

Influence of Selected Yeasts in the Qualitative Characteristics of Wine, Compared to Spontaneous Fermentation.

LAMÇE Fatbardha, SINI Kristaq

Faculty of Biotechnology and Food, Agricultural University of Tirana, Tirana, Albania,

ABSTRACT: In this study, selected yeasts (isolated and identified in the Laboratory of Microbiology and Food Biotechnology at the Agricultural University of Tirana, named KL2 and KB3), commercial oenological yeasts and indigenous yeasts, are compared based on the influence on qualitative characteristics of wine. Strains KL2 and KB3 are *Saccharomyces cerevisiae* species. The variety used for the wine production is Merlot. The wines were chemically analysed for sulphurous anhydride, pH, total acidity, alcoholic degree, reducing sugars, content of anthocyanins and tannins and colour parameters. The experiments showed that the wines produced by the selected yeasts KB3 and KL2 gave the best results in all analysed indicators, especially the wine produced by the selected yeast KB3. The data showed that the quality of wines from selected yeasts was higher than the quality of the wines fermented by commercial yeast and indigenous yeasts.

KEYWORDS: Wine, Fermentation, Selected yeast, Commercial yeast, Indigenous yeast.

I. INTRODUCTION

The diversity and composition of oenological yeasts significantly contribute to the characteristics of the wine. The growth of each species of wine yeast is characterized by a specific metabolic activity, which influences the concentration of specific components in the final product [1]. One of the most important decisions of the winemakers is whether to use indigenous yeast or selected yeast for the fermentation process [10]. Fermentation is a vitally important stage in the process of vinification. The most important elements in the winemaking process are the grapes and the yeasts. Yeasts contribute to the qualitative characteristics of wine and also have a strong impact on the character of wine [2]. Extraction of flavor and color from the grape skins (for red wines) also occurs during fermentation. Indigenous yeasts of the grapes start fermentation process after a week because their initial population is smaller compared to an inoculated fermentation. Yeasts used in fermentation process are selected for their technological characteristics (ability of fermentation, fermentation power, energy of fermentation etc.), but no less important are the qualitative characteristics like volatile acidity and sulphides production. A high value of volatile acidity is correlated to a presence of large amounts of acetic acid. Sulphides are components that in the right levels may be desirable in the wine but in high concentrations they give to the wine, flavours like rubber, rotten eggs, etc [4]. The use of selected yeasts in wine production technology is very important because they provide a controlled fermentation, reducing the risk of slowing or stopping fermentations as well as microbial contamination [5]. The use of selected yeasts [3, 12] improved the quality of wine, also helped the winemakers to control the fermentation process and to obtain products with desirable characteristics.

II. MATERIALS AND METHODS

Yeasts

The experimental protocol includes four variants of fermentation. In the first and second variants, fermentations were carried out by KB3 and KL2 strains, which have been isolated and identified in the Laboratory of Microbiology and Food Biotechnology at the Agricultural University of Tirana [6] and studied for their technological characteristics [7]. In the third experimental variant were used commercial oenological yeast (*Saccharomyces cerevisiae* (Bayanus)),

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 10, October 2015

which were used and stored according to the manufacturer's recommendations. The fourth variant of the experiment was indigenous yeasts.

Winemaking

The Merlot variety grapes were harvested at 21.9 °Brix (vintage 2014), transported to the Food Research Centre of Agricultural University of Tirana. Grapes were separately processed (crushed and destemmed) in the same way, and were divided into 4 lots (25 kg each). Crushed grapes were collected in fermentation tanks and were added a dose of 50 mg/L SO₂. All the tanks, with the exception of one that was fermented with indigenous yeasts (spontaneous fermentation), were inoculated with 10⁶ cells/ml of selected yeasts KL2 and KB3, and commercial yeast. The musts were manually plunged once a day until completion of fermentation. During the fermentation temperature ranged from 23°C to 25°C. After completion of the fermentation, the wines were bottled with the addition of 30 mg/L SO₂ and stored in a cellar.

After 6 months of storage, wines were analysed and results were compared in order to check the influence of the yeasts in the qualitative characteristics of wine.

