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INFLUENCE OF THE TYPE OF FERMENTER ON THE CHEMICAL COMPOSITION OF VRANEC AND MERLOT WINES

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Abstract

In this study, red wines from *V. vinifera* L. cv. Vranec and Merlot (harvest 2021) have been produced by three different fermentation methods, applying classical, roto and punch down fermenters, in order to study their influence on the wine quality. The chemical parameters that confirm the basic wine quality have been determined, including alcohol, dry extract, specific density, reducing sugars, total and volatile acidity, pH, free and total SO_2. The Vranec wines contained relatively higher content of alcohol compared to the Merlot wines, due to the higher content of sugars in the grapes. Wines produced with roto fermenter presented higher values of dry extract and total acidity, compared to the wine produced with classical and punch down fermenters, which confirms that roto fermentation is most suitable for production of complex and structured wines. The alcohol content in all wines ranged from 12.08 to 12.4%, total acidity was between 5.8 to 6.3 g/L and the dry extract from 39 to 45 g/L, while the content of volatile acidity was in range from 0.41 to 0.45 g/L. All wines were dry wines, with content of reducing sugar lower than 4 g/L and all of them were well protected from the oxidation. It was concluded that the type of fermentation affected the wine quality, confirming that applied wine technology is important for production of quality red wines.

Key words: *fermentation, Vranec, Merlot, chemical parameters*

INTRODUCTION

Wine is a beverage obtained by alcoholic fermentation of carbohydrates in the presence of yeast, which converts sugars into ethyl alcohol and carbon dioxide and releasing energy. The production of quality wine is influenced by numerous factors of which grape quality is one of the most important factors. However, the production of quality wine is not possible without good winemaking techniques and effective quality control. The production of red wine involves several procedures, such as grapes harvesting, grapes crushing, alcoholic fermentation (adding sulphur dioxide, yeast and nutrients to start alcoholic fermentation), maceration, addition of enzymes, cold stabilization, filtration, and bottling of the wine (Ivanova et al. 2012). After the addition of SO_2, the grape mash is inoculated with yeast (*Saccharomyces cerevisiae*) to start the alcoholic fermentation. The choice of the type of yeast depends on the grape variety as well as the characteristics of the wine that the producer wants to obtain (Lin et al. 2012).

The alcoholic fermentation is a complex biochemical process which depends on many factors such as: temperature, content of sugars, pH, acidity, presence of phenolic compounds as well as the content of the produced alcohol (Fleet, 2003, Divol et al. 2012, Mamolar-Domenech et al. 2023, Vion et al. 2023). Temperature is one of the most important factors for the start of alcoholic fermentation as well as for its duration. If the temperature is higher than necessary, the alcoholic fermentation starts earlier and lasts shorter. Therefore, the fermentation temperature should not be higher than 32°C. The sugars content in the must also affects the alcoholic fermentation. At low concentrations of sugars, the fermentation takes place slowly, while at concentrations higher than 250 g/L,

the fermentation is laboured and even may stop (Fleet, 2003).

The vessels in which the alcoholic fermentation process is carried out are called fermenters, which have a variety of shapes, sizes and technical designs. Today's fermenters are made of inox, stainless material and have a straight cylindrical or inverted cone shape. Classic fermenters are cylindrical stainless-steel vessels with a conical bottom, into which the crushed grapes enter from the upper opening. These fermenters are supplied with a pump that transports the fluid from the bottom to the top as well as temperature regulation, cooling with circulating water. Roto fermenters are cylindrical vessels in a horizontal position, supplied with a rotation programme. The contact between the must, skins and seeds is better and the maceration is more effective. Punch-down fermenters are cylindrical vessels in a vertical position, equipped with a cap submerging system, which consists of a punch-down agitator, pneumatic cylinder and a control unit. The punch-down mechanism makes it possible to break down the cap that was created during fermentation and submerge it into the must. The pneumatic cylinder continuously pushes the shovel-shaped punch-down agitator up and down, which makes the agitator gently pierce the cap and submerge it in the must.

This study is focused on the production of wines from Vranec and Merlot grapes, with three different types of fermenters: classic, roto and punch-down. The aim was to study the influence of the type of fermentation on the principal chemical parameters of wines from both varieties, such as alcohol, dry extract, specific density, reducing sugars, total and volatile acidity, pH, free and total SO_2.

MATERIAL AND METHODS

Chemicals and reagents

The following chemicals and reagents have been used: NaOH, concentrated H_2SO_4, $Na_2S_2O_3$, phenolphthalein, bromothymol blue, starch, KI, Feling I, Feling II and buffers (pH: 4, 7 and 7), all of them purchased from Alkaloid (Skopje). All reagents used were with analytical grade of purity. Ultra-pure deionized water with 0.0005 μS conductivity was obtained with a membrane filtration unit (Millipore, Molsheim France).

