THE EFFECT OF PECTOLYTIC ENZYME TREATMENTS ON WHITE GRAPE MASHES OF SMEDEREVKA ON THE MICROBIOLOGICAL QUALITY OF WINES

Kiro Mojsov[1], Meri Petreska[2], Jugoslav Ziberoski[3]

Abstract

The paper investigates effects of pectolytic enzyme treatments on white grape mashes on the microbiological quality of wines and wine stability with counting of the presence yeast cells (*Saccharomyces cerevisiae*), after incubation on Sabourald-maltose agar. Also wine samples were investigates of the presence moulds and other bacteria, as and dangerous bacteria *Salmonella and Shigella, Staphylococus aureus, Proteus spp., Sulphite-reducing clostridy and Escherichia coli.*In all wine samples the results showed that have yeasts *Saccharomyces cerevisiae*. *Saccharomyces* is not regard as spoilage organism only if it is found in the wrong place at the wrong time (e.g. in a bottle of semi-sweet wine) causing re-fermentation, meanwhile the better is to have less. Pectinolytic enzyme preparation Trenolin Mash DF showed the best results.

Key words: pectolytic enzymes, white grape *Smederevka,* microbiological quality of wines, microbial spoilage in wine.

Introduction

Smederevka is a variety of white grape cultivated in Republic of Macedonia. It is capable of producing high quality white table wines in this country. Enzymes play a definite role in the ancient and complex process of winemaking. From the pre-fermentation stage, through fermentation, post-fermentation and aging, enzymes are the major driving forces catalysing various biotransformation reactions (Van Rensburg and Pretorius, 2000). Pectolytic enzyme preparations have been used for over 60 years in fruit juice production. The largest industrial application of pectinases is in fruit juice extraction and clarification. Enzymatically treated white wines presented chromatic characteristics, which are considered better than the control wines. These wines also showed greater stability as compared to the control (Revilla and Gonzalez-san jose, 2003).

The winemaking process is a complex ecological niche where the biochemistry and interaction of yeasts, bacteria, fungi and the viruses play a pivotal role in the final

1

[1]*Technological-Technical Faculty,University "Goce Delcev" Stip, Republic of Macedonia* (kiro.mojsov@ugd.edu.mk)
[2]*Ministry of Agriculture, Forestry and Water Economy, Jurij Gagarin 15,1000 Skopje, Republic of Macedonia*
[3]*Faculty of Agricultural Sciences and Food,"Ss Cyril and Methodius University", Skopje, Republic of Macedonia*[1]

product. These microorganisms involved are at the core of the winemaking process, whether for good or ill (Du Toit and Pretorius, 2000). The main microorganisms associated with wine spoilage are yeasts, acetic acid bacteria and lactic acid bacteria. The major spoilage organisms of the yeast genera include *Brettanomyces, Candida, Hanseniaspora, Pichia* and *Zygosaccharomyces*. The genera of lactic acid bacteria include *Lactobacillus, Leuconostoc* and *Pediococcus*, while the acetic acid bacteria genera are *Acetobacter* and *Gluconobacter* (Du Toit and Pretorius, 2002).

The spoilage caused by yeasts is important because they cause refermentation, ester formation, hydrogen sulphide and volatile sulphur compounds, volatile acidity, the formation of volatile phenols, mousiness, film formation, deacidification and the formation of ethyl carbamate. *Saccharomyces* is regarded as spoilage organism only if it is found in the wrong place at the wrong time (e.g. in a bottle of semi-sweet wine) causing re-fermentation. Schizosaccharomyces pombe has been associated with wine spoilage when growing in bottled wine and forming a sediment at the bottom of the bottle (Boulton et al., 1996).

The spoilage caused in wine by lactic acid bacteria is associated particularly with acetification of the wine through the production of acetic acid, mousy taints, bitterness, ropiness, buttery flavour and increased viscosity of the wine (Shimazu and Watanobe, 1981, Zoecklein et al., 1995).

The main spoilage caused by acetic acid bacteria is associated with oxidation of the ethanol to acetaldehyde and eventually acetic acid. Gram-negative acetic acid bacteria require oxygen for growth. They carry out incomplete oxidation of alcohols, leading to the accumulation of organic acids as end products (Bartowsky and Henschke, 2004; Amerine et al., 1980).

Yeasts play a central role in the spoilage of beverages. A few species are capable of spoiling beverages. These can survive and grow under stress conditionswhere other microorganisms are not competitive. This investigates uses the wine industry as a case study where serious microbiological problems are caused by yeasts. The effect of pectolytic enzyme treatments on white grape mashes are discussed on the microbiological quality of wines, the susceptibility of wine to spoilage yeasts and wine stability based on scientific knowledge and industrial practices for monitoring yeast contamination i.e. for monitoring the presence of yeast.

