Original scientific paper
10.7251/AGSY1404447L

STUDY OF TECHNOLOGICAL CHARACTERISTICS OF SELECTED OENOLOGICAL YEASTS FOR DIRECTED FERMENTATION

Fatbardha LAMÇE, Kristaq SINI

Faculty of Biotechnology and Food, Agricultural University of Tirana, Tirana, ALBANIA
*Corresponding authors: fatbardha_meta@yahoo.com; kristaqsini@yahoo.com

Abstract

In this study, 9 strains of yeast (8 strains *Saccharomyces cerevisiae*, 1 strain *Kloeckera apiculata*) selected by authors in 2012 - 2013, were tested to study their technological characteristics by densimetric method. The experiment was carried out in Erlenmeyer flasks containing 100 mL of grape juice (variety Merlot) with 265 g/L sugar. Studied technological characteristics were fermentation power, fermentation energy, and resistance to sulphur dioxide, fermentative ability at low temperatures as well as the dynamics of fermentation. Regarding fermentative power of yeasts 5 strains were distinguished with high alcohol production capability over 12 %vol., 1 strain with average alcohol production capability about 11 %vol. and 3 strains showed low alcohol production capability. Regarding the energy fermentation, 6 strains were distinguished to have high fermentation energy over 1.3 %vol. alcohols/day during the first week of fermentation. Also, 2 yeast strains were distinguished with high fermentative power (over 12 %vol.) in presence of SO_2 and 1 yeast strain with good fermentative ability (over 12 %vol.) at low temperatures. In conclusion, the strains that showed good fermentative ability, will serve to further work for microvinifications with directed fermentation to determine the most competitive and productive strains aimed at standardizing the wine.

Keywords: *selected yeast, oenology, and fermentation.*

Introduction

The conversion into wine of the grape must is a fermentative process performed by the microorganisms, whose composition notably contributes to the quality of the wine. Spontaneous fermentations produce wine characterized by peculiar aromas and flavour due to complexity of the natural micro-flora, which depends upon a number of external environmental factors like temperature, humidity etc, mainly composed by non-*Saccharomyces* yeasts (FAO). Before, wine production was the result of spontaneous fermentation carried out by the micro flora of the grapes but nowadays the winemaking practices are modified by using starter cultures, especially for large quantity wine productions (Rainieri *et al.*, 2000). The selection of yeasts for winemaking consists of identifying those cultures that can ferment grape juice efficiently and produce good quality wines. The use of selected yeasts in wine production technology is very important, because they provide a quick fermentation and safe, and reduce the risk of slowing or stopping fermentation and microbial pollution (Maifreni *et al.*, 1999). Wine fermentation is not carried out by single yeast but from a set of selected yeast which being combined in the right way can provide a qualitative product and sustainable wine (Fugelsang, 1997; Fleet & Heard, 1993). Selection of yeast destined for wine production, depends on their technological features such as fermentation power, fermentation energy, resistance to sulphur dioxide, the ability to ferment at low temperatures (Delfini, 1982). The aim of this study was to the individuation of selected yeast strains useful in the improvement of the quality of the wine. In this work we study the characteristics of these strains, which attribute the improvement of wine quality and the possibility of winemakers to control the fermentation process and the acquisition of some specific characteristics of the wine.

Materials and methods

Microorganisms

In this study, eight (8) strains of *Saccharomyces cerevisiae*, isolated and identified in the Laboratory of Microbiology and Food Biotechnology in 2012 – 2013 at the Agricultural University of Tirana labelled: KS1’, MP4, MS5’, KS7’, KB2, KB1, KL1, M1 and one (1) strain of *Kloeckera apiculata* labelled MS5, are used(Lamçe, F. and Sini, K. 2013).

Microfermentation

The study of technological characteristics of yeasts was determined using the densimetric methods as described by Zambonelli (1998) in Merlot grape variety with 26.5% sugar content. Fermentation tests were carried out in 250 ml Erlenmeyer flasks equipped with corks conceived by Pasteur pipette, which contained 100 ml of must. After steam – sterilizing the flasks at 90 ºC for 15 minutes, 10^6 cells/ ml culture 48 hour PD Broth (potato-dextrose) were inoculated, in sterile conditions and each yeast strain was carried out in triplicate. Tests were placed in different fermentation conditions: microfermentation at 25 ºC, microfermentation at temperatures 12-19 ºC, microfermentation in the presence of SO_2.To control weight loss by CO_2 liberation, weights were made every day until no more weight change, and that means the end of fermentation.