Winemaking

Grapes from *V. vinifera* L. *cv*., Vranec and Merlot were grown in Ovcepole wine region, harvested at optimal technological maturity (Vranec grapes: 24.5° Brix and Merlot grapes: 22.9 °Brix) in September 2021 and transported (~ 5 000 kg of each variety) to the wine cellar IMAKO, Stip, Republic of N. Macedonia. Both grape varieties were processed separately, applying the same technological procedures. Thus, the crushing of the grapes and the separation of the stems was carried out with a mechanical crusher/destimer (Della Toffola Treviso-Italy). The produced grape mash of both varieties was then transported to a cooling system at a temperature of 15 ^{0}C. Cooling was done using a heat exchanger (Della Toffola Treviso-Italy). To prevent oxidation, the cooled grape mash was treated with a 5% solution of sulphur dioxide, to reach a maximum concentration of free SO_2 of 40 mg/L. After cooling and sulfiting, the grape mash was transported to a suitable fermenter: classic, roto and punch down. All three types of fermenters have a volume of 9000 L.

A pectolytic enzyme (Sodinal, Speed up Rouge, 20g/100L) was added to each fermenter. After three hours, the grape mash in each fermenter was inoculated with commercial yeast *Saccharomyces cerevisiae* (Sodinal FERMCTIVE ROUGE, 20g/100L pre-activated and rehydrated in water at 30 ^{0}C), followed by the addition of nitrogen-based nutrients (ammonium dihydrogen phosphate, 10g/100L), necessary for the yeast to carry out the fermentation completely and successfully. Alcoholic fermentation in all three fermenters lasted 9 days for both varieties, and the temperature was constantly controlled at 24 ^{0}C by a cooling system.

The maceration for both grape varieties was carried out in a different way depending on the type of fermenter. In the classical fermenter the contact between the must, skins and seeds was achieved by mixing with a centrifugal pump that pumps the liquid together with the seeds and skins (from the lower part to the upper part of the fermenter) and thus caused mixing and improving the contact between the lees and the hard parts of the grapes. The mixing was programmed by turning on the pump 4 times a day. In the roto fermenter, maceration was performed by rotating the mash 4 times a day.

During this rotation, there was a mixing of the must and the solid parts of the grapes along the entire length of the fermenter. In the punch-down fermenter, the crushing of the formed cap of seeds and skins and their return to the liquid part was performed with the help of a piston that was programmed to push 4 times a day.

After completion of the alcoholic fermentation and maceration (the reducing sugar content was lower than 5 g/L), the wine from each of the three fermenters was separated from the pomace by pressing. The wine obtained from each fermenter was separately transported in a clean stainless-steel tank (7000 L). The wines were decanted and left to rest for a period of two weeks. After completion of the malolactic fermentation, the wines were sulphated again with a 5% solution of SO_2 to a concentration of free SO_2 to 40 mg/L. The wines were then left at a low outdoor temperature for a period of two months (December and January) to carry out natural tartrate stabilization. After stabilization, the wines from both varieties and fermented in the three different fermenters, were bottled in 0.75 L bottles under an inert nitrogen atmosphere to prevent the oxidation process. The wine bottles were stored in a cellar at a temperature of 2-8 °C for a period of one month before the analysis. Table 1 contains the labels of the produced wine samples.

Table 1. Labels of the wine samples.

Type of fermenter	Label of wine	
	Vranec	Merlot
Classical	V-C	M-C
Roto	V-R	M-R
Punchdown	V-P	M-P

Principal chemical composition

The following principal chemical parameters have been determined using the official methods of analysis of wines (OIV, 2022): alcohol (OIVMA-AS312-01 A), dry extract (OIV-MA-AS2-03B), specific density (OIV-MA-AS2-01 A), total acidity (OIV-MAAS313–01), volatile acidity (OIV-MA-AS313–02) and pH (OIV-MA-AS313-15).

Determination of reducing sugars, free SO_2 and total SO_2 was performed according to the methods published by Ivanova-Petropulos & Mitrev, 2014.

RESULTS AND DISCUSSION

The basic chemical parameters that are important for wine quality are: alcohol, dry extract, specific density, reducing sugars, total acidity, volatile acidity, pH, free SO_2 and total SO_2. These parameters determined for wine samples from Vranec and Merlot varieties obtained with three different types of fermenters (classic, roto and punch-down) are shown in Table 2.

Table 2. Basic chemical composition of Vranec and Merlot wines obtained under different fermentation methods.

