Influence of selected yeast during alcoholic fermentation on red wine production

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Abstract— The process of fermentation in wine making turns grape juice into an alcoholic beverage. During fermentation, yeasts transform sugars present in the juice into ethanol and carbon dioxide (as a by-product). In wine making, the temperature and speed of fermentation are important considerations as well as the levels of oxygen present in the must at the start of the fermentation. The most common yeast associated with wine making is *Saccharomyces cerevisiae* which has been favored due to its predictable and vigorous fermentation capabilities, tolerance of relatively high levels of alcohol and sulfur dioxide as well as its ability to thrive in normal wine pH between 2.8 and 4. This research is done by the curiosity of how selected yeast affect in the alcoholic fermentation process for red wine production and how fermentation can be more regular and controlled with the use of selected yeasts than with natural yeast in spontaneous fermentation. During this research, was followed the entire process of fermentation of selected Saccharomyces cerevisiae yeast, of these varieties of red grapes: Pinot Noire, Game, Vranac, Cabernet Sauvignon, Merlot and Shiraz. Every morning was taken a sample of each variety and by aerometer was measured the temperature and sugar to see how is going with fermentation process, how is decreased the sugar level and increased the alcohol and temperature level, because the fermentation process is an exothermic process that mean releases energy. When the aerometer shows 0^0O_e the level of sugar, we take a sample for each variety and sent to the lab for complete analysis that includes: the alcohol grade, SO_2, total acidity, volatile acids, residual sugar, specific weight, extract and color intensity. Each parameter has its own measurement method. The final results of each sample are within allowed limits according to the Wine Law in Kosovo.

Index Terms—Alcohol, Fermentation, Grape, Red wine, Saccharomyces cerevisiae, Samples, Sugar.

1 INTRODUCTION

Grapes, the wonderful fruit of the vineyard, have attracted human attention since ancient times.It is used for consumption and wine production, depending on the variety. Ripened, it contains significant amounts of sugar and acidity that help it to ferment and produce enough alcohol to make a delicious wine.Wine is an alcoholic beverage made from fermented grapes.Yeast consumes grape sugar and converts it into ethanol, carbon dioxide and releases heat.Different varieties of grapes and different strains of yeast produce different styles of wine.The process of red wine production involves the extraction of color and aromatic compounds from the grape skin.Red wine is produced from dark grape varieties.The actual color of the wine can range from purple, typical of new wine, red of matured wine, to brown of old red wine.The red color comes from the anthocyanine pigment present in the grape skin.The role of yeast in wine production is the most important element that distinguishes wine from grape juice.The most common yeast associated with wine production is Saccharomyces cerevisiae which has been favored due to its predictable and potent fermentation ability, tolerance to relatively high levels of alcohol and sulfur dioxide, and the ability to be activated in the normal wine pH between 2.8 and 4.Harvesting grapes usually bring a variety of wild yeasts, of which we can mention Kloeckera and Candida genera.In addition to alcohol, during the fermentation process, yeast can also produce many other secondary products.Wine can be classified by the year of harvest. For most of the wine planned to be aged, are used grapes and best care, so often this type of wine is more expensive than the variety produced for immediate use.The color of wine is determined by the presence or absence of grape skin during fermentation, since most part of grape pulp have transparent juice.Red wine is produced from red (or black) grapes, but its red color is due to the presence of grape skin during fermentation.Compounds known as polyphenols are found in large amount in wine and there is evidence that these compounds are extremely good for health.An interesting polyphenol is resveratrol, which is given numerous beneficial properties.We can conclude by saying that wine, following the advic to consume it properly, may be part of the human nutrition mosaic list, and exceptions are in some cases pathologies, such as: pregnancy or breastfeeding, or advanced states of various diseases.