Analytical determinations:

Free SO₂ was determined through iodometric method which is based on oxidation of free SO₂ with iodine in acid environment. **Total SO₂** was determined with the iodometric method, by titration with iodine in the presence of starch as indicator. pH was measured by pH - meter, Denver Instrument product. **Titrateable acidity** was determined by titration of the wine with NaOH 0.1 N solution in the presence of bromthymol blue as indicator. **Volatile acidity** was determined by the method Dyklo – Gejon, which is based on withdraw volatile acids with water vapour. **Alcohol** content was measured by the ebulliometric method. **Reducing sugars** were determined by the method Reblin [8].

Polyphenols

Tannins were determined by spectrophotometric method. Samples were diluted in a mixture of butanol, chlorhydric acid 12 N (37%) and ferric sulphate, closed hermetically; one of them was placed in 80 °C and the other in a dark place. After the reaction, the absorbance was measured at 550 nm, using distilled water as blank [13]. **Total anthocyanins** were determined by Puissant - Leon method, by the reaction with HCl 1N [9]. **The total polyphenols index** was determined by spectrophotometric method. Samples were diluted and the absorbance was measured at 280 nm, characteristic of benzoic acid present in all polyphenols [14].

Wine chromatic characteristics

Chromatic characteristics of the wines were determined by spectrophotometric method. Due to the colloidal structure of the colouring substances, the wines were diluted with a mixture of tartaric acid, NaOH 1 N and distilled water. After the reaction the absorbance was measured at 420 nm and 520 nm. **The colour intensity** was calculated as the sum of the absorbances measured at 420 nm and 520 nm, according to Sudraud. **The Hue** of wine was calculated as the ratio of absorbance at 420 nm and absorbance at 520 nm. **The colour index** was calculated as the ratio of absorbance at 520 nm and absorbance at 420 nm [11].

Samples were made in triplicate and analysed statistically by Microsoft Excel 07.

III. RESULTS AND DISCUSSIONS

A strictly standard fermentation protocol was followed during the vinifications, the same for the four experimental variants, with the only difference, the inoculated yeasts. The dynamics of fermentation is graphically presented in Figure 1.

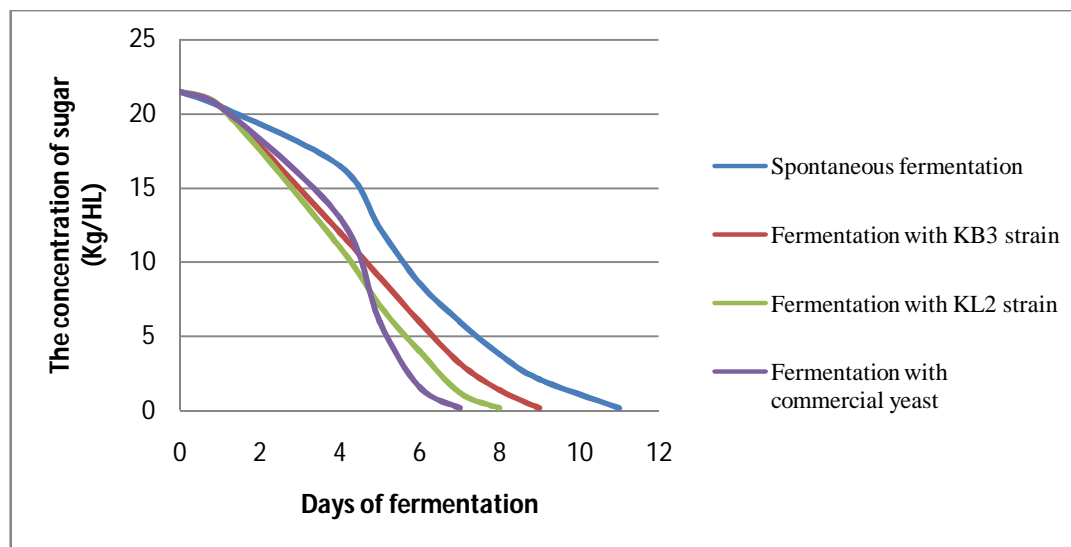


Figure 1. The dynamics of fermentation

As seen from the graphic above the fermentation conducted by commercial yeast ends sooner than the fermentations of selected strains KL2 and KB3. The last to finish was the spontaneous fermentation with 4 days of delay from the fermentation conducted by commercial yeast.

