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Comparison of effects of three commercial pectolytic enzyme preparations in white winemaking

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White grape mashes of *Smederevka* were treated with different pectolytic enzyme preparations. These treatments resulted in increases in clarity of the wine, filterability, amounts of solids that settled and wine quality. The results of the experiments provide comparison of the efficiency of preparations applicable in white winemaking. The time of filtration was two times shorter when using the enzyme preparations Trenolin Mash DF and Rohavin LX compared to the control sample. By using the enzyme preparations Vinozym Process, the speed of desliming was threefold faster, and in the case of Trenolin Mash and Rohavin LX twofold faster compared to the control sample. These enzyme treatments resulted in clarified the white grape juice, degraded the grape mash, reduced juice viscosity and elicited an improvement in the visual aspect of the wine.

Keywords: Pectolytic enzymes, white grape *Smederevka*, filterability, settling rate.

Introduction

Smederevka is a variety of white grape cultivated in Macedonia and it is used for production of quality wines. Although the composition of the grape depends on its variety, soil and climatic conditions, there is little variation in the actual cell structure of the plant. As pectic polysaccharides play a major role in cell walls rigidity they are the main limiting factor (Vidal et al., 2001). To the main polysaccharide chains other shorter or longer, straight or branched, saccharide chains are attached. Pectic enzyme, or pectinase, is an enzyme that breaks down the bonds of the large pectin molecules, which clears the haze and releases more of the fruit`s characteristics. The main enzymes used during winemaking are pectinases. Pectinases occur naturally in all fruit (including grapes) and are partly responsible for the ripening process. However, grape pectinases are 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 pectic enzymes play important role in braking down grape pulp and skin cells and are able to split those chains and saccharide bonds between the chains (Whitaker, 1984). Enzymes cannot act on grapes if they are whole. Therefore, grapes should always be crushed before enzymes are added to enhance extraction. For all pectic enzymes, the wine should be at least 27 °C for the enzyme to work. Pectic enzyme can be added at different times before and after fermentation to break down the pectin. Winemaking is a biotechnological process in which enzymes play a fundamental role. 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 (Ough and Crowell, 1979; Brown and Ough, 1981; Sreenath and Krishnaswamy, 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 and Crowell, 1979). White grape musts of eight different varieties were treated with pectic enzyme preparations. These treatments resulted in increases in total juice yields, clarity of the wine, filterability, methanol production, wine quality, browning capacity and amounts of solids that settled (Brown and Ough, 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%. 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 mushes 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.

Materials and methods

Commercial pectolytic enzyme preparations. This study 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).

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 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 (Macedonia).

Vinification. The grapes were weighed, destemmed, crushed and divided in 5 liters plastic reservoirs for laboratory trials. All laboratory treatments were performed in triplicate. White grape mashes made from Smederevka were macerated for 4 hours at 18 to 20 ^{o}C with addition on one commercial pectolytic enzyme preparation. Control laboratory trials were similar to experimental trials, only without added pectolytic enzyme preparation.

After maceration the pomace was removed and each must were poured into the funnel and collected in 5 liter plastic reservoirs (3 for enzyme treatments musts and 1 for control must (no-treatment). 30 ppm SO_2 were added in each reservoir, musts were kept cool (15-16 ^{o}C) and stood overnight to make suspended material fallen to the bottom. The clear must was then racked from the lees without problems. In each musts (reservoir) there was added yeast (*Saccharomyces cerevisiae*) NEUTRE SC (Lallemand) (200 mg/kg grapes) at ~25 ^{o}C to provide the completion of fermentation.

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 after one day pre-fermentation of mash.

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) after one day pre-fermentation of mash.

Results and discussion

The structure of insoluble pectin in grape skin cell walls is more complex than pulp soluble pectins. Grape cell walls form a physical barrier between the juice in the vacuole of berry cells and the outside medium. Since grape cell walls contain about 30% of pectin, pectinases help to break this physical barrier and therefore increase the yield per ton of grapes obtained.

With enzymes, winemakers can enhance aroma, improve colour, clarity of the wine, filterability and amounts of solids that settled. Enzymes are very popular in white 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 help with reducing viscosity, releasing juice easily, and facilitating filtration.

Effects of enzyme treatments on the filtration speed of white 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.