2 VINIFICATION

2.1 Alcoholic fermentation

Alcoholic fermentation is due to the action of yeast. This fermentation is a biochemical phenomenon, during which many chemical and physical phenomena are noticed. In the first days yeast multiplication occurs and fermentation begins, which is observed with the release of carbon gas. Then begins the rapid outflow of carbon gas to give the impression of a boiling phenomenon. During this process cider sugar is converted into alcohol, carbon gas and other secondary products, under the action of alcoholic yeast. Alcoholic fermentation is associated with a rapid increase of temperature in the fermentation mass because this process is exothermic. The density decreases as a result of the conversion of sugar into alcohol. During the fermentation process, due to the benefit of alcohol, the digestion of dyestuff and tannins occurs and the digestion of a certain amount of minerals. With the onset of alcoholic fermentation, the carbon bubbles take away the solid parts that float inside the cider and stick them high, forming a solid, porous mass, but less dense than the fermenting cider, called "caps". In the "cap" and juice meeting area, fermentation is rapid because yeasts are in much greater amount in the skin than in the juice. The cap also favors the formation of fluoric acids because it is in contact with air. The total acidity at the beginning of fermentation increases, while at the end it decreases to the level of the initial value due to the lowering of tartaric salts. The sinking of

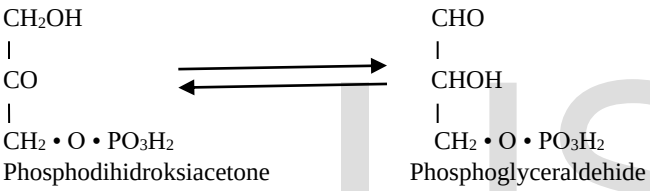
tartric salts occurs by reducing their solubility in the formed alcoholic solution. During alcoholic fermentation the cider blurs and the taste changes from sweet to pungent, due to the extraction of tannic substances from the skin. [1]

2.2 Chemistry and mechanism of alcoholic fermentation

Like any living organism, yeast needs certain energy for living. In the first case the yeast uses sugar in the presence of oxygen and in the other case only the sugar:

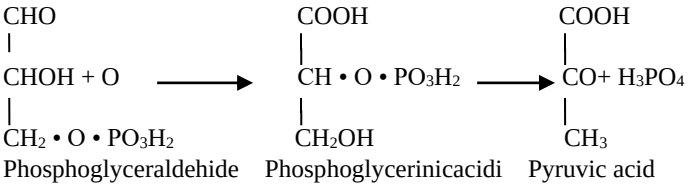
1. $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 673$ cal
2. $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 33$ cal

The above reactions represent the chemistry of one and the other process in yeast metabolism. Based on this material, alcoholic fermentation occurs in two directions: one leading to the formation of ethyl alcohol and carbon dioxide as the major products, and the other leading to the formation of secondary sugar transformation products. Initially glucose with phosphoric acid decomposes into two trios, and this is the first bridge to sugar decomposition:



Phosphoglyceraldehyde is an oxidizing-reducing process, where one part is being oxidized and the other being reduced.

1. Oxidation of phosphoglyceraldehyde:



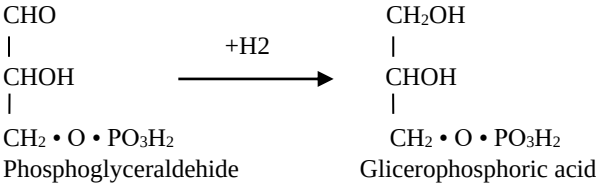
In the following reactions, pyruvic acid causes decarboxylation and this is the moment when carbon dioxide is formed by the formation of acetaldehyde in the process of alcoholic fermentation:

$$CH_3 \bullet CO \bullet COOH \xrightarrow{\text{dekarboksilaza}} CH_3 \bullet CHO + CO_2$$

Formed acetaldehyde is further reduced where ethyl alcohol is produced:

$$CH_3 \bullet CHO \xrightarrow[+2H]{\text{kodehidraza}} CH_3 \bullet CH_2OH$$

1. Reduction of phosphoglyceraldehyde:



Due to the formation of glycerol and pyruvic acid at the same time, prior to the formation of alcohol, the process of alcoholic fermentation proceeds in two stages, one of which parks the fermentation of glycerin pyruvate and the other directly alcoholic fermentation:

1. $C_6H_{12}O_6 = CH_2OH \bullet CHOH \bullet CH_2 \bullet OH + CH_3 \bullet CO \bullet COOH$
2. $C_6H_{12}O_6 = 2CH_3 \bullet CH_2OH + 2CO_2$ [2]

