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The Impact of Oenological Means on Glycerol Content in Montenegrin Wines Vranac and Kratošija

Sanja Radonjić[1], Vesna Maraš[1], Vesna Kodžulović[1], Tjaša Jug[2], Tatjana Košmerl[3]

[1]"13. Jul Plantaže" a.d., Podgorica, Montenegro
[2]Agriculture and Forestry Institute, Nova Gorica, Slovenia
[3]Biotechnical Faculty, University of Ljubljana, Slovenia

Abstract

The influence of three different commercial yeasts (BDX, BM4X4 and ICV D21) and two different lactic acid bacteria (LAB), on glycerol content in Montenegrin wines Vranac and Kratošija were studied during 2012 vintage. Basic quality parameters of grape must (sugar content, total acidity, pH, tartaric and malic acid) were determined, as well as glycerol content after alcoholic and malolactic fermentation. It was found that within both wines, the highest content of glycerol was reached when BDX yeast was used. There were no significant differences within glycerol content when different LAB was used. In Kratošija wine glycerol content for BDX treatment varied between 9.02 – 9.09 g L^{-1}, while in Vranac wine varied from 8.88 – 8.95 g L^{-1}. Slightly higher glycerol content in wines with BM4X4 and ICV D21 commercial yeast were achieved also in Kratošija wine.

Key words: wine, yeast, lactic acid bacteria, glycerol

Introduction

Glycerol, 1,2,3–propantriol, is the chemical compound with the highest concentration in wine after water and ethanol. It is the most important by-product of alcoholic fermentation which significantly contributes to wine quality by providing sweetness and fullness (Ribereau-Gayon et al., 1972).

Pure glycerol is an odourless, colourless, high viscosity liquid and due to its non-volatile nature, this polyalcohol does not contribute to wine aroma (Taherzadeh et al., 2002). Typical glycerol levels in wine are given as 1–15 g L^{-1}, with average values approximately 7 g L^{-1} (Taherzadeh et al., 2002). Depending on the sugar content in must undergoing fermentation and on the biological and technological factors acting in primary winemaking, glycerol contents in wines range between 5 and 20 g L^{-1} (Gheorghiţă et al., 2006). Besides, according to Cotea et al. (1982) wines made from grapes attacked by noble rot (*Botrytis cinereae*) carry larger amounts of glycerol, even more than 20 g L^{-1}. A well-known yeast *Saccharomyces cerevisiae* is the most important glycerol producing yeast. In *S. cerevisiae* glycerol is a by-product of the fermentation of sugar to ethanol in a redox-neutral process (Grossmann et al., 2001).

It is reported that glycerol concentration in wine is variable depending on the yeast strain, medium and process conditions. It has also been shown that the proportion of glycerol in wine depends on: the initial concentration in sugar of must, the amount of sulphur dioxide used to protect must, the fermentation temperature maintained during the alcoholic fermentation, the yeasts which completed the alcoholic fermentation of the must (Popa, 1996). The amount of glycerol produced varies also with the degree of ripeness of grapes (i.e. sugar content), health condition and the grape cultivar (i.e. micronutrients) (Ough et al., 1972; Radler and Schutz, 1982, Cotea et al., 1982). Glycerol degradation during malolactic fermentation is influenced by the content of fermentable sugars in the medium, the pH of the medium and the lactic acid bacteria strains involved in the fermentation (Popa et al., 2004).

Vranac and Kratošija are the most important grapevine cultivars for production of red wines in Montenegro. According to many literature data, Kratošija cultivar appeared earlier and was introduced into cultivation quite earlier than Vranac (Maraš et al., 2012). Work on autochthonous grapevine cultivars is of huge interest considering the fact that it is shown that Vranac and Kratošija are in close relationship (parent–offspring) and it is proposed that Montenegro is the best candidate for the origin and spreading centre of Kratošija and its group of synonyms (Primitivo, Zinfandel and Crljenak Kaštelanski) (Maraš et al., 2014). Wines of these cultivars are of premium quality, Vranac has dark red ruby colour, full body, fruity taste and pleasant astringency with aging potential in oak barrels. Kratošija wine is characterized by an intense ruby-red colour and aroma of red berry fruits and an extremely pleasant taste; it has a light and harmonious structure and smooth finish.

For the purposes of this paper, glycerol content was determined after alcoholic and after malolactic fermentations in wine made of Montenegrin autochthonous grape cultivars Vranac and Kratošija.

