),* cultivated in the Veles vineyard, the Povardarie region, Republic of Macedonia, were harvested at optimal maturity (2009 vintage), at 180-200 g L^{-1} sugar, 6.0-7.0 g L^{-1} total acids, and pH from 3.0 to 3.2, and transported to the private winery "Tristo" Veles, Republic of Macedonia.

Vinification

The grapes were weighed, destemmed, crushed and divided in 5 liters plastic reservoirs for laboratory trials, and for industrial trials were placed in a stainless steel fermentor (4 t.). All laboratory treatments were performed in triplicate and industrial in duplicate. White grape mash made from *Smederevka* were macerated for 4 hours at 18 to 20 °C with addition on one commercial pectolytic enzyme preparation. Control laboratory trials were in all same with experimental trials only whitout added pectolytic enzyme preparation. After maceration the pomace was removed and each must are pour into the funnel and collect musts in 5 liters plastic reservoirs (3 for enzyme treatments musts and 1 for control must (no-treatment). In each reservoir are add 30 ppm SO_2 and are keept musts cool (15-16 °C) and allow to stand overnight, so that suspended material will fall to the bottom. The clear must is then racked from the lees without problems. In each musts (reservoir) are add yeast (*Saccharomyces cerevisiae*) NEUTRE SC (Lallemand) (200 mg/kg grapes) at ~25 °C to completion of fermentation. The bottled wines (0.5 l) were stored at 4 - 6 °C, and phenolic compounds were measurmed after 6 months wine maturation.

Instrumentation and reagents

Analysis of polyphenolic components was carried out with a Aglient 8453 UV-Vis spectrophotometer. All analyses were performed in duplicate. The reagent p-(dimethylamino)cinnamaldehyde (*p*-DMACA), standards of gallic acid and (+)-catechin were from Fluka, and the Folin-Ciocalteu reagent was from Merck. All other used reagents were of analytical purity grade.

Total phenolics assay

The Folin-Ciocalteu method was used for the determination of the total phenolics (Ivanova et al., 2010).

Total flavonoids assay

Total flavonoids were determined using the colorimetric assay with aluminium chloride and (+)-catechin as standard for calibration according to Zhishen et al., (1999).

Total catechins assay

The concentration of total catechins in wines was determined using the method of Di Stefano et al. (1989) with the reagent p-DMACA.

Results and discussion

Spectrophotometric determinations of total phenolics (TP), total flavonoids (TF), total catechins (TC), were performed for the *Smederevka* wines obtained with pre-fermentation enzyme maceration (4 hours) on grape mashs, and after alcoholic fermentation to completion of fermentation. Measurements were performed in order to study the effects of three different macerating enzymes on polyphenol contents of *Smederevka* wines from Macedonia compared to control wines no added enzyme. The obtained results of laboratory trials (Table 1) showed decreased concentration of total phenolics (TP) from 699.1 mg L^{-1} to 422.5 mg L^{-1} (37.8%) compared to the control samples from 679.1 mg L^{-1}. Pectolytic enzyme preparation Vinozym Process (from 699.1 mg L^{-1} to 558.8 mg L^{-1}, decreased 17.7%) Trenolin Mash DF (from 550.2 mg L^{-1} to 488.1 mg L^{-1}, decreased 28.1%) and Rohavin LX (from 483.7 mg L^{-1} to 422.5 mg L^{-1}, decreased 37.7%), depend of used doses.

Table 1. Effects of pectolytic enzyme treatments on white grape mashs of *Smederevka* on phenolic content of wines (TP: total phenolics, TF: total flavonoids, TC: total catechins). Laboratory trials: 5 kg grape

Enzyme preparations	Dose	aTP mg L-1	aTF mg L-1	aTC mg L-1
Vinozym Process	I-1	558.8 ± 0.25	54.83 ± 0.02	5.70 ± 0.06
3;4;5;6 g/100kg grapes	I-2	699.1 ± 1.35	91.46 ± 0.01	8.49 ± 0.15
	I-3	595.2 ± 0.00	83.06 ± 0.17	7.39 ± 0.00
	I-4	604.0 ± 0.45	64.91 ± 0.03	6.37 ± 0.28
Trenolin Mash DF	II-1	550.2 ± 0.50	66.17 ± 0.08	6.99 ± 0.03
1;2;3;4 ml/100kg grapes	II-2	488.1 ± 0.95	39.86 ± 0.02	4.26 ± 0.02
	II-3	521.9 ± 1.40	40.30 ± 0.02	4.06 ± 0.03
	II-4	512.5 ± 1.75	62.05 ± 0.03	7.21 ± 0.02
Rohavin LX	III-1	449.6 ± 0.35	38.63 ± 0.03	3.65 ± 0.01
2;3;4;5 ml/100kg grapes	III-2	422.5 ± 1.30	33.40 ± 0.02	3.48 ± 0.07
	III-3	483.7 ± 0.55	38.43 ± 0.12	4.49 ± 0.01
	III-4	453.5 ± 1.25	33.33 ± 0.02	3.84 ± 0.00
Control-no added enzyme	IV	679.1 ± 2.55	80.12 ± 0.99	8.74 ± 0.03

