JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 21, No. 1, 2023

Manuscript received 17.05.2023
Accepted: 21.06.2023

In print: ISSN 2545-4447
On line: ISSN 2545-4455
doi: https://doi.org/10.46763/JAPS23211041i
Original scientific paper

THE QUALITY OF RED WINE VRANEC, MERLOT AND FRANKOVKA FERMENTED BY COMMERCIAL AND BAKER'S YEASTS

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Abstract

The aim of this study is determination of the quality of red wines from grape varieties Vranec, Merlot and Frankovka fermented by wine yeasts from the French manufacturer SELECTYS® LA DÉLICIEUSE and baker's yeast seeded in a ratio of 0.25 g/L. The research was done during 2020 and 2021 in the Vinica vineyard, where the three grape varieties are grown at an altitude from 400 to 520 m. Regarding enological parameters, the highest amount of alcohol was measured for Merlot wines fermented by French yeast (13.07%), Frankovka wine fermented by baker's yeast had the highest amount of sugar (9.71 g/L), Vranec wine produced by baker's yeast had the highest total acidity (7.37 g/L) while the highest concentration of volatile acids was determined for Frankovka wine fermented by French yeast SELECTYS® LA DÉLICIEUSE (1.58 g/L). The impact of wine yeast to the quantity of particular elements was the most statistically significant for Fe57 in Merlot and Vranec wines, Cu65 in Frankovka wines and Pb208 in Merlot. Vranec and Merlot wines fermented by baker's yeast indicated higher amounts of phenolic compounds while Frankovka wines produced by SELECTYS® LA DÉLICIEUSE showed opposite tendency with significantly higher amounts of polyphenols.

Key words: *yeasts, wine, fermentation, Vranec, Merlot, Frankovka, winegrapes*

INTRODUCTION

Wines are classified as red, white and rosé on the basis of grape variety, sugar content, alcohol content, carbon dioxide content, color, fermentation and ageing process or geographical origin (Jackson RS., 2000).

Wines can be differentiated by the geographical location of the vineyards, the different methods of vinification, the use of yeasts of different origins (wine yeasts, indigenous yeasts or baker's yeasts) and etc.

Red wines are obtained by alcoholic fermentation of the must in the presence of the solid parts of the berry (skin and seeds), unlike white wines, which are produced exclusively by fermentation of the grape juice (Ribéreau-Gayon P., et al. 2006).

The use of yeasts in wine fermentation is of great importance as they are largely responsible for the complexity and sensory quality of wines. For this reason, current studies are mainly focused on the search for new yeast species to be used in winemaking technology. Isolation, morphological and physiological characterization of *Saccharomyces cerevisiae* yeast strains from 15 microregions of Tikveš wine-growing region of Macedonia as well as their impact of the organoleptic profile of wines produced from Vranec and Cabernet Sauvignon grape variety were studied by Ilieva et al., (2019).

Wine fermentation is a series of ordered and complex processes of biochemical transformation of yeasts and bacteria. To facilitate control of fermentation conditions and reduce the risk of spoilage and unpredictable changes in wine taste, commercial active dry yeast is commonly used in current wine production (Liu et al., 2021; Liu et al., 2016). The increasing usage of traded selected yeasts for winemaking leads

inevitably to a loss of the autochthonous yeast populations naturally present in regional grapes and, consequently, drives to the potential loss of genetic diversity and heritage. Largely, the specificities, authenticity, uniqueness and, most importantly, the quality characteristics of the wine are dependent on the natural microbiota found in the grapes of each viticulture region (Ilieva et al., 2021).

Domestication of wine yeast, while inadvertent until recent decades, has generated strains that differ considerably from "wild" *S. cerevisiae* strains. Inoculations of "wild" *S. cerevisiae* yeasts can influence the process of fermentation and greatly affect the quality of the wine. Isolating strains from successful fermentations for inoculation in subsequent vintages was being practiced during winemaking in order to avoid unwanted malolactic or acetic fermentation. Largely, the specifics and the most important quality characteristics of the wine are due to the natural microflora of the grape of the viticulture region (Ilieva et al., 2017). In the work of Petrov et al. the correlation between wine yeast SELECTYS® LA DÉLICIEUSE and spontaneous fermentation by wild yeast was examined (Petrov, Ilieva, Kostadinović Veličkovska & Dimovska, 2022).