The influence of three different commercial yeasts and two different lactic acid bacteria on glycerol content in Montenegrin wines Vranac and Kratošija were studied during 2012 vintage.

Material and Methods

Autochthonous grape cultivars Vranac and Kratošija were planted in the vineyard of the company "13. Jul Plantaže" in the Ćemovsko field in sub-region Podgorica. At harvest, grapes from examined cultivars were harvested manually and transported to the experimental cellar. During the harvest only healthy bunches were picked and the grapes that were used were of good health condition. Wines were produced at microvinification scale in the experimental cellar at the company "13. Jul Plantaže". Alcoholic fermentation of trials was performed in PVC barrels using traditional method with temperature of 25-27 °C. Potassium metabisulphite, purchased from Agroterm KFT, Hungary was added; 8 g 100 kg^{-1} of grapes from both cultivars. All enzyme, wine yeasts, lactic acid bacteria and yeast nutrients were obtained from Lallemand, Australia.

Within all cultivars commercial yeasts that were used to induce alcoholic fermentation are: Enoferm BDX, Lalvin BM4x4 and Lalvin ICV D21 (30 g hL^{-1}). After alcoholic fermentation wines were racked from the pomace. Then after two days wines were racked again, lees were removed and wine was divided into three identical volumes for malolactic fermentation; two with different commercial lactic acid bacteria (ALPHA and VP41) and the third without addition of lactic acid bacteria (control wine). All alcoholic and malolactic fermentation trials were performed in three repetitions. Malolactic fermentation was monitored by the determination of malic and lactic acid after inoculation with lactic acid bacteria twice per week.

Malolactic fermentations last about three-four weeks and when malic acid was totally consumed by lactic acid bacteria, we considered it finished. The content of malic and lactic acid after alcoholic and malolactic fermentation are presented in Table 1.

For determination of basic chemical parameters of grape must: malic acid, tartaric acid, sugar, total acidity and pH value and for determination of malic and lactic acid in wine the reference methods of European Union were used (Commission regulation (EEC) No. 2676/90). Glycerol content in wine was determined using wine analysis instrument WineScanTM FT 120 (FOSS).

Tab. 1. The content of malic and lactic acid in Vranac and Kratošija wines, after alcoholic and malolactic fermentation
Садржај јабучне и млијечне киселине у винима Вранац и Кратошија након алкохолне и јабучно-млијечне ферментације

Grape cultivar *Сорта*	Yeast *Квасац*	Lactic acid bacteria *Бактерије млијечне киселине*	After *Након*	L-malic acid (g L^{-1}) L *-јабучна кис. (g L^{-1})*	L- lactic acid (g L^{-1}) L *-млијечна кис. (g L^{-1})*
Vranac	CTRL	CTRL	Alcoholic fermenation *Алкохолне ферментације*	0.16	0.98
	BDX	CTRL		1.54	0.37
	ICV D21	CTRL		1.43	0.27
	BM4X4	CTRL		1.42	0.26
	CTRL	CTRL	Malolactic fermentation *Јабучно-млијечне ферментације*	0.05	1.16
	BDX	CTRL		0.08	1.03
		VP41		0.07	1.02
		ALPHA		0.02	1.03
	BM4X4	CTRL		0.04	0.89
		VP41		0.01	0.88
		ALPHA		0.02	0.88
	ICV D21	CTRL		0.09	0.87
		VP41		0.01	0.89
		ALPHA		0.04	0.87
Kratošija	CTRL	CTRL	Alcoholic Fermenation *Алкохолне ферментације*	0.01	1.24
	BDX	CTRL		1.82	0.29
	ICV D21	CTRL		0.33	1.01
	BM4X4	CTRL		1.77	0.21
	CTRL	CTRL	Malolactic fermentation *Јабучно-млијечне ферментације*	0.00	1.39
	BDX	CTRL		0.11	1.12
		VP41		0.13	1.18
		ALPHA		0.17	1.13
	BM4X4	CTRL		0.12	0.99
		VP41		0.13	0.93
		ALPHA		0.15	0.97
	ICV D21	CTRL		0.17	0.22
		VP41		0.11	1.08
		ALPHA		0.20	1.10

Mann-Whitney test was used to compare differences of examined parameters between wines with grape cultivar as an independent variable. IBM statistics 20.0 software is used.