➤ **The analytical indicators of wines.**

Wine samples were analyzed initially for basic parameters such as total and free sulphur dioxide, pH, titratable and volatile acidity expressed in Table 1. The alcoholic degrees and reducing sugars are shown in Figure 2.

Table 1. Analytical indicators of wines

	Wine from KL2 strain	Wine from KB3 strain	Wine from spontaneous fermentation	Wine from commercial yeast
Free SO ₂ (mg/l)	16.00 ¹ ± 0.60	19.21 ± 0.01	14.41 ± 0.02	18.40 ± 0.80
Total SO ₂ (mg/l)	57.60 ± 0.64	66.56 ± 0.33	75.20 ± 0.32	78.40 ± 0.37
pH	3.72 ± 0.02	3.66 ± 0.02	3.41 ± 0.10	3.84 ± 0.01
Titratable Acidity(gr/L)	6.38 ± 0.06	5.86 ± 0.02	6.37 ± 0.08	5.86 ± 0.06
Volatile Acidity (gr/L)	0.22 ± 0.02	0.18 ± 0.01	0.35 ± 0.02	0.21 0.01

1. The average and standard deviation

From Table 1 it is noticed that the wine fermented by the strain KB3 and the commercial yeast have a higher content of free SO₂ compared to the other wines, even though the dose of treatment with SO₂ has been the same for all four variants of experiments. Wines which are distinguished for higher content of the total SO₂ are spontaneous fermentation and the one with commercial yeast. pH values in these wines ranges from 3.41 (spontaneous fermentation) to 3.84 (commercial oenological yeast fermentation). The wines fermented by KL2 strain and spontaneous fermentation showed the highest content of total acidity. Spontaneous fermentation also showed a high content of volatile acidity, this because of the longer time of fermentation while the wine fermented by KB3 strain results in a very low value of volatile acidity.

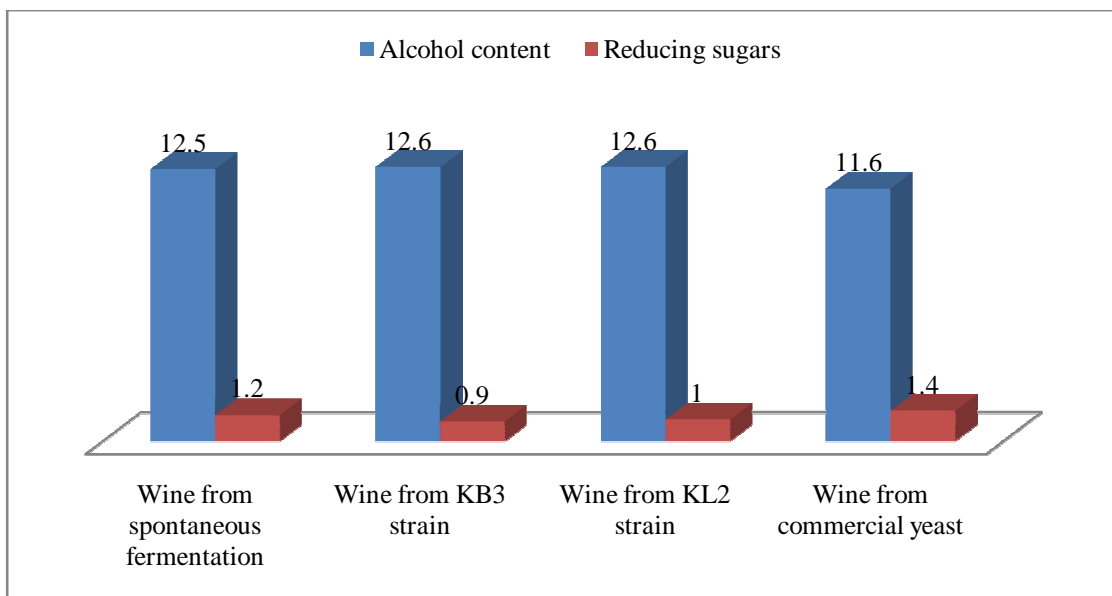


Figure 2. The content of alcoholic degree and reducing sugars in wines.