Analytical determinations

The dynamics of the fermentation was determined as the amount of alcohol produced during fermentation, in all the days until the end. The fermentation energy is related to the speed with which a yeast strain begins fermentation and ends it. The fermentation energy was determined as the sum of the amount of CO_2 produced in 7 and 11 first days of fermentation converted to alcoholic degrees (%vol.). Resistance to SO_2 expresses the ability of yeast strains to give fermentation in the presence of activity of this antiseptic, which was calculated with the total amount of CO_2 produced, converted into alcoholic degrees. Fermentation at low temperature is related to the ability of yeasts to ferment the musts at low temperatures. Alcohol produced during fermentation was determined indirectly by multiplying the weight loss (in %) with 1.292 factor (Zenelaj 2004).

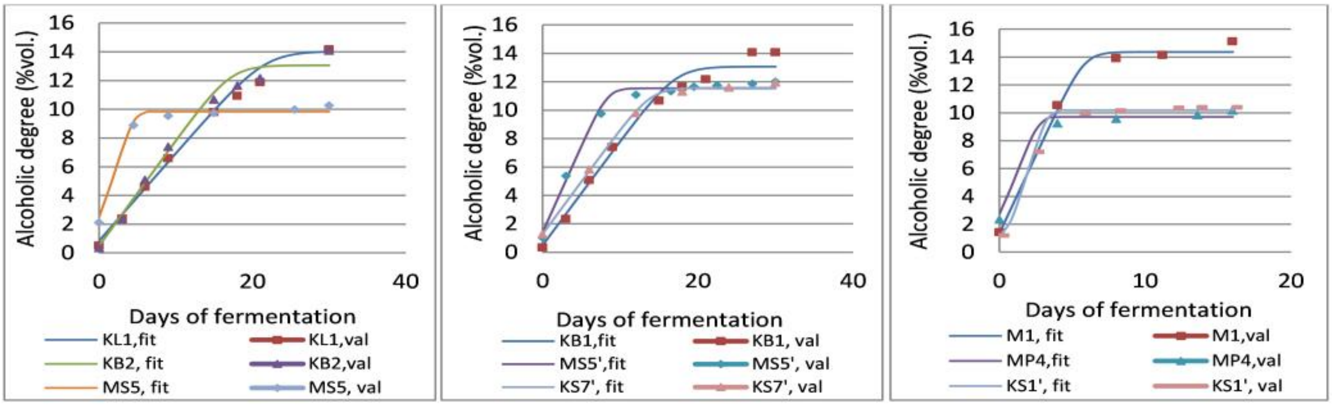
Results and Discussion

The following are the results which refer to the technological indicators studied as dynamics of fermentation, fermentation energy, fermentation power, resistance to SO_2 and the ability to ferment at low temperatures. Results of the work are processed by Baranyi model.

Performance of fermentation dynamics (fermentation curve)

Fermentation dynamics,which means the performance fermentation in time, is presented graphically in Figure 1.

Figure.1. Performance of fermentation dynamics



a) b) c)

Table 1.Performance of fermentation dynamics

Strains	yDatMin	yDatMax	rate	lag	y0	yEnd	se(fit)	R^2_stat
KL1	0,51	14,18	0,60		0,85	14,03	0,54	0,98
KB2	0,38	14,39	0,62		0,66	14,24	0,56	0,98
KB1	0,35	14,07	0,73		0,49	13,06	0,61	0,97
M1	1,44	15,12	2,23		1,36	14,36	0,35	0,99
MS5’	1,03	12,01	1,82		1,40	11,54	0,43	0,98
MP4	2,36	10,18	3,51	0,70	1,49	9,70	0,43	0,96
MS5	2,11	10,27	3,79	0,71	1,19	9,83	0,38	0,97
KS7’	1,28	11,96	1,47		1,28	11,57	0,29	0,99
KS1’	1,2	10,39	3,75	0,46	1,11	10,18	0,26	0,99

From the fermentation performance in Figure 1 and Table 1, it is clear that some strains as MP4, MS5 and KS1’, have adaptation phase and their fermentation began with delay, compared with other strains. The alcohol produced by these strains is in small amounts. The strains KL1, KB1 and KK2 develop gradually fermentation being extended from 20 to 22 days, associated with high alcoholic degrees and small residual sugar. The strains KS7’and M1 develop a fast fermentation which last up to 15 days, strain M1 gives a high alcoholic degree with a small residual sugar and the strain KS7’ ends alcoholic fermentation with an average alcoholic degree, leaving a higher residue of sugar than strain M1.

The fermentation energy

The speed of fermentation is presented as a very important indicator of oenological skills of yeast strains which helps in selection of proper strains which will be used for fermentation in relation to competitiveness with other microorganisms present in the fermentation substrate. In our tests, this indicator is calculated to strains after 7 days and 11 days of fermentation. The data are presented in the table below.