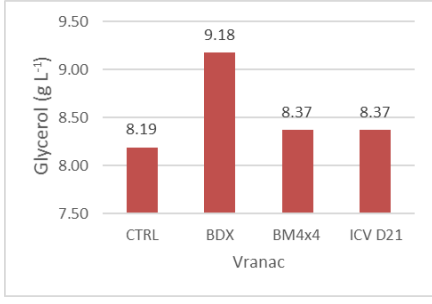
Results and Discussion

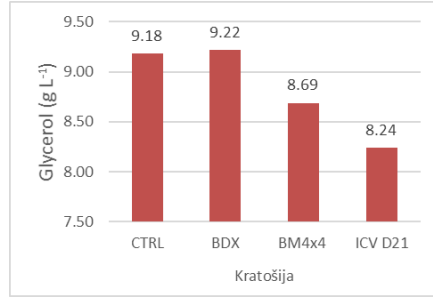
Basic quality parameters of grape must are shown in Table 2. Vranac is a grape cultivar which usually accumulates a higher content of sugar than was achieved in this paper. Šućur et al., (2015) reported that during grape ripening in vintage 2012, average air temperatures were quite low, which can influence the accumulated sugar content. Kratošija accumulates high content of sugar and high total acidity is its varietal characteristics, compared to Vranac, which has low total acidity content and high pH.

Tab. 2. Basic quality parameters of grape must of Vranac and Kratošija cultivars
Основни параметри квалитета шире сорти Вранац и Кратошија

Cultivar *Сорта*	Malic acid (g L^{-1}) *Јабучна киселина* (g L^{-1})	Tartaric acid (g L^{-1}) *Винска киселина* (g L^{-1})	Sugar (%) *Шећер* (%)	Total acidity (g L^{-1}) *Ук. киселине* (g L^{-1})	pH
Vranac	1.10	4.84	18.8	5.61	3.70
Kratošija	1.80	3.64	21.5	5.73	3.63

Glycerol content in Vranac and Kratošija wine after alcoholic fermentation is presented at Graphs 1 and 2. Average value of glycerol content and range was higher in the wine of Kratošija cultivar. In both examined wines, BDX yeast gave wine with the highest glycerol content. In Vranac wine the same glycerol content was reached in wines with BM4x4 and ICVD21 yeast; while wine with spontaneous alcoholic fermentation have the lowest content. In Kratošija wine, the lowest glycerol content was reached in wine with ICV D21 yeast.





Graphs 1, 2. Glycerol content in Vranac and Kratošija wines after alcoholic fermentation
Садржај глицерола у винима Вранац и Кратошија након алкохолне ферментације

However, in wines of both examined cultivars there were no significant differences in glycerol content between wines with applied different commercial yeast.

Using Mann-Whitney test with grape cultivar as independent variable, it can be concluded that there are not statistically significant differences in glycerol content between Vranac and Kratošija wine after alcoholic fermentation ($p > 0.05$).

An almost constant feature of malolactic fermentation observed both in white and red wines is represented by the increase in corpulence and the improvement of aftertaste. According to Dittrich et al. (1987) lactic acid bacteria, particularly lactobacilli, are able to metabolise glycerol in whole or in part. Dittrich et al. (1980) compared 8 wines with and without spontaneous malolactic fermentation and found that 6 wines which underwent malolactic fermentation showed a decrease in glycerol content, which also applied to wine inoculated with *Lactobacillus plantarum* to achieve malolactic fermentation. Popescu-Mitroi et al. (2014) showed that glycerol degradation occurs during malolactic fermentation, especially in wine samples uninoculated with malolactic bacteria, where malolactic fermentation takes place spontaneously and uncontrollably based on indigenous microflora. In similar experiments performed on wines produced under controlled conditions and using *Leuconostoc oenos* starter cultures, no change was noted in the glycerol content of wines (Henick-Kling et al. 1993, 1994, 1998; Nielsen and Richelieu, 1999). In our research similar observations were achieved, after malolactic fermentation there were no significant changes in glycerol content. In Vranac wine, after malolactic fermentation glycerol content in wines inoculated with commercial yeast and lactic acid bacteria decreased in average for 0.15 g L^{-1} (BM4x4), 0.20 g L^{-1} (ICV D21) and 0.27 g L^{-1} (BDX), while in control wine glycerol content increased for 0.06 g L^{-1}.