As seen in Figure 2, alcoholic degree is higher in the wines produced by selected strains KL2 and KB3. These wines also have a lower content of reducing sugars, which means that the yeast had consumed all the sugar founded in musts. While the wine, produced by commercial yeast had a lower alcoholic degree and a higher value of reducing sugars which means that the fermentation has stopped before consuming the sugars in the wine. The wine produced by spontaneous fermentation shows similar values like the wines from the strains KB3 and KL2.

Table 2. The polyphenolic values.

Analysis	Wine from strain KL2	Wine from strain KB3	Wine from spontaneous fermentation	Wine from commercial yeast
I.P.T ¹	49.87 ² ± 0.50	60.67 ± 0.12	49.27 ± 0.46	41.33 ± 0.12
Total Anthocyanins (mg/L)	252.61 ± 0.70	315.45 ± 0.68	270.39 ± 0.64	249.87 ± 0.75
Tannins (g/L)	1.26 ± 0.01	1.38 ± 0.00	1.23 ± 0.01	0.93 ± 0.00

1. Total polyphenols index.
2. The average and standard deviation.

The polyphenols are an important indicator of the quality of the red wine, which affect the taste, colour etc. The wine fermented by the KB3 strain had the highest values of IPT, total tannins and anthocyanins. The wine fermented by commercial yeast had the lowest values of these indicators.

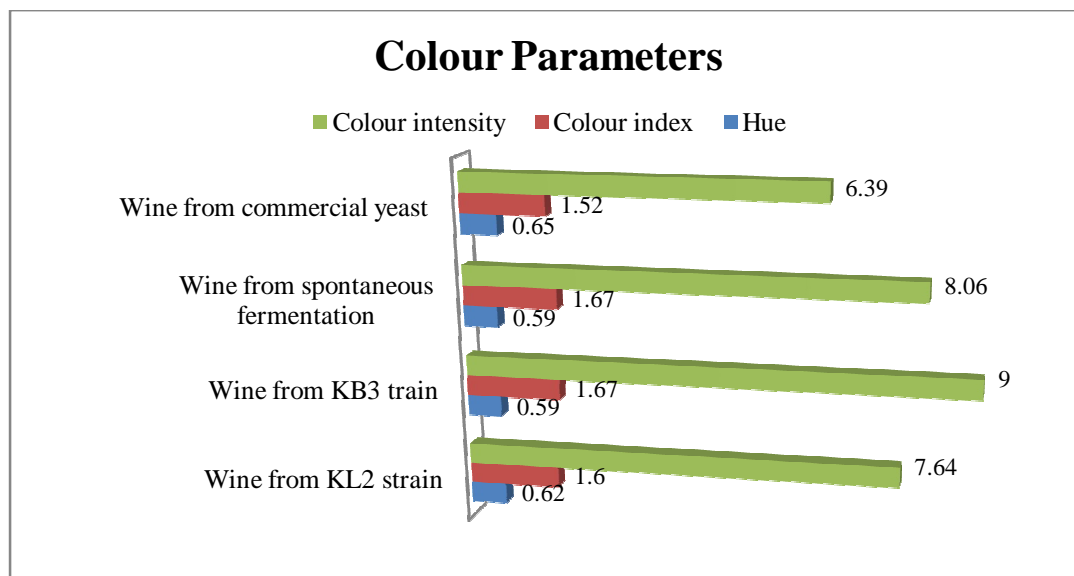


Figure 3. Colour parameters in wine

Regarding the intensity of colour and colour index, the highest values belongs to the wines produced by the strain KB3 and spontaneous fermentation, and the hue had higher values in the wines fermented by the commercial yeast and indigenous yeasts.