Wine	V-C	V-R	V-P	M-C	M-R	M-P
Alcohol (%)	12.40	12.28	12.36	12.10	12.08	12.16
Dry extract (g/L)	40	45	40	39	44	39
Specific density	0.9957	0.9956	0.9959	0.9946	0.9946	0.9951
Reducing sugars (g/L)	2.7	2.4	2.6	2.2	2.5	2.4
Total acidity (g/L)*	6.1	6.3	6.2	5.9	6.0	5.8
Volatile acidity (g/L)**	0.44	0.44	0.42	0.45	0.41	0.44
pH	3.3	3.4	3.4	3.2	3.2	3.3
Free SO_2 (mg/L)	42	44	40	42	44	39
Total SO_2 (mg/L)	98	89	92	102	87	94

V-C: wine Vranec produced with classical fermentation, V-R: wine Vranec produced with roto fermentation, V-P: wine Vranec produced with punchdown fermentation, M-C: wine Merlot produced with classical fermentation, M-R: wine Merlot produced with roto fermentation, M-P: wine Merlot produced with punchdown fermentation.

*Total acidity expresses as g/L tartaric acid

**Volatile acidity expressed as g/L acetic acid

The alcohol content in the analysed wine samples from both varieties ranged from 12.08 to 12.4% (mean value 12.23%). The Vranec wines contained a slightly higher content of alcohol compared to the Merlot wines, regardless of the type of fermentation applied, because Vranec grapes had a higher content of reducing sugars than Merlot grapes (24.5° Brix in Vranec grapes and 22.9 °Brix in Merlot grapes). It is already known that according to the sugar content of the grapes/must, the alcohol content expected to be produced during fermentation can be approximately calculated. Thus, a wine with about 10% alcohol (*V/V*) should be obtained from must with 180 g/L of sugar. Considering the influence of fermentation, wines of both varieties obtained by roto fermentation presented the lowest alcohol value, while the highest value was measured for the Vranec wine obtained by the classic fermentation method (V-C) and the Merlot wine obtained by the punch-down method (M-P).

The wine dry extract consists of non-volatile soluble components, including sugars, non-volatile acids, glycerol, 2,3-butyl glycol, and phenols. In this study, the wines of both varieties presented high dry extract values, ranging from 39 to 45 g/L, which indicates that produced wines are rich and structured. Regarding the influence of the type of fermentation, the wines obtained by roto fermentation have the highest extract content, regardless of the variety, while the wines obtained by classical fermentation and punch-down fermentation have lower extract values. In fact, by the roto method greater extraction of the non-volatile components from the grapes in the wine has been achieved, confirming that the most complex and structured wines, more stable and richer wines have been obtained applying the roto fermenter.

Regarding the specific gravity of the wines, values ranging from 0.9946 to 0.9959 were obtained. These values are expected for red wines, according to the alcohol content and dry extract.

The main carbohydrates in grapes are glucose and fructose, which are usually called "reducing sugars". The content of sugars in grapes depends on the variety, ripeness, health of the grapes and the growing conditions. During alcoholic fermentation, the content of sugars decreases as a result of their conversion to ethyl alcohol, which usually results in dry wines with a low sugar content (< 5 g/L) (Neceva & Ivanova Petropulos, 2016). In this study, the analysed wines had a low content of reducing sugars ranging from 2.2 g/L (M-C) to 2.7 g/L (V-C), which means that all wines were dry and in all of them the fermentation ended successfully.

According to the wine acidity, it is very important to distinguish between the several types of wine acidity: total acidity, pH and volatile acidity, because all of them affect the wine's sensory characteristics. Total acidity is the sum of "non-volatile and volatile acidity", and includes all types of acids, such as formic acid, organic acids (tartaric, malic and citric), as well as amino acids. Since the tartaric acid is the predominant component in must and wine, the total acidity of the wine is expressed in tartaric acid equivalents. In this study, the concentration of total acidity ranges from 5.8 (M-P) to 6.3 g/L (V-R). These values are relatively high and sufficient to ensure the chemical and microbiological stability of the wines, as well as a sufficiently optimal freshness. Red wines are stable even at lower acidity due to the presence of phenols that increase acidity and enable stability of the wines during maturation. Taking into account the influence of fermentation, the wines obtained by the roto method presented slightly higher values of total acidity compared to the wines obtained by classical and punch-down fermentation.

Another factor that protects the wine and influences its stability is the pH. The pH values ranged between 3.2 to 3.4, typical for red wines. It is generally known that the lower the pH, the more difficult it is for microorganisms to survive. Comparing to Montenegrin wines (Pajović-Šćepanović, et al. 2016), the content of total acidy was slightly higher, and the value of pH was slightly lower in the analysed Vranec and Merlot wines.