TABLE 1. EFFECTS OF ENZYME TREATMENTS ON THE TIME OF FILTRATION OF THE WINE SAMPLES AFTER ONE DAY PRE-FERMENTATION OF MASH

Enzyme preparations	Dose	[a] Time of filtration, (min)	Time of filtration Vs control	Speed of filtration, (ml/min)
Vinozym Process				
I-1=3g/100kg grape	I-1	26.333 ± 0.136	0.792	0.379
I-2=4g/100kg grape	I-2	20.555 ± 0.207	0.618	0.486
I-3=5g/100kg grape	I-3	20.555 ± 0.207	0.618	0.486
I-4=6g/100kg grape	I-4	20.555 ± 0.207	0.618	0.486
Trenolin Mash DF				
II-1=1ml/100kg grape	II-1	19.333 ± 0.136	0.582	0.517
II-2=2ml/100kg grape	II-2	18.166 ± 0.135	0.546	0.550
II-3=3ml/100kg grape	II-3	18.166 ± 0.135	0.546	0.550
II-4=4ml/100kg grape	II-4	18.166 ± 0.135	0.546	0.550
Rohavin LX				
III-1=2ml/100kg grape	III-1	20.166 ± 0.135	0.607	0.495
III-2=3ml/100kg grape	III-2	19.166 ± 0.135	0.577	0.521
III-3=4ml/100kg grape	III-3	19.166 ± 0.135	0.577	0.521
III-4=5ml/100kg grape	III-4	19.166 ± 0.135	0.577	0.521
Control-no added enzyme	0	33.222 ± 0.207	1.000	0.301

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

Table 1 shows results for the effect of the use of pectolytic enzymes on the filtration speed of white *Smederevka* wines and control trials "no-enzyme addition". In Table 1 it can be seen decreased time of filtration by 18.166 min (control 33.222 min). By using the enzyme preparation the Vinozym Process was 1.26-1.61 times shorter.

The time of filtration in the case of the Trenolin Mash DF was 1.72-1.83 and with Rohavin LX 1.65-1.73 times shorter, depend of used doses. The obtained results for the analyzed wines were in agreement with previously published (Canal-Llauberes, 1993; Villettaz and Dubourdien, 1991; Plank and Zent, 1993; Rogerson et al., 2000; Čapounova and Drdak, 2002; Revilla and Gonzalez-SanJose, 2002, Trepo, 2008).

Effects of enzyme treatments on the speed of sedimentation of white wines

Table 2 shows results for the effect of the use of pectolytic enzymes on the speed of sedimentation of white *Smederevka* wines and contol trials "no-enzyme addition". By using the enzyme preparation Vinozym Process, the thickness of the sediment was by 4.3 cm, i.e. the speed of desliming was threefold faster compared to the control sample (1.5 cm). The speed of desliming was in the case of Trenolin Mash and Rohavin LX was twofold faster (sediment by 2.9 cm) compared to the control sample. The obtained results for the analyzed wines were in agreement with previously published (Sims and Bates, 1994; Ribereau-Gayon et al., 2000; Čapounova and Drdak, 2002).

TABLE 2. EFFECTS OF ENZYME TREATMENTS ON THE SPEED OF SEDIMENTATION OF THE WINE SAMPLES AFTER ONE DAY PRE-FERMENTATION OF MASH

Enzyme preparations	Dose	The thickness of the sediment, (cm)
Vinozym Process		
I-1=3g/100kg grape	I-1	2.10 ± 0.081
I-2=4g/100kg grape	I-2	2.86 ± 0.047
I-3=5g/100kg grape	I-3	3.97 ± 0.081
I-4=6g/100kg grape	I-4	4.32 ± 0.047
Trenolin Mash DF		
II-1=1ml/100kg grape	II-1	1.83 ± 0.047
II-2=2ml/100kg grape	II-2	2.46 ± 0.047
II-3=3ml/100kg grape	II-3	2.83 ± 0.047
II-4=4ml/100kg grape	II-4	2.96 ± 0.047
Rohavin LX		
III-1=2ml/100kg grape	III-1	1.86 ± 0.047
III-2=3ml/100kg grape	III-2	2.33 ± 0.047
III-3=4ml/100kg grape	III-3	2.66 ± 0.047
III-4=5ml/100kg grape	III-4	2.81 ± 0.047
Control-no added enzyme	0	1.50 ± 0.081

Note: aThe 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).