In Kratošija wines, decrease of glycerol content was evident only in BDX wine (0.16 g L^{-1} in average), while in control wine and wines inoculated with BM4x4 and ICV D21 glycerol content increased in average for 0.03, 0.06 and 0.01 g L^{-1}, respectively. Besides, after malolactic fermentation, wines in which alcoholic fermentation was performed by BDX yeast had the highest content of glycerol particularly in wines without addition of lactic acid bacteria (Table 3). However, the differences in glycerol content in wines depending on applied commercial yeast, as well as, on commercial lactic acid bacteria were not statistically significant.

Tab. 3. Glycerol content (g L^{-1}) in Vranac and Kratošija wines after malolactic fermentation
Садржај глицерола (g L^{-1}) у винима Вранац и Кратошија након малолактичке ферментације

Grape cultivar *Сорта*	Yeast *Квасац*	Lactic acid bacteria *Бактерије млијечне киселине*		
		CTRL	VP41	ALPHA
Vranac	CTRL	8.25	-	-
	BDX	8.95	8.88	8.89
	BM4x4	8.18	8.23	8.24
	ICV D21	8.16	8.16	8.18
Kratošija	CTRL	9.21	-	-
	BDX	9.09	9.02	9.06
	BM4x4	8.77	8.74	8.76
	ICV D21	8.32	8.19	8.26

Results of Mann-Whitney test with grape cultivars as independent variables showed that there are statistically significant differences for glycerol content ($p < 0.05$). Therefore, glycerol content was not dependent on different commercial yeast and lactic acid bacteria, but it is shown that it depends on grape cultivar and on initial sugar content in must (Table 4).

Tab. 4. Ratio of average glycerol content in wine after MLF and initial sugar content in grape must for examined wines.
Однос просјечне вриједности глицерола у винима након МЛФ и садржаја шећера у шири за испитивана вина

Grape cultivar *Сорта*	CTRL wine *Контролно вино*	Average value of wines inoculated with yeast and LAB *Просјек за вина инокулисана са квасцима и бактеријама млијечне киселине*	Standard deviation *Стандардна девијација*
Vranac	4.07	4.16	0.18
Kratošija	3.93	3.93	0.16

Conclusion

Based on achieved results it can be concluded that after alcoholic and malolactic fermentation, within both cultivars, the highest content of glycerol was achieved when BDX yeast was used.

However, the differences in glycerol content in wines depending on applied commercial yeast, as well as, on commercial lactic acid bacteria were not statistically significant in wines of both examined varieties. Besides, it is noticed that glycerol content did not significantly change after malolactic fermentation implicating that commercial lactic acid bacteria did not metabolise glycerol. After malolactic fermentation it is shown that there was a statistically significant difference for glycerol content between wines of examined grape cultivar which implies that beside dependency on initial sugar content of must glycerol content is also a cultivar characteristic.

References

Cotea, V., Pomohaci, N. and Gheorghiţă, M. (1982). *Oenologia* (p. 65). Bucharest: Didactică şi Pedagogică.

Commission regulation (EEC) No. 2676/90 determining (1990). Community methods for the analyses of wines. *Official Journal of the European Union*, L 272. p. 192.

Dittrich, H.H., Sponholz, W.R., Wunsch, B. and Wiplefr, M. (1980). Zur Veranderung des Weines durch den bakteriellen. *Saurebbau Wein Win*, 35, 421-428.

Dittrich, H.H. (1987). *Mikrobiologie des Weines. Handbuck der Lebensmietteltechnologie* 2nd Edition (p. 101). Stuttgart.

Gheorghiţă, M., Băducă Câmpeanu, C., Muntean, C. and Giugea, N. (2006). *Oenologie vol I* (pp. 346-348). Craiova: Sitech.

Grossmann, K. M., Schneider, I., Huehn, T., Remize, F. and Dequin, S. (2001). Effets de la surproduction de glycerol sur l'activite des levures et les aromes du vin. *Bull. O.I.V., 74* (843-844), 347-362.

Henick-Kling, T., Acree, T.E., Gavitt, B.K., Krieger, S.A. and Laurent, M.H. (1993). Sensory aspect of malolactic fermentation. In: *Proceedings of the Eight Australian Wine Industry Technological Conference, Melbourne, Australia* (pp. 148-152).

