The Effect of Pectolytic Enzyme Treatments on Red Grapes Mash of Vranec on Grape Juice Yields

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 depends on its variety, the soil and the climatic conditions, there is little variation in the actual cell structure of the plant.

Pre-fermentation maceration or skin contact besides aroma release and improve wine color, increase juices yields and eases pressing and filtration operations (Cruess et al., 1954; Haight and Gump, 1994; Ganga et al., 2001; Canal-Llauberes and Pouns, 2002). Grape skin cell walls are a physical barrier on this aroma and juice diffusion. 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.

The pectic enzymes play an important role in breaking 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.

By weakening the cell walls of the pulp and hydrolyzing the soluble pectin, the enzymes in red grape maceration facilitate juice release and thus increase the free run juice yield, which avoids excessively harsh pressing (Brown and Ough, 1981; Ribereau-Gayon et al., 2000; Plank and Zent, 1993).

First of all, as the must is less viscous thanks to the degradation of pectins and other cell wall components such as cellulose, hemicellulose, and pressure applied to the grapes can be lower.

Materials and methods

Commercial pectolytic enzyme preparations. In this study were used for laboratory trials three commercial macerating pectolytic enzyme preparations with corresponding quantities suggested of producer:

- Vinozym Vintage FCE, Novozymes A/S, Bagsvaerd, Denmark; dose - 4 g/100 kg grape;
- Rohapect VR-C, AB Enzymes GmbH, Darmstadt, Germany; dose - 4 g/100 kg grape;
- Trenolin Rot DF, Erbslöh Geisenheim AG, Geisenheim, Germany); dose - 20 ml/100 kg grape.

For industrial trials we used one commercial pectolytic enzyme preparation (Vinozym Vintage FCE, 4 g/100 kg grape) along with controls with no added enzyme.

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 grape cultivars Vranec (Vitis vinifera), cultivated in the Ovce Pole vineyard, the Povardarie region, Republic of Macedonia, were harvested at optimal maturity (2009 vintage), at 200-220 g/l sugar, 6.5-7.5 g/l total acids, and pH from 3.1 to 3.3, and transported to the private winery “Imako Vino” Stip, Republic of Macedonia.
Grape samples for industrial trials. The grape cultivars Vranec (*Vitis vinifera*), cultivated in the Veles vineyard, the Povardarie region, Republic of Macedonia, were harvested at optimal maturity (2009 vintage), at 210-230 g/l sugar, 6.5-7.5 g/l total acids, and pH from 3.1 to 3.3, and transported to the private winery “Tristo” Veles, Republic of Macedonia.

Wine samples. Microvinification. Laboratory trials were conducted in laboratory of private winery “Imako Vino” Stip. Grapes for laboratory trials were weighed, crushed/destemmed and divided in 5 liters plastic fermentation tanks. Red grape mash were macerated for 6 hours (18-20 °C), with addition on one commercial pectolytic enzyme preparation. After addition of SO₂ (50 ppm) and yeast (*Saccharomyces cerevisiae*) NEUTRE SC (Lallemand, 2009) (200 mg/kg grape), maceration time of 5 days (~25 °C) was applied in order to study the effect of macerating enzymes on grape juice yields. After the maceration, the pomace was removed, in the obtained 4 different variations. Control trial was in all same with experimental trials only no added pectolytic enzyme preparation. All treatments were performed in duplicate.

Vinification. Industrial trials were conducted in private winery “Tristo” Veles. Grapes for industrial trials were weighed, crushed/destemmed and were placed in a vertical fermentor (4 t). Red grape mash were macerated for 6 hours (18-20 °C), with addition on one commercial pectolytic enzyme preparation. After addition of SO₂ (50 ppm) and yeast (*Saccharomyces cerevisiae*) NEUTRE SC (Lallemand, 2009) (200 mg/kg grape), maceration time of 5 days (~25 °C) was applied in order to study the effect of macerating enzymes on grape juice yields. After the maceration, the pomace was removed. Control trial was in all same with experimental trials only no added pectolytic enzyme preparation. Control trial was in all same with experimental trials only no added pectolytic enzyme preparation. All treatments were performed in duplicate.