2.3 Causes of alcoholic fermentation

Yeast - A yeast is a unicellular fungus that reproduces asexually by flowering or division, especially Saccharomyces genus which is important in food fermentations. The most well-known examples of yeast fermentation are in the production of alcoholic beverages and the fermentation of bread. Saccharomyces cervisiae varieties are the most common yeast in fermented foods and drinks based on fruits and vegetables. All species of this genus ferments glucose and many ferment other plant carbohydrates such as sucrose, maltose and raffinose.
Necessary Conditions for Fermentation - Most yeasts require an abundance of oxygen for growth, so by controlling oxygen supply, their growth can be controlled. In addition to oxygen, they require a basic substrate such as sugar. Some yeast can ferment sugars in alcohol and carbon dioxide in the absence of air, but require oxygen for growth. They produce ethyl alcohol and carbon dioxide from sugar such as glucose and fructose.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{Yeast}} \underset{\text{Ethyl alcohol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

Yeasts are active in a wide temperature range of 0-50 °C, with an optimum temperature range of 20-30°C. The optimum pH for most microorganisms is near neutral point (pH 7.0). Molds and yeast are usually acid-tolerant and are therefore associated with the breakdown of acidic foods. Yeast can grow in a pH range of 4 to 4.5 and molds can grow from pH 2 to 8.5 but favor an acidic pH. In terms of water requirements, yeasts are between bacteria and molds. Bacteria have the highest water requirements, while molds require less water. Normal yeasts require a minimum water activity of 0.85 or a relative humidity of 88%. Yeasts are quite tolerant of high sugar concentrations and grow well in a solution containing 40% sugar. At concentrations higher than this, only a certain group of yeast - the osmophilic type - can survive. [3]

2.4 Selected yeast and nutritional requirements of yeast

One of the most important discoveries in wine production is that alcoholic fermentation can be better encouraged and controlled through inoculation of grape juice with selected cultures of Saccharomyces cerevisiae. From an ecological point of view, it is expected that the inoculated strain of Saccharomyces cerevisiae will suppress and eradicate indigenous non-Saccharomyces species and indigenous strains of Saccharomyces cerevisiae, and predominate fermentation. Although these expectations and assumptions have been widely accepted by winemakers. Inoculation of grape juice with high initial population of Saccharomyces cerevisiae will not necessarily prevent the growth of indigenous non-saccharomyces yeast and that these species may also contribute to general

fermentation, so dominance of the inoculated species was not always assured and depends on the specific conditions of the vinification.[4] Yeast needs sugar to turn into alcohol, amino acids to build proteins and ultimately new cells, vitamins and minerals to make enzymes work correctly and they need phosphorus to create new DNA. Typical vitamin requirements for yeast include biotin, nicotinic acid, vitamin B, and pantothenic acid. Vitamins can also play an antioxidant role. Phosphorus is an essential component of deoxyribonucleic acid (DNA), as well as phospholipids within cell membranes. 3-5% of the dry cell weight material of yeast is phosphorus, most of which is stored in vacuoles inside the yeast cell. Minerals include calcium (Ca), potassium (K), magnesium (Mg), and many more trace metal ions. [6]

2.5 Grape harvest time and maceration process

The time of harvest is determined primarily by the ripeness of the grape as measured by sugar, acid and tannin levels with winemakers basing their decision to pick based on the style of wine they wish to produce. The harvest season typically falls between August & October in the Northern Hemisphere and February & April in the Southern Hemisphere. [5] Throughout the history of wine, winemakers would use the sugar and acid levels of the grape as a guide in determining ripeness. Early winemakers tasted the grapes to gauge ripeness. Modern winemakers use a refractometer to measure high sugar levels and °Brix or titration tests (using an indicator such as phenolphthalein) to determine the titratable acidity within the grape. In recent times there has been more of an emphasis on the "physiological" ripeness of the grape, usually in the form of tannins and other phenolics. [8] The harvest time of the varieties sampled is:
Pinot Noir – End of August
Game - Begining of September
Merlot - End of September
Cabernet Sauvigon– End of September
Shiraz - Beginning of October
Vranac - End of October [9]
Maceration is the winemaking process where the phenolic materials of the grape—tannins, coloring agents (anthocyanins) and flavor compounds—are leached from the grape skins, seeds and stems into the must. Temperature is the guiding force, with higher temperatures encouraging more breakdown and extraction of phenols from the skins and other grape materials. Maceration continues during the fermentation period, and can last well past the point when the yeast has converted all sugars into alcohol. The process itself is a slow one with compounds such as the anthocyanins needing to pass through the cell membrane of the skins to come into contact with the wine. [7] Maceration process is influenced by numerous factors such as temperature, duration, alcohol content, SO_2 where the extraction of the volatile and phenolic compounds varies according to variety, maturation conditions and other factors. Consequently, the maceration should be modulated and fractioned according to the grape variety and quality and also to the style of wine desired. [10] This technological operation implies the contact between the juice and the solid parts of the grapes for a specific time. [11]