IV. CONCLUSIONS

The vinifications showed that wines produced by selected strains KB3 and KL2 gave better results compared to the fermentation with commercial yeasts and indigenous yeasts.

The fermentation with selected yeast ended in 8-9 days, all the sugar was assimilated, giving a high alcoholic degree. The polyphenols values were higher in these wines compared with the wines produced with indigenous yeasts and commercial yeast.

Commercial yeast ended the fermentation in 7 days, but this was followed with a lower alcoholic degree, lower content of polyphenols value, while reducing sugars showed a higher value compared with other experimental variants.

Spontaneous fermentation ended in about 11 days, this followed with a higher content of volatile acidity.

From the results obtained it was observed that KB3 strain represents the best oenological qualities (technological and quality). The KB3 strain is recommended to be used as starter culture in directed fermentation.

REFERENCES

- [1] ROMANO, P., FIORE, C., PARAGGIO, M., CARUSO, M., CAPECE, A. Function of yeast species and strains in wine flavour. International Journal of Food Microbiology 86, p169– 180, 2003.
- [2] ROSS, J. P., Wild Yeast in Winemaking. Wine & Vines, 1997.
- [3] SZUCS, E., LEHOCZKI-TORNAL, J., PETER, G., HAJDÚ, C.S., SZARVAS, J. : Selection of indigenous *Saccharomyces cerevisiae* strains in Eger Wine District, XXXth World Congress of Vine and Wine, Budapest, (OIV Konferencia Kiadványban), 2007.
- [4] KARLSSON, P.: Using native yeast or selected yeast at Quevedo Wines in the Douro. Winemaking & Viticulture, 2013.
- [5] MAIFRENI, M., COMI, G., RONDININI, G.: "Selection and oenological characterization of *Saccharomyces cerevisiae* strains isolated from Tocai, Pinot and Malvasia grapes and musts of the Collio area". Annali di Microbiologia ed Enzimologia, 49: 33-43, 1999.
- [6] LAMÇE, F. & SINI, K.: "Isolation and characterization of oenological yeasts". Albanian Journal of Agricultural Sciences; Vol. 12 Issue 4, p669, 2013.
- [7] LAMÇE, F. & SINI, K.: "Study of the oenological characteristics of selected yeasts". In 4th International Conference of Ecosystems (ICE2014), Tirana – Albania, p 199, 2014.
- [8] PIJANOWSKI, J.C., GUERRERA, A., SCHUBERT, E. VWT 172: Laboratory analysis of musts and wines, standard operating procedures, Napa Valley College, 2007.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 10, October 2015

- [9] PUISSANT, A. & LÉON, H.: La matière colorante des grains de raisins de certains cépages cultivés en Anjou en 1965. *Ann. Technol. Agric.*, 16(3), pp.217–225, 1967.
- [10] REGODON, J. A., PEREZ, F., VALDES, M. E., DE MIGUEL, C. AND RAMIREZ, M.: A simple and effective procedure for selection of wine yeast strains *Food Microbiology* 14, 247–254, 1997.
- [11] BECCHETI, R.: “Metodi di analisi dei vini e delle bevande spiritose”, sesta edizione, 1999.
- [12] ORLIĆ, S., OČIĆ, N., JEROMEL, A., HUIĆ, K., REDŽEPOVIĆ, S.: “Selection of Indigenous *Saccharomyces cerevisiae* Strains from Kutjevo Wine Growing Area at the Laboratory Scale”. *Agriculturae Conspectus Scientificus*, Vol.70, No.3 (93-97), 2005.
- [13] PORTER, L. J., HRSTICH, L. N., & CHAN, B. G.: The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 25(1), 223–230, 1985.
- [14] CETÓ, X., GUTIÉRREZ, J. M., GUTIÉRREZ, M., CÉSPEDES, F., CAPDEVILA, J., MÍNGUEZ, S., JIMÉNEZ-JORQUERA, C., VALLE M.: “Determination of total polyphenol index in wines employing a voltammetric electronic tongue”. *Analytica Chimica Acta* 732: 172–179, 2012.