The main object of this study is comparison of the quality of the wines from Vranec, Merlot and Frankovka fermented by wine yeast SELECTYS® LA DÉLICIEUSE and baker's yeast. For this purpose, after fermentation of wines by both yeasts, the oenological parameters (% of alcohol, sugar, total and volatile acids, pH and free SO_2 were compared as well as the amount of 8 elements (Li7, Be9, Mn55, Fe57, Ni60, Cu65, Ga71 and Pb208).

MATERIAL AND METHODS

The research was carried out during 2020 and 2021, during which 3 varieties of grapes were used: Vranec, Merlot and Frankovka, produced in the Vinica vineyard is found at the altitude of 400 to 520 m.

Fermentation technology

The tested grape varieties were harvested at technological maturity and processed with an electric grape mill. The grape mash was placed in 6 barrels of 75L each, for fermentation, then grapes were added in an amount of 10 mg/kg. After two hours, all samples were inoculated with the respective yeasts. Three of the vessels were seeded with yeasts for general use (baker's yeast), three of the vessels were seeded with pure cultured wine yeasts from the French manufacturer SELECTYS® LA DÉLICIEUSE. In the samples with inoculated yeasts this was done at a ratio of 0.25 g/L. The maceration period is 5 days, during which the mixture is stirred 3 times a day. The temperature in the rooms was constant from 22 °C to 25 °C.

Reagents and standards

The following chemicals were used in this study: Acetonitrile CHROMASOLV™ gradient grade ≥99.9% (Honeywell Riedel-de Haën™, Seelze, Germany), acetic and nitric acids Optima® LC/MS glacial (Fisher Chemical, Geel, Belgium), acetone Reag. Ph. Eur. 100% (VWR, Fontenay-sous-Bois Cedex, France), methanol ≥99.8% HPLC grade (Fischer Scientific, Loughborough, United Kingdom), water (deionized, nanopure®, Werner, Leverkusen, Germany).

Determination of oenological parameters, macro and micro elements and total phenolic compounds (TPC)

Determination of oenological parameters of wines fermented by two type of yeasts were performed with FOSS WINESCAN. Determination of the amount of alcohol was performed ebuliometrically by a Dujardin-Salleron ebulliometer (GW Kent, Ypsilanti, USA) method (Zoecklein et al., 1995), and for the determination of total reducing sugars, the Luff-Schoorl method (ISI 28-1e: Determination of Reducing Sugar, DE by Luff-Schoorl's method) was used and the results expressed as g/L. Quantification of titratable (TA) and volatile (VA) organic acidity in wines was performed according to the methodologies described by Ilieva et al. (2017), and both are expressed as g/L. total phenolic content was performed by the AOAC International Method (AOAC SMPR 2015.009: Estimation of Total Phenolic Content Using the Folin–Ciocalteu Assay, 2015) and expressed as mg/L. The pH values of the wines were determined by the International Standard Method according to OIV-MA-AS313-01.

Macro and micro elements in wine were performed with ICP/MS (mass spectrometry, which uses inductively coupled plasma that ionizes the sample). Inductively coupled

plasma with mass spectrometry (ICP-MS, model 7500cx Agilent Technologies, USA) with a glass concentric nebulizer was used for analyses of the elements content. In this study, five step set or combination of power, pressure, and time conditions for microwave-assisted digestion were applied. Microwave-assisted digestion conditions involved the digestion of 0.5 g of the sample with 5 mL HNO_3 and 2 mL of H_2O_2 in the microwave digestion system CEM model MARS 5 (CEM Corporation, Matthews, NC, USA). After digestion, the vessels were allowed to cool until the pressure of the vessel was reduced to below 50 psi and temperature was below 40 °C. The caps of each vessel were then carefully removed and the contents were filtered using 2µm filter paper diluted to 25 mL in a volumetric flask using deionized water, and stored in polyethylene vial prior to the final determination of the elements' concentration.

RESULTS AND DISCUSSION

The process of fermentation in wine-making turns grape juice into an alcoholic beverage (Amerine et al., 1980). In our research, the highest alcohol percentage of 13.07% was measured for Merlot wine obtained by fermentation using commercial wine yeast from the manufacturer SELECTYS® LA DÉLICIEUSE, while the lowest percentage of 11.36% is the Vranec variety obtained by fermentation in which baker's yeast is used. Generally speaking, the percentage of the alcohol in wine depends from the percentage of sugar and activity of the wine yeasts. The higher alcohol content is associated with the "vinous" aroma of fermented must, the intensity depending on the type of alcohols present and their concentrations (Lambrechts & Pretorius, 2000).