3 METHODOLOGY

Samples were taken at Stone Castle Winery located in Rahovec, Kosovo where the whole fermentation process was controlled, respectively by fermentation tanks with their varieties: T-1 (Pinot Noir), T-2 (Game), T-3 (Vranac), T-4 (Merlot), T-5 (Shiraz) and T-6 (Cabernet sauvignon). All samples were measured for sugar and temperature every morning during the fermentation process by an aerometer (as shown in fig 1). If the temperature rises too high during fermentation, each tank has a water cooling system running continuously around the tank.

Fig 1. a)Fermentation tanks b) Aerometer c) Sugar and temperature measurement

4 RESULTS OBTAINED FROM THE FERMENTATION PROCESS

Each grape variety begins and finishes the fermentation process depending on the time of ripening and harvesting of the grape and the amount of sugar. All grape varieties, for fermentation, have been treated with 5% SO_2, extractive enzymes, yeast rehydration, selected Saccharomyces cerevisiae yeast and yeast food. Below we present the fermentation process of each grape variety that was taken as sample.

Table 1. The fermentation process of the Pinot noir grape

Variety	Date of measurement	Sugar rate ^oO_e	Temperature rate^oC	Laboratory analysis
Pinot noir	28.08.2017	100	20	Alcohol 13,9%
	29.08.2017	97	22	SO_2 free/total 42/90
	30.08.2017	83	25	Volatile/total acids 0,4/4,9
	31.08.2017	65	26	Sugar residue 1,8 g/l
	01.09.2017	45	27	Specific weight 0,9938
	02.09.2017	10	26	Extract 30,5g/cm^3
	03.09.2017	0	25	Color Intensity 4,2