Materials 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

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

Doses: 1, 2, 3, and 4 mL/100 kg grapes

- Rohavin LX, AB Enzymes GmbH, Darmstadt, Germany;

Doses: 2, 3, 4, and 5 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) (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.

Wine samles. Microvinification. 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 mash 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 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 ^{o}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 ^{o}C to completion of fermentation. The bottled wines (0.5 l) were stored at 4 – 6 ^{o}C.

Determination of the total yeast cells. The effect of pectolytic enzyme treatments on red grape mashes on the microbiological quality of wines and wine stability were investigated with counting of the presence yeast cells (*Saccharomyces cerevisiae*), after incubation on Sabourald-maltose agar.

1 ml wine sample was added to Sabourald-maltose agar base for yeasts and moulds in petri dish. After keeping the samples at room temperature (thermostat) for 3-5 days were counted the presence yeast cells (cells/ml wine).

Sabouraud-maltose agar: peptone (1.0%), maltose (4.0%), agar (2.0%).

Determination of bacteria *Salmonella and Shigella, Staphylococus aureus, Proteus spp., Sulphite-reducing clostridy and Escherichia coli.*

***Salmonella and Shigella* in 25 ml.** 25 ml wine sample was added in Erlenmeyer with 225 ml Selenite broth. After keeping the samples at 37 °C (thermostat) for 24 hours with eza were transplanted at SS base for *Salmonella and Shigella* and placed 24-48 hours at 37 °C.

Selenite broth: peptone (o.5%), lactose (0.4%), Na-selenite (0.4%), Na-phosphate (1.0%).

***Staphylococus aureus* in 0.1 ml.** 1 ml wine sample + 9 ml physiological solution=10 ml wine solution

1 ml from wine solution are put at BAIRD PARKER base for *Staphylococus aureus*, and are keep in thermostat 24 hours at 37 °C.

BAIRD PARKER AGAR: tryptone (1.0%), meat extract (0.5%), LiCl (0.5%), yeast extract (0.1%), agar (2.0%).

***Proteus spp.* in 0.1 ml.** 1 ml from wine solution are put at SS base for *Proteus spp.*, and are keep in thermostat 24-48 hours at 37 °C.

SS agar: peptone (5.0 g), meat extract (5.0 g), lactose (10.0 g), egg salts (8.5 g), natrium citrate (8.5 g), natrium thiosulphate (3.5 g), ferric citrate (1.0 g), agar (13.0 g), neutral red (0.023 g), brilliant green (0.00033 g).

***Sulphite-reducing clostridy* in 0.1 ml.** 1 ml from wine solution are put at sulphite agar base for *Sulphite-reducing clostridy*, and are keep in thermostat 24-48 hours at 37 °C.

Sulphite-reducing bacteria usually produce black colonies as a result of the reduction of sulphite to sulphide, which reacts with the iron(III)salt.

Sulphite agar: tryptone (15.0 g), yeast extract (10.0 g), distilled water (750 ml), water (aqua fontis) (250 ml).

***Escherichia coli* in 10 ml**. 10 ml from wine solution are put at liquid MAC CONKEY base (5 ml) for *Escherichia coli*, and are keep in thermostat 24-48 hours at 44 °C. After this, with eza are transplants at pink red egg yolk agar, and are keep in thermostat 34-48 hours at 44 °C. *Escherichia coli* grow as red or pink colonies.

MAC CONKEY AGAR: peptone (20.0 g), synthetic detergent (5.0 g), sodium chloride (5.0 g), lactose (10.0 g), neutral red (0.07), agar (12.0).

Results and discussion

Yeasts and bacteria are part of the natural microbial ecosystem of wine and play an important role in winemakingby reducing wine acidity and contributing to aroma and flavour. They can cause numerous unwelcome wine spoilage problems, which reduce wine quality and value.

Enzymes play an important role in winemaking. The application of industrial enzyme preparations in the wine industry is a common practice. They have been used to increase juice yield, filtration rate, rate of settling, and clarity of wines besides some microbiological implications.

The largest industrial application of pectinases is in fruit juice extraction and clarification. Pectinolytic enzymes added to macerated fruits before the addition of wine yeast in the process of producing red wine resulted in improved visual characteristics (colour and turbidity) as compared to the untreated wines. These wines also showed greater stability as compared to the control (Revilla and Gonzalez-san jose, 2003). The concepts of the susceptibility of wine to spoilage yeasts and wine stability are based on scientific knowledge and industrial practices for monitoring yeast contamination. A discussion on acceptable levels of yeasts and microbiological criteria in the wine industry is supported by data obtained from wineries, wholesalers, and the scientific literature.