TABLE 3. COMPARISON OF THE EFFECTS OF PECTOLYTIC ENZYME PREPARATIONS ON MASH AND WINE SAMPLES

Enzyme preparations	Clarity	Speed of filtration	Speed of settling
Vinozym Process	+ +	+	+ +
Trenolin Mash DF	+ +	+ +	+
Rohavin LX	+ +	+ +	+

Note: + + = distinct increase; + = slight increase.

After the application of pectolytic enzyme preparations during pre-fermentation of mash of white grape *Smederevka,* we obtained the results that are demostrated in Table 3.

Conclusion

Commercial pectolytic enzyme preparations offer significant processing improvements (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 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 with cost effective method. 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 .

References

Brown, M. and Ough, C., 1981. "A comparison of Activity and Effects of Two Commercial Pectic Enzyme Preparations on White Grape Musts and Wines," Am. J. Enol. Vitic., Vol. 32(4), pp.272-276.

Canal-Llaubères, R.M., 1993. "Enzymes in winemaking," In: Fleet, G., (Ed.), Wine microbiology and biotechnology, Harwood Academic Publishers, Philadelphia, pp.477-506.

Cruess, W., Neal, R., Chong, G., and Dan Uchimoto, 1951. "The effect of pectic enzymes in wine making," Am. J. Enol. Vitic., Vol. 2(1), pp.59-75.

Čapounova, D., and Drdak, M., 2002. "Comparison of some commercial pectic enzyme preparations applicable in wine technology," Czech J. Food Sci., Vol. 20, pp.131-34.

Höhn, A., Daqing, S., and Nolle, F., 2005. "Enzymes in fruit juice and wine industry," Ch.5, In: Barrett, D. et al., Processing fruits, science and technology, 2nd ed., CRC Press LLC, pp.98-112.

Lao, C., López-Tamames, E., Lamuela-Raventós, R., Buxaderas, S., and Del Carmen de la Torre-Boronat, M., 1997. "Pectic enzyme treatment effects on quality of white grape musts and wines," Journal of Food Science., Vol. 62(6), pp.1142-149.

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

Plank, P., and Zent, J., 1993. "Use of enzymes in wine making and grape processing," Ch.10, In: Gump, B. and Pruett, D. (Eds.), Beer and wine production analysis, characterization, and technological advances, American Chemical Society, Washington, D.C., pp.191-196.

Revilla, I., and Gonzàlez-SanJosé, M.L., 2002. "Addition of pectolytic enzymes: an enological practice which improves the chromaticity and stability of red wines," International Journal of Food Science & Technology., Vol. 38(1), pp.29-36.

Ribereau-Gayon, P., Glories, Y., Maujean, A., Dubourdien, D., 2000. Handbook of enology, Wiley, Chichester, UK, Vol. 2, pp. 129-185.

Rogerson, F.., Vale, E., Grande, H., and Silva, M., 2000. "Alternative processing of port-wine using pectolytic enzymes," Cienc. Technol. Aliment., Vol.2(5), pp.222-27.

Sims, C. and Bates, R., 1994. "Challenges to processing tropical fruit juices, banana as an example," Proc. Fla. State Hort. Soc., Vol.107, pp.315-19.

Sreenath, H., Krishnaswamy, S., 1992. "The use of commercial enzymes in white grape juice clarification," Journal of Fermentation and Bioengineering, Vol. 73(3), pp.241-43.

Trepo, G., 2008. "Enzymes for short maturation wines," Biotimes, No 4, XI-XII.

Vidal, S., Williams, P., O`Neil, M., Pellerin, P., 2001. Carbohydrate Polymers, Vol.45, pp.315-23.

Villettaz, J., Dubourdieu, D., 1991. Enzymes in wine-making. In: Fox, P. (Ed.), Food Enzymology, Elsevier Science Publishers Ltd., pp.427-51.

Whitaker, J., 1984. "Pectin supstances, pectin enzymes and haze formation in fruit juices," Enzyme Microb. Technol., Vol. 6, pp.341-49.

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