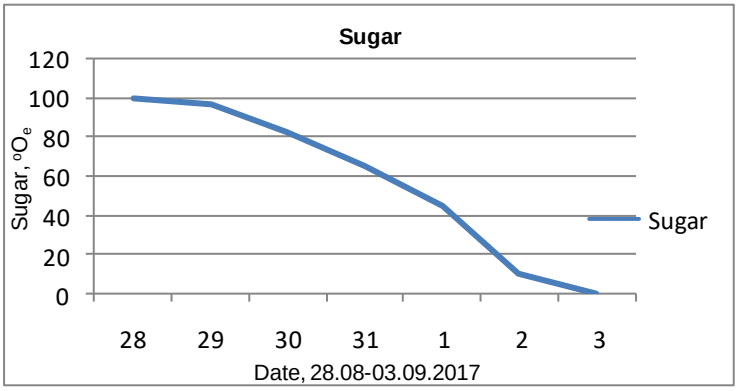


Fig 2. Amount of sugar during the fermentation process of Pinot noir grape

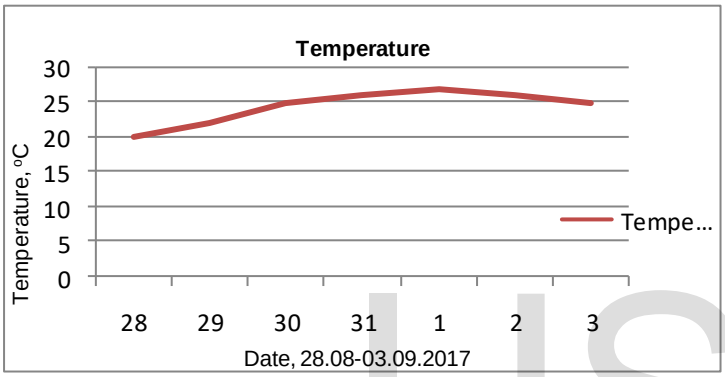


Fig 3. Temperature rate during the fermentation process of Pinot noir grape

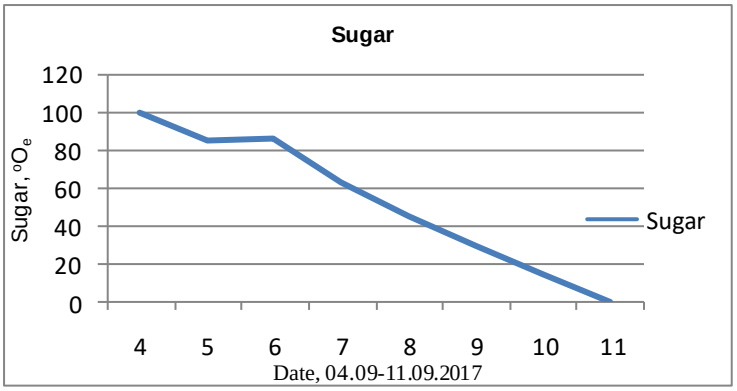


Fig 4. Amount of sugar during the fermentation process of Game grape

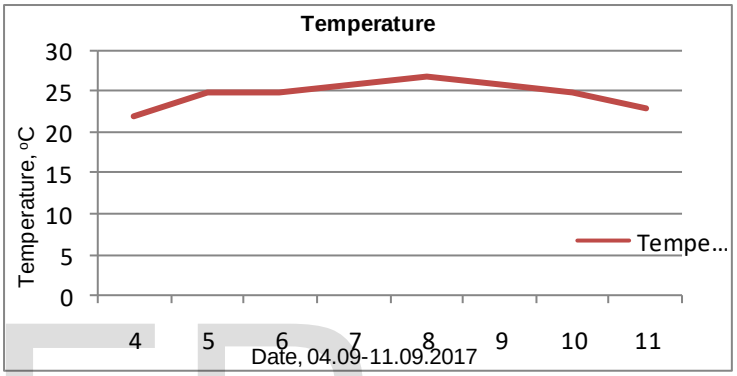


Fig 5. Temperature rate during the fermentation process of Game grape

Table 2. The fermentation process of Game grape

Variety	Date of measurement	Sugar rate °O_e	Temperature rate °C	Laboratory analysis
Game	04.09.2017	100	22	Alcohol 13,9 %
	05.09.2017	85	25	SO_2 free/total 43/78
	06.09.2017	86	25	Volatile/total acids 0,47/5,2
	07.09.2017	63	26	Sugar residue 2,1 g/l
	08.09.2017	45	27	Specific weight 0,9945
	09.09.2017	29	26	Extract 32 g/cm^3
	10.09.2017	15	25	Color Intensity 9,0
	11.09.2017	0	23	

Table 3. Fermentation process of the Merlot grape

Variety	Date of measurement	Sugar rate °O_e	Temperature rate °C	Laboratory analysis
Merlot	23.09.2017	102	17	Alcohol 14,2 %
	24.09.2017	101	18	SO_2 free/total 47/80
	25.09.2017	100	19	Volatile/total acids 0,45/4,6
	26.09.2017	90	23	Sugar residue 2,3 g/l
	27.09.2017	63	26	Specific weight 0,9945
	28.09.2017	40	27	Extract 31 g/cm^3
	29.09.2017	20	26	Color Intensity 7,2
	30.09.2017	10	24	
	01.10.2017	2	23	
	02.10.2017	0	22	

