

# The impact of different commercial yeasts on quality parameters of Montenegrin red wine – Vranac and Kratošija

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## Abstract

The influence of three different commercial yeasts (BDX, BM4X4 and ICV D21), on quality parameters of wines from varieties Vranac and Kratošija were studied during two consecutive vintage years. The basic quality parameters of grape must (sugar content, total acidity, pH, tartaric and malic acid) and the wine quality parameters (alcohol content, total dry extract, glycerol, pH, total polyphenols and total anthocyanins) were determined after each phase of vinifications: after alcoholic and malolactic fermentation as well as after three months of wine maturation. There are significant differences in quality parameters of grapes and wines between vintages 2012 and 2013. Higher content of total dry extract and glycerol in wines after completion of alcoholic fermentation was achieved in vintage 2013. In vintage 2013, the highest content of total polyphenols (3.75 and 2.98 g L<sup>-1</sup>) and anthocyanins (1247 and 713 mg L<sup>-1</sup>) in Vranac wine after malolactic fermentation and after three months of maturation, were achieved by yeast BM4X4, while the highest total polyphenols (2.14 and 1.92 g L<sup>-1</sup>) and anthocyanins content (527 and 262 mg L<sup>-1</sup>, respectively) was achieved by BDX strain in Kratošija wine. The results of sensory analysis showed no significant differences between the used yeast strains and studied. Surprisingly, the wines of vintage 2012 are even slightly better sensory evaluated.

## Introduction

Wine styles are defined by complex and highly diverse chemical compositions. Amongst the myriad of grape and wine processing options known to influence wine style, choice of yeast strain represents a low-cost opportunity to broaden the spectrum of wine flavour profiles possible from a single vineyard or variety [1]. There are hundreds of different commercially available wine yeast strains that, potentially, provide a means by which winemakers can tailor their wines for different consumer market segments. *Saccharomyces cerevisiae* is a yeast species specialized in metabolizing media with high sugar contents and small quantities of nitrogenous compounds. In the past, musts were fermented by yeast indigenous to the grape microbiota, but nowadays most are inoculated with selected yeast strains preserved in dried form. Traditionally, yeasts have been selected for their fermentative power, suitable fermentative kinetics at different temperatures, low acetic production and resistance to sulphur dioxide. However, new selection criteria emerged and role of yeasts in winemaking involved improvement of wine in terms of their colour, aroma, structure and other technological properties [2,3] concluded that the role of yeast is complex and strongly associated with wine quality, and that it is becoming even more important to select yeast that are right for each kind of wine, region and even microclimate.

Wine phenolics are extracted from grape skins and seeds during fermentation, interact with insoluble cell wall material from the grapes [4,5], and undergo a range of chemical transformations during maturation. Selection of yeast strain has been shown to impact on the concentration of anthocyanins [6,7] and other phenolics [6,8,9] in finished wine. The work combined analyses of wine total polyphenols,

total anthocyanins, total dry extract, alcohol and sensory analysis. The impact of yeast upon wine sensory properties in red wine play a role in the perception of red and dark fruit aromas [10,11]. Also, yeasts have great influence on glycerol production which is influenced by many growth and environmental factors [12]. Several studies have described the effect of yeast strain on glycerol production [13,14] and it appears to be one of the key factors impacting glycerol production. The amount of glycerol produced varies with the type of yeast used, with sugar content and the grape variety. This manuscript describes the results of four fermentation experiments with different commercial yeasts, across two vintages and within two grape varieties, which emphasize the significant impact that choice of yeast strain can have upon red wine chemical composition.

Current viticulture and wine production in Montenegro is based mainly on presumed autochthonous grapevine cultivars, such as Vranac, Kratošija, Krstač, and Žižak. The dominant cultivars for red wine production are Vranac and Kratošija. Significantly less prevalent is Krstač, used for white wine production, while no commercial wine is produced from Žižak. Vranac and Kratošija are the most important grapevine varieties for production of red wines in Montenegro.

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Vranac wine became a national brand as well as the most recognizable and the best product of the company “13. Jul Plantaže”. Kratošija is a Montenegrin autochthonous variety for production of red wines, grown for centuries in Montenegro, which falls into the category of recommended varieties for quality wine. According to many literature data, Kratošija variety appeared earlier and was introduced into cultivation quite earlier than Vranac [15]. Wines of these varieties are of premium quality, Vranac has dark red ruby colour, full body, fruity taste and pleasant astringency and it has potential of lying down and maturing. 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.

## Material and methods

The trial was carried out during the 2012 and 2013 growing seasons. Autochthonous grapevine varieties Vranac and Kratošija were planted in the commercial vineyard of the company “13. Jul Plantaže” in the Cemovsko field in sub-region Podgorica (Montenegro).