Henick-Kling, T., Acree, T.E., Krieger, S.A., Laurent, M.H. and Edinger, W.D. (1994). Modificazzioni del gusto indotte dalla fermentazione malolattica. *Vignevini, 21* (4), 41-47.

Henick-Kling, T. and Acree, T. E. (1998). Modificazioni dell'aroma del vino con la fermentazione malolattica ed uso in colture selezionate negli USA. *Vignevini, 25* (7-8), 44-50.

Maraš, V., Tomić, M., Kodžulović, V., Šućur, S., Raičević, J., Raičević, D. and Čizmović, M. (2012). Researh of origin and work on clonal selection of Montenegrin grapevine varieties cv. Vranac and cv.Kratošija. *Agroznanje, 13* (1), 103-112.

Maraš, V., Bozović, V., Giannetto, S. and Crespan, M. (2014). SSR molecular marker analysis of the grapevine germplasm of Montenegro. *OENO One, 48* (2), 87-97.

Nielsen, J. C. and Richelieu, M. (1999). Control of flavour development in wine during and after malolactic fermentation by *Oenococcus oeni*. *Appl. Environ. Microbiol*., *65* (2), 740-745.

Popa, A., Popa, D. and Dragomir, F. (2004). *Microbiologie oenologică* (pp. 142-193). Editura Universitaria Craiova.

Popa, A. (1996). *Vinul. Importanţă socială. Posibilităţi de apreciere* (p. 42). Bucureşti: Editura Didactică şi Pedagogică.

Popescu-Mitroi, I., Radu, D. and Stoica F. (2014). The study of glycerol metabolism in the malolactic fermentation of red wines. *Romanian Biotechnological Letters, 19* (1), 9019-9027.

Radler, F. and Schutz, H. (1982). Glycerol production of various strains of *Saccharomyces*. *Am. J. Enol. Vitic., 33* (1), 36–40.

Ribereau-Gayon, P., Peynaud, E., Sudraud, P. and Ribereau-Gayon, P. (1972). In: *Traite´ D'oenologie. Sciences et Techniques du Vin* (pp. 353–361). Paris: Dunod.

Šućur, S., Maraš, V., Kodžulović, V., Raičević, J., Gazivoda, A., Mugoša, M., Savović, A. and Košmerl, T. (2015). *Effect of microbiological and technological parameters on Montenegrin red wines quality*. Book of proceedings. Sixth International Scientific Agricultural Symposium "Agrosym 2015" Jahorina, Bosnia and Herzegovina. 260-266.

Taherzadeh, M.J., Adler, L. and Lidén, G. (2002). Strategies for enhancing fermentative production of glycerol – A review, *Enzyme Microb. Technol., 31* (1), 53–66.

Утицај енолошких средстава на садржај глицерола у црногорским винима Вранац и Кратошија

Сања Радоњић[1], Весна Мараш[1], Весна Коџуловић[1], Тјаша Југ[2], Татјана Кошмерл[3]

[1]"13. Јул Плантаже" а.д., Подгорица, Црна Гора
[2]Пољоприовредни и шумарски институт, Нова Горица, Словенија
[3]Биотехнички факултет, Универзитет у Љубљани, Словенија

Сажетак

Утицај три различита комерцијална квасца (BDX, BM4X4 i ICV D21) и двије врсте комерцијалних бактерија млијечне киселине на садржај глицерола у црногорским црвеним винима Вранац и Кратошија је проучаван (берба 2012). Поред садржаја глицерола, одређени су и основни параметри квалитета шире (садржај шећера, укупне киселине, pH, винска и јабучна киселина). Након алкохолне и малолактичке ферментације, у винима обје сорте, највећи садржај глицерола је одређен у вину гдје је BDX квасац коришћен. Није било значајних разлика у садржају глицерола у третманима гдје су бактерије млијечне киселине коришћене. Садржај глицерола у винима са BDX квасцима у Кратошије је био између 9.02 и 9.09 g L^{-1}, док се у винима Вранац кретао од 8.88 – 8.95 g L^{-1}. Мало већи садржај глицерола је одређен у винима сорте Кратошија инокулисаним са BM4X4 и ICV D21 комерцијалним квасцима.

Кључне ријечи: вино, квасци, бактерија млијечне киселине

Sanja Radonjić
E-mail address: *sanja.radonjic511@gmail.com*

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