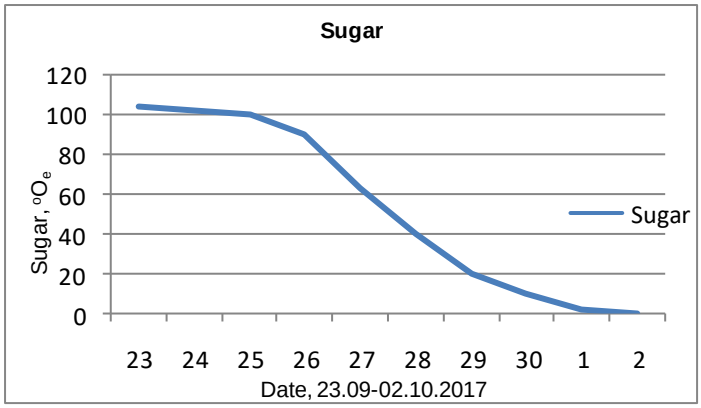


Fig 6. Amount of sugar during the fermentation process of Merlot grape

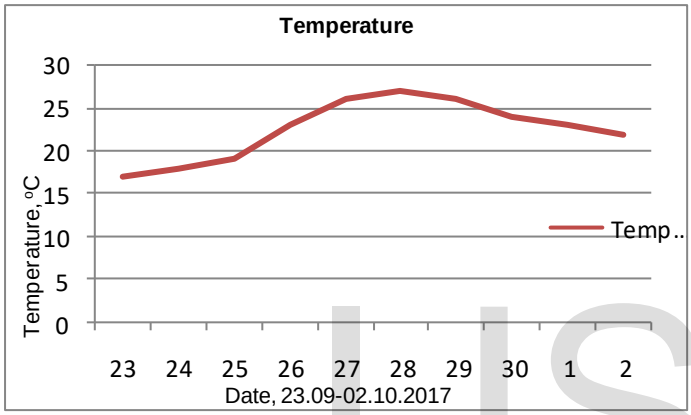


Fig 7. Temperature rate during the fermentation process of Merlot grape

Table 4. Fermentation process of Cabernet sauvignon grape

Variety	Date of measurement	Sugar rate °Oe	Temperature rate °C	Laboratory analysis
Cabernet sauvignon	26.09.2017	101	17	Alcohol 14,1 %
	27.09.2017	96	19	SO_2 free/total 47/80
	28.09.2017	85	23	Volatile/total acids 0,45/4,6
	29.09.2017	65	26	Sugar resi-due 2,3 g/l
	30.09.2017	40	26	Specific weight 0,9952
	01.09.2017	21	25	Extract 32g/cm^3
	02.09.2017	12	24	Color Inten-sity 8,5
	03.09.2017	5	23	
	04.10.2017	2	21	
	05.10.2017	0	20	

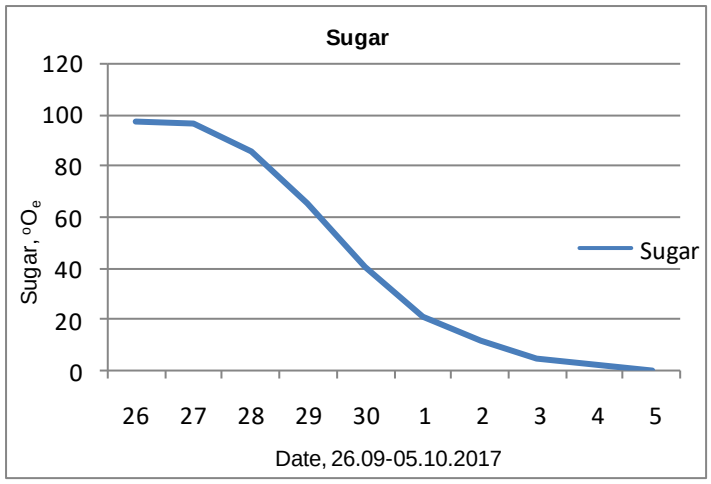


Fig 8. Amount of sugar during the fermentation process of Cabernet Sauvignon grape

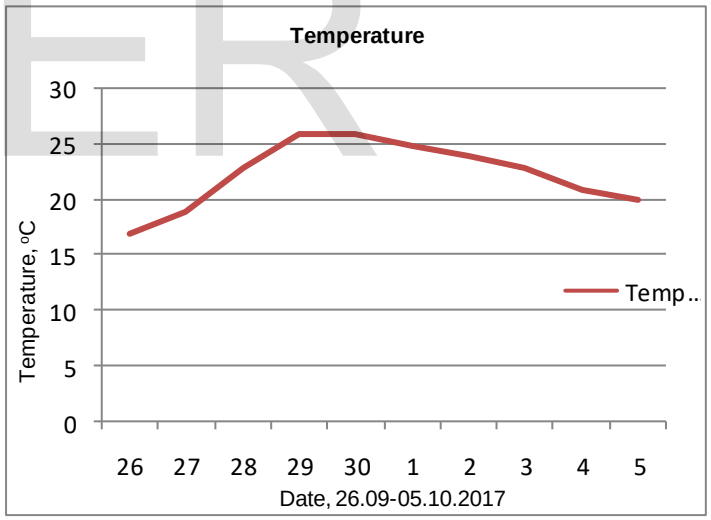


Fig 9. Temperature rate during the fermentation process of Cabernet Sauvignon grape

Table 5. Fermentation process of Shiraz grape

Variety	Date of measurement	Sugar rate ^oO_e	Temperature rate ^oC	Laboratory analysis
Shiraz	06.10.2017	98	20	Alcohol 13,6%
	07.10.2017	95	22	SO_2 free/total 57/79
	08.10.2017	65	27	Volatile/total acids 0,44/4,8
	09.10.2017	42	28	Sugar resi-due 2,1 g/l
	10.10.2017	21	27	Specific weight 0,9942
	11.10.2017	13	26	Extract 30g/cm^3
	12.10.2017	0	24	Color Inten-sity 8,3