The residual sugar in red wine usually is related to the type of yeast and environmental conditions during the process of fermentation. The highest and the lowest values of residual sugars were obtained in samples which were fermented using baker's yeast, 9.71 g/L in Frankovka, and 0.59 g/L in Vranec, respectively. The significant difference in the residual sugars between those two wines depends on the amount of sugar in grapes and different ability of wine yeast SELECTYS® LA DÉLICIEUSE and baker's yeast to consume the sugar and convert in alcohol (Bisson, 1999; Branco et al., 2014; Maicas, 2020). The use of *S. cerevisiae* as starter culture is the most widespread practice in winemaking but not always inoculation of musts using selected *Saccharomyces* strains does ensure their dominance at the final stages of fermentation (Capece et al., 2010).

Total acids showed a strong negative correlation with alcohol and sugars in red wines. The data obtained in the research showed the lowest value of 4.80 g/L for Frankovka wine fermented with beer yeast, while the highest value of 7.37 g/L for Vranec fermented with baker's yeast. There is a strong positive correlation between total acids and volatile acids.

Volatile acids are strongly correlated with alcohol, sugars and total acids. Frankovka fermented with commercial wine yeasts has the highest value of volatile acids of 1.58 g/L, while Vranec fermented with baker's yeast has the lowest value of 0.23 g/L.

Wine pH is strictly intertwined with the microbiological and physiochemical stability of the product, as it affects the selection of microorganisms as well as some key chemical reactions, including sulphur dioxide balance (Comuzzo & Battistutta, 2019). Also, acidity and pH play an important role on the sensory characteristics and balance of wines (Comuzzo & Battistutta, 2019).

The pH data obtained in our research ranged from 2.97 for Vranec fermented with baker's yeast to 3.44 for Merlot fermented with commercial wine yeast. The correlation coefficient showed that there is a strong positive correlation between pH and the content of alcohol, sugar and free SO_2, while there is a strong negative correlation with total acids.

The amount of SO_2 in wines has a very important role in preventing oxidation and maintaining freshness in wine. The highest SO_2 value of 17.32 mg/L was obtained for Merlot fermented with baker's yeast, and the lowest 7.27 mg/L for Vranec fermented with baker's yeast.

Macro- and macroelements in wine are very important for its quality. The obtained data indicated that the highest level of Li7, Ni60, Cu65, Ga71 and Pb208 has the Merlot variety fermented with selected wine yeasts, while Fe57 has the Vranec fermented with baker's yeast, Mn

55 has the Merlot fermented with baker's yeast and the highest level of Be 9 has Frankovka fermented with commercial wine yeast. However, significant difference was obtained by presence of Li 7 in Vranec wines fermented by both yeasts. In addition, Vranec wine produced by bakery yeast had more than double amount of Fe 56 (7868.90 ppb) in comparison with the same wine fermented by wine yeast SELECTYS® LA DÉLICIEUSE (3947.09 ppb). Opposite, Merlot wine produced by wine yeast had almost double of the amount of Mn55 in comparison to the same wine produced by bakery yeast (Table 2). Frankovka wine produced by wine yeast SELECTYS® LA DÉLICIEUSE had 63,24 ppb Cu65 which is almost double in comparison to the same wine produced by bakery yeast (37.82 ppb). Finally, Merlot wine produced by wine yeast SELECTYS® LA DÉLICIEUSE had more than three times higher amount of Pb208 (21.96 ppb) in comparison to the same wine produced by bakery yeast (6.98 ppb) (Table 2). According to the Blackwell and Tobin, greater amounts of metal may be accumulated by yeasts due to their mechanisms for transport of metal ions into microbial cells include lipid peroxidation, complex permeation, carrier mediation, ion channels/pumps and endocytosis (Blackwell and Tobin, 1995). Most mechanisms of metal transport appear to rely on the electrochemical proton gradient across the cell membrane, which has a chemical and electrical potential, both of which are responsible for driving transport of ionized solutes across membranes (Gadd, 1993).

Tab. 1. Oenological parameters of Vranec, Frankovka and Merlot wines fermented by wine and baker's yeasts.