a the values are average from 2 replicates±SD

Table 2. Effects of pectolytic enzyme treatments on white grape mashs of *Smederevka* on phenolic content of wines (TP: total phenolics, TF: total flavonoids, TC: total catechins). Industrial trials: 1220 kg grape. Private winery "Tristo" Veles

Enzyme preparations	Dose	aTP mg L-1	aTF mg L-1	aTC mg L-1
Trenolin Mash DF	II-2	341.6 ± 2.65	40.81 ± 0.12	4.09 ± 0.03
2 ml/100kg grapes				
Control-no added enzyme	IV	562.3 ± 2.50	87.63 ± 0.16	11.55 ± 0.07

a the values are average from 2 replicates±SD

The obtained results of industry trials(Table 2) showed decreased concentration of total phenolic (TP) from 341.6 mg L^{-1} compared to the control sample from 562.3 mg L^{-1} (decreased 39.2%). These enzyme treatments resulted in clarified the white grape juice, degraded the grape mash, reduced juice viscosity and total phenols and elicited an improvement in the visual aspect of the wine. The obtained results for the analysed wines were in agreement with previously published data (Ough C. and Crowell E., 1979; Brown M. and Ough C., 1981; Sreenath H. and Krishnaswamy S., 1992; Lao et al., 1997).

Conclusion

The application of pectolytic enzymes would facilitate break up of the grape cell wall and aid juice and wine clarification by breaking-down the released grape pectins. Enzyme treatment also resulted in white wines with reduced juice viscosity and total phenols and elicited an improvement in the visual aspect of the wine.

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References

Brown M.R., Ough C.S. (1981) A Comparison of Activity and Effects of Two Commercial Pectic Enzyme Preparations on White Grape Musts and Wines. American Journal of Enology and Viticulture. 32:4: 272-276

Canal-Llauberes R.M. (1993) Enzymes in winemaking. In: Wine Microbiology and Biotechnology. Fleet G.H.,(Ed.). 477-506, Philadelphia, Harwood Academic Publishers

Cruess, W. V., O'Neal, R., Chong, G., Uchimoto, D. (1951) The Effect of Pectic Enzymes in Wine Making. Am. J. Enol. Vitic., 2:1: 59-75

Di Stefano R., Cravero M.C., Gentilini N. (1989): Metodi per lo studio deipolifenoli dei vini. L'enotecnico I. Maggio. 25:5: 83-89

Höhn, A., Daqing, S., and Nolle, F. (2005) Enzymes in Fruit Juice and Wine Industry.,Ch.5. In: Processing Fruits, Science and Technology, Second Edition, Barrett, D.M., Somogyi, L.P., and Ramaswamy, H.S., 98-112, CRC Press LLC.

Ivanova V., Stefova M., Chinnici F. (2010) Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods. J. Serb. Chem. Soc. 75:1: 45-59.

Lao,C., López-Tamames,E., Lamuela-Raventós,R.M., Buxaderas,S., Del Carmen de la Torre-Boronat,M. (1997) Pectic Enzyme Treatment Effects on Quality of White Grape Musts and Wines. Journal of Food Science. 62:6: 1142-1149

Ough,C.S., Crowell,E.A. (1979) Pectic-enzyme treatment of white grapes: Temperature, variety and skincontact time factors. Am.J.Enol.Vitic. 30:1: 22-27

Ribéreau-Gayon P., Glories Y., Maujean A., Dubourdieu D. (2000). Handbook of Enology, Volume 2: The Chemistry of Wine and Stabilization and Treatments. 141-205, England, John Wiley&Sons Ltd

Sreenath,H.K., Krishnaswamy,S. (1992) The use of commercial enzymes in white grape juice clarification. Journal of Fermentation and Bioengineering. 73:3: 241-243

Trepo, G. (2008) Enzymes for short maturation wines., Biotimes. 4: XI-XII

Zhishen J., Mengcheeneg T., Jianming W. (1999). The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555-559

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