Grapes of both varieties were planted in 2003, grafted onto Paulsen 1103 rootstock, trained to a modified single Guyot training system, rows spaced 2.6 m apart and with 0.7 m between plants in the row. All standard agro-technical operations were applied and vineyards were in good and healthy condition. Wines were produced at microvinification scale in the experimental cellar at the company “13. Jul Plantaže”. At harvest, grapes from both examined varieties were harvested manually and transported to the experimental cellar. Alcoholic fermentation of trials was performed in PVC barrels using traditional method. For the vinification of control wines, an average grape sample of both varieties was 100 kg of grapes. For trials with yeast addition we used an average grape sample of 400 kg for both varieties. Potassium metabisulphite, purchased from Agroterm KFT, Hungary was added; 8 g 100 kg<sup>-1</sup> of grapes from both varieties. All enzyme, wine yeasts, lactic acid bacteria and yeast nutrients were obtained from Lallemend, Australia. Three commercial yeasts, dominantly used for production of red wines, were chosen to induce alcoholic fermentation. Within all varieties commercial yeasts that were used are: Enoferm BDX, Lalvin BM4x4 and Lalvin ICV D21 (30 g hL<sup>-1</sup>). Enzyme Lalvin EX-V for maceration (2 g 100 kg<sup>-1</sup>) and yeast nutrient Go-Ferm Protect (30 g hL<sup>-1</sup>) were added during vinification, while yeast nutrient Fermaid E (25 g hL<sup>-1</sup>) was added during fermentation. After alcoholic fermentation; wines

were racked and malolactic fermentation without addition of lactic acid bacteria (LAB) was performed. After completion of malolactic fermentation wines were racked and potassium metabisulphite was added in amount depending of free SO<sub>2</sub> in analysed wine samples.

For determination of basic wine chemical parameters: alcohol, total dry extract and pH value, the reference methods of European Union [16] were used. Total polyphenols and anthocyanins content were determined by spectrophotometer. Total polyphenols were quantified by Folin-Ciocalteu index method (Compendium of international methods of wine and must analysis-OIV, 2014). The total anthocyanins were determined using the pH differential method [17]. Content of alcohol, total dry extract and glycerol in wine was determined using wine analysis instrument WineScan™ FT 120 (FOSS). Each sample of wine was judged by the experienced sensory panel for colour, aroma and taste intensity and quality, body, aftertaste, harmony and general sensation using an official OIV 100-points scale.

## Results and discussion

### Grape quality

Quality parameters of examined grape varieties are shown in Table 1. Vranac variety is known as variety with high total anthocyanin content, what is confirmed in this research. Vranac accumulated higher content of sugar in 2013, with higher total acidity and tartaric acid content. There are no differences between pH and malic acid content between years. There are significant differences of total polyphenols and anthocyanins content between years, which can be explained by different climate conditions during vintages time. [18] reported that lower temperatures were during grape ripening in vintage 2012, than in 2013, that can influence total polyphenols and anthocyanins content in grape, as well as and sugar content. Similar situation is noticed within Kratošija variety. Kratošija also accumulated higher content of sugar in 2013, with higher total acidity, tartaric and malic acid content. High acidity is varietal characteristic of Kratošija variety. In average, Kratošija accumulates high content of sugar and low anthocyanins content.

### After alcoholic fermentation

Higher content of total dry extract and glycerol in wines after completion of alcoholic fermentation was achieved in vintage 2013 (Table 2).

**Table 1.** Quality parameters of Vranac and Kratošija grapes.

Grape variety	Harvest season	Sugar (%)	Total acidity (g L <sup>-1</sup> )	Tartaric acid (g L <sup>-1</sup> )	pH	Malic acid (g L <sup>-1</sup> )	Total polyphenols (g L <sup>-1</sup> )	Total anthocyanins (mg L <sup>-1</sup> )
Vranac	2012	18.8	3.61	4.84	3.70	1.10	0.12	19.5
Vranac	2013	21.2	4.63	5.49	3.70	1.00	0.93	205.5
Kratošija	2012	21.5	5.73	3.64	3.60	1.80	0.49	60.0
Kratošija	2013	22.6	6.23	4.05	3.59	3.00	0.45	21.0

**Table 2.** Chemical parameters after alcoholic fermentation for Vranac and Kratošija wine.

Variety	Yeast	Alcohol (vol. %)		Total dry extract (g L <sup>-1</sup> )		Glycerol (g L <sup>-1</sup> )	
		2012	2013	2012	2013	2012	2013
Vranac	Control	12.59	13.38	24.20	29.62	8.19	11.21
	BDX	12.41	13.69	25.03	30.40	9.18	10.56
	D21	12.48	14.04	24.07	31.16	8.37	10.44
	BM4x4	12.48	13.98	24.57	30.52	8.37	10.07
Kratošija	Control	13.58	13.01	24.28	28.69	9.18	10.58
	BDX	13.84	13.08	26.28	28.37	9.22	9.11
	D21	13.84	13.24	24.69	29.30	8.24	9.17
	BM4x4	13.77	13.11	26.04	28.15	8.69	8.34

**Table 3.** Total polyphenols and total anthocyanins contents after alcoholic fermentation for Vranac and Kratošija wine.