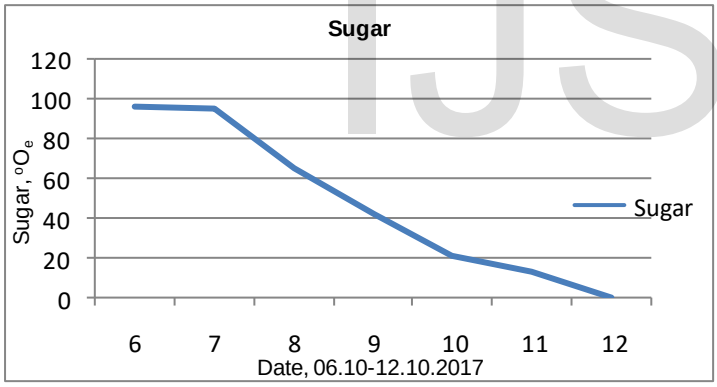


Fig 10. The amount of sugar during the fermentation process of Shiraz grape

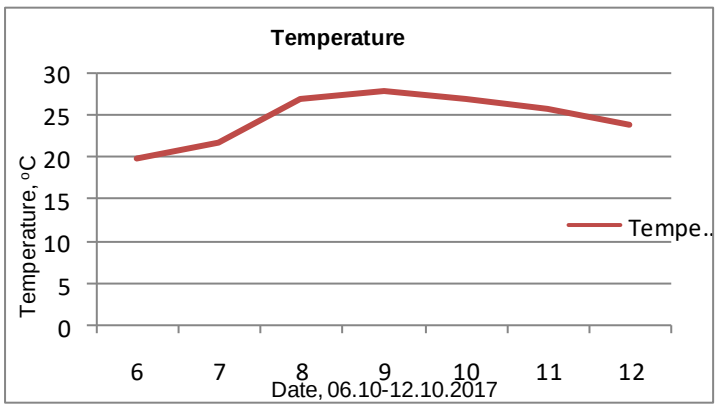


Fig 11. Temperature rate during the fermentation process of Shiraz grape

Table 6. Fermentation process of Vranac grape

Variety	Date of measurement	Sugar rate ^oO_e	Temperature rate ^oC	Laboratory analysis
Vranac	01.10.2017	101	23	Alcohol 14,1 %
	02.10.2017	80	25	SO_2 free/total 51/70
	03.10.2017	61	26	Volatile/total acids 0,44/5,2
	04.10.2017	42	27	Sugar resi-due 2,15 g/l
	05.10.2017	30	26	Specific weight 0,9942
	06.10.2017	20	25	Extract 31g/cm^3
	07.10.2017	12	23	Color Inten-sity 10,6
	08.10.2017	7	21	
	09.10.2017	2	20	
	10.10.2017	0	20	