Wine	Yeast	Alcohol %	Sugar (g/l)	Total acids (g/L)	Volatile Acidity (g/L)	pH	Free SO_2 (mg/L)
Vranec	SELECTYS® LA DÉLICIEUSE	12.15	2.3	5.91	0.34	3.25	9.24
Vranec	baker's yeasts	11.36	0.59	7.37	0.23	2.97	7.27
Frankovka	SELECTYS® LA DÉLICIEUSE	12.35	4.42	6.58	1.58	3.38	8.20
Frankovka	baker's yeasts	12.54	9.71	4.80	0.40	3.40	9.02
Merlot	SELECTYS® LA DÉLICIEUSE	13.07	4.67	5.15	0.78	3.44	7.58
Merlot	baker's yeasts	12.93	3.13	5.18	0.37	3.32	17.32

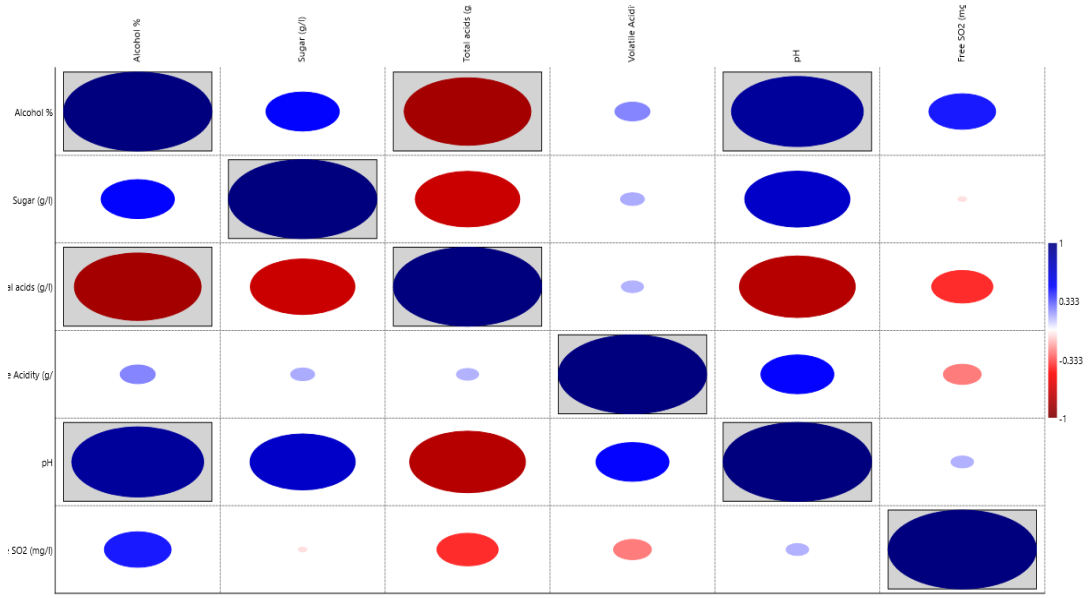

Figure 1. Correlation between investigated chemical characteristics of red wines

Table. 2. Multielement analysis of Vranec, Frankovka and Merlot wines fermented by wine and baker's yeasts.

Wine	Yeast	Li7 ppb ug/L	Be9 ppb ug/L	Mn55 ppb ug/L	Fe57 ppb ug/L	Ni60 ppb ug/L	Cu65 ppb ug/L	Ga71 ppb ug/L	Pb208 ppb ug/L
Vranec	SELECTYS® LA DÉLICIEUSE	16.64	0.29	1 311.18	3 947.09	30.93	50.31	3.03	5.86
Vranec	baker's yeasts	25.25	0.27	1 272.26	7 868.90	38.28	42.99	2.97	4.18
Frankovka	SELECTYS® LA DÉLICIEUSE	20.48	1 233.57	2 117.81	4 343.72	71.14	63.24	9.88	3.43
Frankovka	baker's yeasts	21.69	1 145.76	2 300.88	3 239.29	72.47	37.82	8.46	2.77
Merlot	SELECTYS® LA DÉLICIEUSE	53.44	0.39	3 000.61	5 037.64	97.82	163.08	15.47	21.96
Merlot	baker's yeasts	50.31	0.41	3 001.54	3 624.718	79.00	147.79	14.43	6.98

Polyphenols in grapes are phenolic acids, anthocyanins and flavonoids and their composition and content can vary depending on the location of grape cultivation (Leifert W.R. et. al. 2008).