The effect of pectolytic enzyme treatments on white grape mashes on the microbiological quality of wines and wine stability were investigated with counting of the presence yeast cells (*Saccharomyces cerevisiae*), after incubation on Sabouraldmaltose agar (Table 1).

Tabela 1. Efekt pektolitičkih enzimskih tretmana kaši od belog grožđja Smederevke na mikrobiološki kvalitet vina

Table 1. The effect of pectolytic enzyme treatments on white grape mashes of Smederevka on the microbiological quality of wines

Enzimski preparati *Enzyme preparations*	Kolicine (g ili ml 100kg grožđe^{-1}) *Dose (g or ml 100kg grape^{-1})*	[a]Kvasci (*Saccharomyces cerevisiae),* (ćelije ml^{-1}) [a]Yeasts (*Saccharomyces cerevisiae), (cells ml^{-1})*	Bakterii (ćelije ml^{-1}) Bacteria *Salmonella and Shigella; Staphylococus aureus; Escherichia coli; Sulphite-reducing clostridy;Moulds and other bacteria (cells ml^{-1})*
Vinozym Process	3 g	1920±86	0
	4 g	1940±43	0
	5 g	1893±82	0
	6 g	1900±16	0
Trenolin Mash DF	1 ml	967±47	0
	2 ml	700±82	0
	3 ml	833±47	0
	4 ml	700±82	0
Rohavin LX	2 ml	933±47	0
	3 ml	900±82	0
	4 ml	953±41	0
	5 ml	1267±94	0
Kontrolen-bez enzim *Control-no added enzyme*	0	140±16	0

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

In all wine samples the results showed that have yeasts *Saccharomyces cerevisiae* from 400 to 2000 cells/ml. *Saccharomyces* is not regard as spoilage organism only if it is found in the wrong place at the wrong time (e.g. in a bottle of semi-sweet wine) causing re-fermentation, meanwhile the better is to have less. Pectinolytic enzyme preparation Vinozym Process showed yeasts *Saccharomyces cerevisiae* from 1893 to 1940 cells ml^{-1} wine, Trenolin Mash DF from 700 to 967 cells ml^{-1}, and Rohavin LX from 900 to 1267 cells ml^{-1} depend of used doses and contol trial "no-enzyme addition" (140 cells ml^{-1}). Pectinolytic enzyme preparation Trenolin Rot DF showed the best results.

In all wine samples the results showed that have not the growth of unwanted bacteria as *Salmonella and Shigella, Staphylococus aureus, Proteus spp., Sulphite-reducing clostridy and Escherichia coli.*

Conclusion

Significance and impact of the study is that pectolytic enzyme treatments on white grape mashes had a pronounced effect on the microbiological quality of wines and wine stability. Results from comparison of effects of pectolytic enzyme preparations in winemaking on the microbiological quality of wines can contribute to a better orientation in the choice of suitable enzyme preparations in wine industry.

References

Amerine, M.A., Kunkee, R., Ough, K.C.S., Singleton, V.L. and Webb, A.D., (1980). The technology of wine making. (4th Ed). AVI, Westport, Connecticut, pp. 185-703.

Bartowsky, E.J. and Henschke, P.A., (2004). "Acetic acid bacteria and wine: all is well until oxygen enters the scene", Winemaking The Australian Wine Research Institute (AWRI), pp.86-91.

Boulton, R.B., Singleton, V.L., Bisson, L.F. and Kunkee, R.E., (1996). Principles and practices of winemaking. Chapman Hall, New York, pp. 75-221.

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

Du Toit, M. and Pretorius, I.S., (2000). "Microbial Spoilage and Preservation of Wine: Using weapons from nature'own arsenal- A review"., South African Journal of Enology and Viticulture, Vol. 21, pp. 74-96.

Du Toit, M. and Pretorius, I.S., (2002). "The occurrence, control and esoteric effects of acetic acid bacteria in winemaking", Annals of Microbiology, Vol. 52, pp. 155-179.

Revilla, I. and Gonzalez-san jose, M.L., (2003). "Addition of pectolytic enzymes: an enological practice which improves the chromaticity and stability of red wines", Int. J. Food Sci. Technol., Vol.38, pp.29-36.

Shimazu, Y. and Watanabe, M., (1981). "Effects of yeast strains and environmental conditions on forming organic acids in must during fermentation", J. Ferment. Technol., Vol.59, pp. 27-32.

Van Rensburg, P. and Pretorius, I.S., (2000). "Enzymes in winemaking: harnessing natural catalysts for efficient biotransformations- A review", South African Journal of Enology and Viticulture, Vol. 21, pp. 52-73.

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H. and Nury, F.S., (1995). Wine analysis and production. Chapman and Hall Enology Library, New York, pp. 97-301.