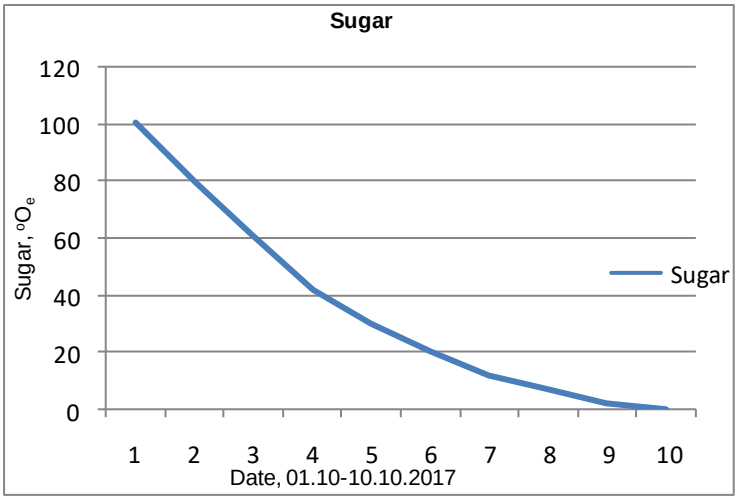


Fig 12. Amount of sugar during the fermentation process of Vranac grape

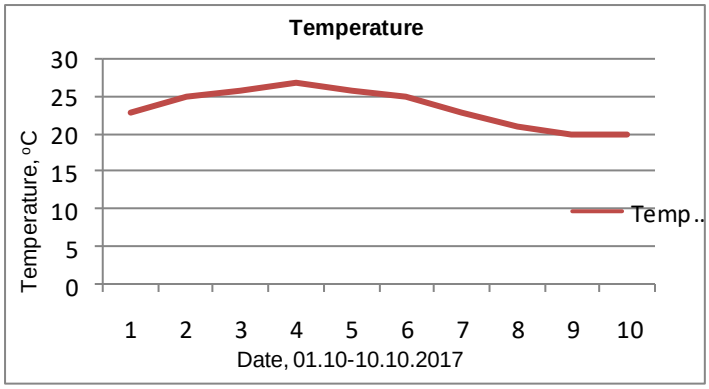


Fig 13. Temperature rate during thefermentation process of Vranac grape

Fig 14. Color extraction and its intensity during the alcoholic fermenta tion process

5 Discussion of results

This research was done to see the effect of selected yeast during the process of alcoholic fermentation for red wine production.Although grape itself has natural yeast that can turn grape sugar into alcohol, but during this process, in addition to alcohol, unwanted secondary products are created and the process of alcoholic fermentation can easily be diverted.Today are used selected yeasts Saccharomyces Cerevisiae which necessarily convert sugar to alcohol by releasing CO_2 during this process.With the use of selected yeasts, the fermentation process is more orderly, controlled and we obtain the final product with high quality in color and characteristic aroma of the variety.For research were taken these varieties of red grapes: Pinot Noire, Game, Merlot, Cabernet Sauvignon, Shiraz and Vranac. Harvest time was respected in each variety and each was harvested in a timely manner when it reached its highest sugar level.On the basis of the sugar content of the grapes and according to laboratory analysis was produced wine with this % of alcohol: Pinot Noire 13.9%, Game 13.9%, Merlot 14.2%, Cabernet 14.1%, Shiraz 13.6% and Vranac 14.1%.During the fermentation process the lowest temperature was 17^0C while the highest was 28^0C, so it is within the permissible range.The critical temperature limits of the activity of selected yeast are temperatures below 150 °C and above 280 °C, i.e. at temperatures below 150 °C or above 280 °C the yeast loses its activity and the fermentation process may stop.Fermentation with natural yeast can cause stagnation, may take longer and as a result we may obtain a wine with higher volatile acids (acetic acid) and sugar residues.The fermentation process with selected yeast takes 7-14 days and based on the results we see that we have obtained wine with volatile acids (acetic acid) 0.44-0.47g/l and with sugar residues 1.8-2.3g/l so we obtained a high quality dry wine.On the basis of the grape variety we have also obtained the color intensity ranging from the lightest grape variety Pinot Noire with color intensity 4.2 to the darkest grape variety Vranac with color intensity 10.6. As an important parameter of wine is the extract which represents the wine matter that does not evaporate. The wine that has the most extract counts as a very high quality wine. On the basis of the analysis of the samples and on the basis of the varieties of wines we have obtained wine with this extract value: Pinot Noire 30,5g/cm3, Game 32 g/cm3, Merlot 31 g/cm3, Cabernet sauvignon 32 g/cm3, Shiraz 30 g/cm3 and Vranac 31 g/cm3.

6 Conclusions

During this research, was contolled the alcoholic fermentation process that started on 28.08.2017 with the Pinot Noire variety and ended on 10.10.2017 with the Vranac variety by selected yeasts of Saccharomyces Cerevisiae. This yeast converts grape sugar into alcohol by releasing carbon dioxide-CO^2Using this yeast, the fermentation process is more controlled and lasts 7-14 days, depending on the grape variety and the amount of sugar.The process was completed as intended and the temperature throughout the process was kept within the permissible limits for yeast activity. Measuring the amount of sugar we have seen a gradual decreasing as the temperature has increased.After completion of the alcoholic fermentation process, samples were taken for organoleptic and laboratory analysis.From organoleptic analysis we find that we have obtained a wine with characteristic color and taste of the variety and without any external aromas, while from the laboratory analysis we find that we have obtained high quality wine with 13.6-14.2% alcohol and with extract 30 -32 g/cm3.The results obtained from this research give us a conclusion that if we want to produce a high quality wine, it is preferable to use selected yeasts of Saccharomyces cerevisiae.

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