Variety	Yeast	Total polyphenols (g L <sup>-1</sup> )		Total anthocyanins (mg L <sup>-1</sup> )	
		2012	2013	2012	2013
Vranac	Control	2.62	2.20	862	729
	BDX	3.05	3.75	1247	1229
	D21	2.81	3.73	889	1158
	BM4x4	2.35	3.67	766	1260
Kratošija	Control	1.30	1.28	310	330
	BDX	1.86	2.06	415	516
	D21	1.91	2.14	526	577
	BM4x4	1.92	1.87	453	523

**Table 4.** Chemical parameters and sensory evaluation in Vranac and Kratošija wine.

Variety	Yeast	Alcohol (vol. %)		Total dry extract (g L <sup>-1</sup> )		Total polyphenols (g L <sup>-1</sup> )		Total anthocyanins (mg L <sup>-1</sup> )		Sensory analysis (score)	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Vranac	Control	12.53	12.94	23.70	29.70	1.63	1.95	595.5	522.0	72.33	71.50
	BDX	12.44	13.33	26.10	30.05	1.68	2.32	463.5	574.5	77.00	75.60
	D21	12.43	13.84	24.80	29.40	2.21	2.98	508.5	730.5	77.00	76.90
	BM4x4	12.53	13.84	24.20	30.70	2.31	2.42	427.5	522.0	74.33	75.00
Kratošija	Control	13.44	12.81	24.20	27.04	1.28	1.33	132.0	222.0	71.00	70.00
	BDX	13.69	12.91	26.10	27.60	1.93	1.92	136.5	262.5	72.67	71.50
	D21	13.70	12.95	26.10	28.10	1.37	1.33	180.0	258.0	80.70	79.00
	BM4x4	13.90	12.73	25.50	26.10	1.71	1.75	181.5	186.0	79.60	78.00

After alcoholic fermentation, there were no significant differences in achieved alcohol content between used commercial yeast and control wine as well. In the vintage of 2013 the highest content of total dry extract was achieved by D21 yeast in both varieties, while in 2012 it was by BDX yeast.

Higher content of total polyphenols and anthocyanins is characteristic of Vranac variety, comparing to Kratošija which achieves significantly lower content of these compounds. Higher content of these compounds in wines after completion of alcoholic fermentation was also achieved in vintage 2013 (Table 3). There were no significant differences between BDX and D21 used yeast in total polyphenols content for both vintages in Vranac wines. In the vintage of 2013 there were not also significant differences between used yeast in achieved content of total anthocyanins, while control wine had much lower anthocyanins content. In 2012 vintage BDX yeast significantly stressed out in achieving anthocyanins content, while control wine and wine with other yeast had similar anthocyanins content values. Regarding to Kratošija wine, in this phase wine where D21 commercial yeast were used, showed the highest potential in accumulation of total polyphenols and anthocyanins in both vintages. Also, control wines had significantly lower content of these compounds for both vintages. However, these data are susceptible to changes and in these phase, cannot be considered as final indicator if wine quality.

### After maturation

In vintage 2013, the highest content of total dry extract, total polyphenols and total anthocyanins of Vranac wine after malolactic fermentation and after three months of maturation, were achieved by yeast BM4X4 (Table 4). There were no significant differences between alcohol content within treatments. Wines from 2012 are slightly better sensory evaluated and it is shown that D21 and BDX yeast had similar and significantly higher evaluating marks. The lowest sensory evaluation showed control wine. In Kratošija wine the highest total polyphenols and anthocyanins content was achieved by BDX yeast in

vintage 2013, while in 2012 the best results showed commercial yeast D21. Regarding to sensory evaluation it is shown that the highest marks got wines where D21 and BM4x4 were used.

### Conclusion

Results of this study highlight the importance of different commercial yeast inoculation as a tool to modulate red wine composition. Regarding to Vranac wine, it is noticed that inoculation with yeast BM4X4 gave wine with highest total polyphenol and total anthocyanin content, while wine with commercial yeasts BDX had the highest total dry extract content. As sensory characteristics are the most important, we concluded that D21 and BDX improved Vranac wine flavour and aroma. In Kratošija wine the highest total polyphenols and anthocyanins content was achieved by BDX yeast in vintage 2013, while in 2012 the best results showed commercial yeast D21. Regarding to sensory evaluation it is shown that the highest score got wines produced by D21 and BM4x4 yeasts.

In conclusion, by taking a broad approach to characterising the impacts of different wine yeasts on red wine composition we demonstrate a clear wine yeast impact 'signature' despite using different grapes from different vintages. The use of commercial strains of *S. cerevisiae* is becoming a common practice in winemaking. This practice ensures a reproducible product, reduces the risk of wine spoilage and allows a more predictable control of fermentation and quality.

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