Phenolic compounds can be useful markers for wine quality and its authenticity (Merkytė et al., 2020). Wine quality evaluation is based on taste, aroma and chemical characteristics. The sensory evaluation covers colour and taste, especially astringency which depends from the phenolic compounds presented in the wine. Thus, phenolic compounds are widely used for the wine quality and authenticity assessment (Chira et al., 2011; Heras-Roger et al., 2016; Lukic et al., 2016). In our research, the average polyphenolic profile obtained from three repetitions showed that the lowest value in the Vranec variety fermented with commercial wine yeasts, and the highest value of 43.281 mg/L in the Merlot fermented with baker's yeast. The highest significant differences were obtained for Frankovka wines fermented by wine and bakery yeasts. The total phenolic compounds obtained by wine yeast was 32. 132 mg/L while the same wine fermented by bakery yeast had 26.358 mg/L. This result can be explained by the fact that wine yeasts are more appropriate for fermentation of grape must and accumulation of phenolic compounds in wines (Ilieva et al., 2017).

Table 3. Determination of phenolic compounds in Vranec, Frankovka and Merlot wines fermented by wine and baker's yeasts (mg/L).

Wine	Yeast	First replication	Second replication	Third replication	Average
Vranec	SELECTYS® LA DÉLICIEUSE	24.042	22.763	22.993	23.266
Vranec	baker's yeasts	26.324	26.139	25.333	25.932
Frankovka	SELECTYS® LA DÉLICIEUSE	32.85	32.105	32.105	32.132
Frankovka	baker's yeasts	26.91	26.439	25.726	26.358
Merlot	SELECTYS® LA DÉLICIEUSE	41.475	41.071	40.79	41.112
Merlot	baker's yeasts	43.607	42.91	43.327	43.281

CONCLUDING REMARKS

The results from our study showed that type of the yeast strongly influenced wine fermentation and quality of the final wines. Vranec wine fermented by yeast strain SELECTYS® LA DÉLICIEUSE had higher amount of sugar while Frankovka wine had double amount of sugar fermented by bakery yeast in comparison to the same wine produced by wine yeast. Total and volatile activity was affected to the yeast strain to a lesser extent while amount of SO_2 was almost double in Merlot wine produced by bakery yeast. Regarding phenolic compounds, Frankovka wine was affected the most by the influence of yeast strain with significantly higher content of phenolic compounds produced by wine yeast SELECTYS® LA DÉLICIEUSE. Finally, the results from the element analysis indicated different influence of type of yeast strains to different elements. The most affected was Fe57 in Merlot and Vranec wines, Cu65 in Frankovka wines and Pb208 in Merlot wines.

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КВАЛИТЕТ НА ЦРВЕНИТЕ ВИНА ОД СОРТИТЕ ВРАНЕЦ, МЕРЛО И ФРАНКОВКА, ФЕРМЕНТИРАНИ СО КОМЕРЦИЈАЛЕН ВИНСКИ И ПЕКАРСКИ КВАСЕЦ

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Резиме

Целта на ова истражување е да се утврди квалитетот на црвените вина од сортите грозје Вранец, Мерло и Франковка ферментирани со вински квасец од францускиот производител SELECTYS® LA DÉLICIEUSE и пекарски квасец засеан во сооднос од 0,25 g/L. Во однос на анализираните параметри, најголемо количество алкохол е измерено во виното Мерло ферментирано со француски квасец (13,07 %), виното Франковка ферментирано со пекарски квасец има најголемо количество шеќер (9,71 g/l). Виното Вранец произведено од пекарски квасец има највисока вкупна киселост (7,37 g/L), додека највисока концентрација на испарливи киселини има виното Франковка ферментирано со француски квасец SELECTYS® LA DÉLICIEUSE (1,58 g/L). Влијанието на винскиот квасец врз количината на одредени елементи беше статистички најзначајно за Fe 57 во вината Мерло и Вранец, Cu 65 во виното Франковка и Pb208 во Мерло. Вината од Вранец и Мерло ферментирани со пекарски квасец покажаа повисоки количини на фенолни соединенија, додека виното Франковка произведено со SELECTYS® LA DÉLICIEUSE покажа спротивна тенденција со значително повисоки количини на полифеноли.

Клучни зборови: *квасец, вино, ферментација, грозје, Вранец, Мерло, Франковка.*