Volatile acidity of wine represents a very significant physico-chemical parameter, which should be monitored during the winemaking. Increased volatile acidity in wine negatively affects its organoleptic characteristics, as well as its overall quality. The occurrence of increased content of volatile acids is related to the process of formation of acetic acid in a relatively high concentration (Neceva & Ivanova Petropulos, 2016). All wines, regardless of variety and type of fermentation, contained low and similar volatile

acidity in the range of 0.41 to 0.45 g/L, with no influence on quality. These values were expected, considering the fact that the temperature of fermentation was controlled and the content of added SO_2 in the wines was sufficient to protect the wines from oxidation and also from the creation of a higher concentration of acetic acid. The content of acetic acid is a parameter that is legally controlled and therefore constant and regular monitoring during production is necessary. The maximum allowed content of volatile acidity is 1.2 g/L acetic acid for red wines (Official Gazette of the Republic of Macedonia, 2012).

The use of SO_2 in winemaking is due to its ability to act as an effective antioxidant and antimicrobial agent. SO_2 has the ability to bleach pigments as well as the ability to eliminate unpleasant odours in wine. Moreover, SO_2 can selectively act against wild yeasts originating from grape skins and block their activity. Sulphur dioxide can be added into wine as a solution (5% solution of sulfuric acid) or as a potassium metabisulfite salt ($K_2S_2O_5$) which is ionized in an acidic environment releasing gaseous SO_2 (Ivanova-Petropulos & Mitrev, 2014). In this study, the free SO_2 content ranged from 39 to 44 mg/L, while the total SO_2 content ranged from 87 to 102 mg/L, which is a sufficient SO_2 content for the wines to be protected from oxidation.

Understanding the relationship between pH and SO_2 is of particular importance for winemaking. The effectiveness of the antimicrobial activity of SO_2 depends on the pH of the wine. The higher the pH of the wine, the less SO_2 will be need in a free form. Therefore, it is very important for wines to have a lower pH value and a higher content of total acidity, because in this way the microbiological and chemical stability of the wine would be achieved. If SO_2 is added in a higher concentration, it causes an unpleasant sharp smell of SO_2, and strengthening the metallic taste in the mouth, which has negative effects on the organoleptic characteristics of the wine, and thus on its the quality.

CONCLUDING REMARKS

Vranec and Merlot wines were produced by three types of fermentation, applying classical, roto and punch-down fermenters and the chemical composition was determined. Wines presented relatively high values of alcohol, dry extract, total acidity and low values of volatile acidity. This means that wines from both varieties had satisfactory quality, presenting good chemical and microbiological stability. Alcoholic fermentation was finished in all wines. Wine produces with roto fermenter contained highest dry extract and total acidity, which confirms that this roto fermentation was most suitable for production of more complex and structural wines.

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ВЛИЈАНИЕ НА ТИПОТ НА ФЕРМЕНТАТОР НА ХЕМИСКИОТ СОСТАВ НА ВИНА ОД СОРТИТЕ ВРАНЕЦ И МЕРЛО

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Резиме

Во ова истражување произведени се црвени вина од *V. vinifera* L. *cv*. сортите Вранец и Мерло (година на берба 2021) со примена на три ферментациони методи, со примена на класичен ферментатор, рото ферментатор и ферментатор со потиснување, со цел да се проучи нивното влијание врз квалитетот на винаta. Беа определени хемиските параметри со кои се потврдува основниот квалитет на виното, вклучувајќи алкохол, сув екстракт, специфична тежина, редуцирачки шеќери, вкупна и испарлива киселост, pH и слободен и вкупен SO_2. За винаta од сортата Вранец беше забележано дека имаат повисока содржина на алкохол споредено со винаta од сортата Мерло, поради повисоката содржина на шеќери во грозјето од сортата Вранец. Винаta произведени со рото ферментатор имаат повисока содржина на вкупен екстракт и вкупна киселост, споредено со винаta произведени со класичен ферментатор и ферментатор со потиснување, со што се потврдува дека рото ферментацијата и посоодветна за производство на комплексни и структурни вина. Содржината на алкохол во сите вина се движеше во граници од 12,08 до 12,4 %, вкупната киселост беше во опсег од 5,8 до 6,3 g/L и сувиот екстракт во граници од 39 до 45 g/L, додека содржината на испарлива киселост беше во граници од 0,41 до 0,45 g/L. Сите вина беа суви, со содржина на редуцирачки шеќери пониска од 4 g/L, и сите беа соодветно заштитени од оксидација. Беше заклучено дека начинот на ферментација влијае на квалитетот на виното, при што беше потврдено дека е многу важно каква технологија ќе се примени за производство на квалитетно вино.

Клучни зборови: *ферментација, Вранец, Мерло, основни хемиски параметри.*