![Experimental setup for grape vinification](image)

The enzyme preparations were first diluted to a 10% solution using cool, clean water, and added corresponding quantities (suggested of producer: Vinozym Vintage FCE, 4 g/100 kg; Rohapect VR-C, 4 g /100 kg; Trenolin Rot 20 ml /100 kg) to the freshly crushed grapes. At the “no-enzyme addition” (control trials) were added an equal amount of deionized water as a replacement for the enzyme additions. The contents of each reservoir were stirred thoroughly.

**Materials and procedure to measure free run juice yield**

Materials: Cheesecloth (two squares to fit funnel); Funnels (2); Glass or plastic reservoirs, 3 to 4 lit., (2); Graduated cylinders, 1000 ml or bigger (1); Spatulas or spoons (2).

Procedure: The Cheesecloth are place in a funnel and the funnel into glass or plastic reservoir. The grape mash of each plastic reservoir (5 kg) are pour into the funnel and collect filtrate (free run juice) in glass or plastic reservoir. The amount of free run juice (filtrate) collected are measure with graduated cylinders and it is free run juice yield.

**Results and discussion**

With enzymes, winemakers can enhance aroma, improve color and throughput of their red wines, as well as increase profits by increasing yields. 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 faster release of juice during pressing.

**Table 1. Comparison of free run juice yields obtained with pectolytic enzyme treatments on red grape mash of Vranec 2009 and control trials “no-enzyme addition”**

| Enzyme preparation                | *L* Average, L | % of grape weight | Increase yield, % |
|-----------------------------------|----------------|------------------|-------------------|
| Control - no enzyme               | 2.342 ± 0.068  | 46.85            | 0                 |
| Vinozym Vintage FCE, 4g/100 kg grape | 2.660 ± 0.040  | 53.20            | 6.35              |
| Rohapect VR-C, 4g/100 kg grape    | 2.585 ± 0.015  | 51.70            | 4.85              |
| Trenolin Rot DF, 20mL/100 kg grape| 2.605 ± 0.025  | 52.08            | 5.23              |

Note: *Values represented in the table are averages of results of two separately conducted experiments ±SD; *SD- standard deviation.

**Table 2. Comparison of free run juice yields obtained with pectolytic enzyme treatment on red grape mash of Vranec 2009 and control trials “no-enzyme addition”**

| Enzyme preparation                | *L* Average, L | % of grape weight | Increase yield, % |
|-----------------------------------|----------------|------------------|-------------------|
| Control - no enzyme               | 546 ± 6        | 44.82            | 0                 |
| Vinozym Vintage FCE, 4g/100 kg grape | 614 ± 9        | 50.32            | 5.50              |

Note: *Values represented in the table are averages of results of two separately conducted experiments ±SD; *SD- standard deviation.
Table 1 shows results of free run juice yields obtained with pectolytic enzyme treatments on red grape mash of Vranec and control trials “no-enzyme addition”. Obtained results are of laboratory trials (5 kg grapes).

In Table 1 it can be seen that pectolytic enzyme treatments on red grape mash of Vranec gives increased on free run juice yields by 4.85 % to 6.35 % compared with non-treated mash of control trials. Particularly, treatment with pectolytic enzyme preparation Vinozym Vintage FCE provided 6.35 % juice yield increase, with Trenolin Rot DF - 5.23 %; and with Rohapect VR-C - 4.85 %.

Table 2 shows results of free run juice yields obtained with pectolytic enzyme treatment (Vinozym Vintage FCE) on red grape mash of Vranec and control trial “no-enzyme addition”. Obtained results are of industrial trials (1220 kg grapes). It is seen that pectolytic enzyme treatment on red grape mash of Vranec gave increase of free run juice yield of up 5.50 % compared with non-treated mash of control trial.

Red grape mash of Vranec treated with Pectolytic enzyme provided in average greater yields of free run juice than untreated grape mash.

The obtained results were in agreement with previously published data but of other winegrapes (Ough et al., 1975; Ough and Crowell, 1979; Brown and Ough, 1981; Villetaz and Dubourdieu, 1991; Rogerson et al., 2000; Haight and Gump, 1994; Harbord et al, 1990).

**Conclusion**

Increase of free run juice yield by 6.35% in laboratory trials and by 5.5% in industrial trials gives ground for application of the pectolytic enzymes in wine industry. By increasing juice yields subsequently will lead to improving of wine throughput and increasing profits.

**References**

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