Table 2. The fermentation energy

Strains	Fermentation energy (at first 7 days)	Fermentation energy (at first 11 days)
KS1’	1.44 ± 0.02	0.94 ± 0.02
MP4	1.36 ± 0.02	0.89 ± 0.03
MS5	1.39 ± 0.03	0.90 ± 0.07
MS5’	1.39 ± 0.02	1.04 ± 0.03
KS7’	1.40 ± 0.02	1.04 ± 0.04
KB2	0.62 ± 0.03	0.65 ± 0.02
KB1	0.72 ± 0.02	0.73 ± 0.04
KL1	0.65 ± 0.03	0.65 ± 0.03
M1	**1.92 ± 0.02**	**± 0.03**

Table 2 shows that strains, KL1, KB2, and KB1 present constant value of the fermentation energy compared with other strains, which is distinguished starting even from Figure1. These strains were distinguished even for a longer time of fermentation, compared to other strains.
The strains KS1’, MP4, MS5, MS5’, KS7’ and M1 in the first 7 days of fermentation had a high fermentation energy, compared with the other strains but this fermentation energy declined in coming days. However from Figure 1 and Table 1. It is clear that alcohol produced by strains MS5’, KS7’ and M1 is in average levels. The strains KS1’, MP4, and MS5 despite of having high fermentation energy, the alcohol produced by these strains is low.

Resistance to SO_2.

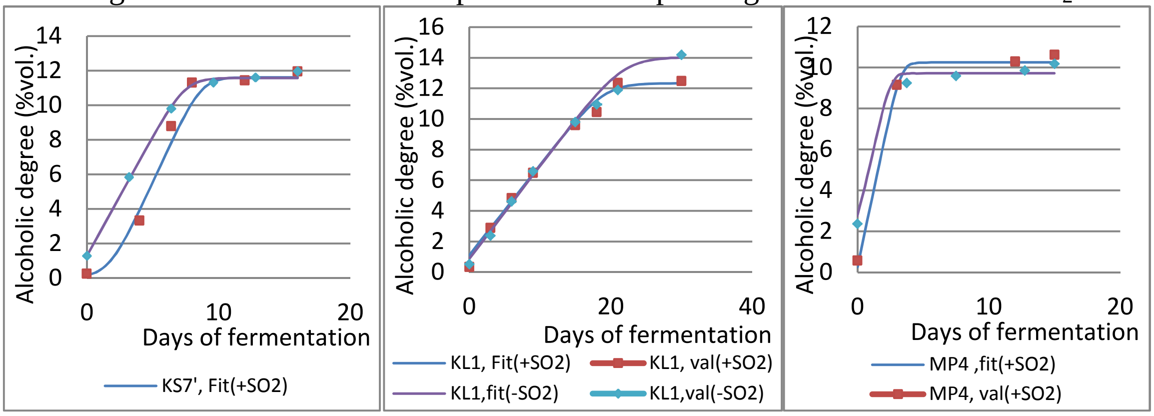
The reaction of yeasts against SO_2 is very heterogeneous. Some species demonstrate a high degree of sensitivity towards this compound, while others are resistant. The following are the results of this study that clearly shows the sensitivity of yeasts to SO_2.

Table 3.Yeast fermentative performance depending on the resistance to SO_2

Strains	yDatMin	[1]yDatMax $+SO_2$	[2]yDatMax $-SO_2$	rate	lag	y0	yEnd	se(fit)	R^2_stat
KS7’	0,24	**11,96**	**11.96**	1,66	1,76	0,15	11,61	0,30	0,995
KL1	0,33	12,47	14.18	0,59		1,09	12,34	0,54	0,981
KB2	0,26	**12,96**	**14.39**	0,60		1,17	12,84	0,59	0,978
KB1	0,06	11,66	14.07	0,73		0,14	11,34	0,39	0,990
MS5’	0,00	0,00	12.01	0,00		0,00	0,00	0,00	0,000
M1	0,00	**0,00**	**15.12**	0,00		0,00	0,00	0,00	0,000
MP4	0,57	10,61	10.18	3,02		0,25	10,24	0,34	0,989
MS5	0,45	10,83	10.28	3,22		0,26	10,42	0,31	0,991
KS1’	0,59	10,01	10.39	3,01		0,41	9,80	0,48	0,973

[1]yDatMax +SO2: maximal value of alcohol produced in presence of SO_2
[2]yDatMax -SO2: maximal value of alcohol produced in absence of SO_2

Figure 2. Yeast fermentative performance depending on the resistance to SO_2



(a) (b) (c)

From Table 3, we can see that six strains show sensitivity to the use of SO_2 in must compared with fermentation in its absence. The strain KS7' resulted slightly sensitive to SO_2, although it had adaptation phase and its fermentation started later than other strains, alcohol produced by this strain was in average level (Figure 2.a). The strains MS5' and M1 exhibited a high degree of sensitivity to $SO_{2,}$ therefore these strains didn't ferment in his presence. The strains KL1, KB1 and KB2 showed that they were less sensitive to SO_2, develop slower fermentation but they provided high alcohol content and a small residual sugar. (Figure 2.b). The strains MP4 and MS5 were appeared more active against SO_2; they provided a higher alcoholic degree than in conditions of its absence. (Figure 2.c).

Influence of temperature to fermentation

An important indicator for the progress of fermentation is the temperature at which it happens. The results of influence of temperature to fermentation are given in Table 4

Table 4. Influence of temperature to fermentation

Strains	**Alcoholic degree (%vol.) in 25 °C**	**Alcoholic degree (% vol.) in 12 - 19 ° C**
KS1'	10.40± 0.03	10.08 ± 0.03
MP4	10.18 ± 0,03	9.89 ± 0.03
MS5	10.28 ± 0,02	9.99 ± 0.03
MS5'	12,01 ± 0,02	11,45 ± 0.02
KS7'	11,96 ± 0,02	11,52 ± 0.04
KB2	14,39 ± 0,03	11,63 ± 0.04
KB1	14,07± 0.02	10,19 ± 0.19
KL1	14,17 ± 0.03	11,81 ± 0.08
M1	15,12 ± 0.02	13,93 ± 0.07

Table 4 shows that all strains are affected by low temperatures in fermentation, because all strains have faster stopped fermentation compared with fermentation in temperature 25 °C. The strains KS1', MP4, MS5, MS5', M1dhe KS7' are less active in low temperatures (they produce about 1% vol. less alcohol compared to fermentation at temperature 25 °C). While the strains KB2, KB1 and KL1 are not very active at low temperatures.

Conclusions

As a result of this study performed in the laboratory and the results obtained from it, we can draw some conclusions:

The strains KS1', MP4 and MS5 were not very active at low temperatures, while regarding to resistance to SO_2, the strains MS5 and MP4 were totally resistant. These strains also showed high fermentation energy but alcohol produced by them, was at low levels and this coupled with a relatively high residual unfermented sugar.

The strain KB1 was distinguished for high fermentation power and medium fermentation energy. This strain was not very active at low temperatures and showed sensitivity to the treatment of must with SO_2 interrupting more quickly the fermentation compared with normal conditions.

The strain M1 was distinguished for high fermentation power and energy. This strain was not very active at low temperatures and showed a very high sensitivity to $SO_{2,}$ not giving fermentation in its presence.

While the strains KB2 and KL1 were distinguished for high fermentation power, with a small residue of unfermented sugar and medium fermentation energy. These strains were easily sensitive to low temperatures and to SO_2, so they can serve for vinification with directed fermentation and in case of a positive result, the extent of directed fermentation in wine industry in our country.

References

LAMÇE, F. & SINI, K. (2013). “Isolation and characterization of oenological yeasts”. Albanian Journal of Agricultural Sciences; Vol. 12 Issue 4, p 669.

MAIFRENI, M., COMI, G., RONDININI.G. (1999) “Selection and oenological characterization of Saccharomyces cerevisiae strains isolated from Tokay, Pinot and Malvasia grapes and musts of the Collio area”.Annali di Microbiologia ed Enzimologia, 49: 33-43.

RAINIERI, S., PRETORIUS.I.S. (2000) “Selection and improvement of wine yeasts”. Annals of Microbiology, 50, 15-31.

FLEET, G.H., HEARD, G.M., (1993). Yeasts: growth during fermentation. In: Fleet, G.H. (Ed.), Wine Microbiology and Biotechnology. Harwood Academic, Chur, Switzerland, pp. 27– 54.

DELFINI, C. (1982) “Tecnica di Microbiologia Enologica” (Techniques of Oenological Microbiology). Luigi Scialpi Editore, Roma.

FUGELSANG, K.C.(1997) “Wine Microbiology” Chapman & Hall International Thomson Publishing, USA.

FAO (1998) CORPORATE DOCUMENT REPOSITORY, “Fermented fruits and vegetables. A global perspective”. Web: http://www.fao.org/docrep/x0560e/x0560e08.htm.

ZAMBONELLI, C. (1998) “Microbiologia e Biotecnologia dei vini” (Wine Microbiology and Biotechnology). Edagricole-Edicione Agricole della Calderini, Bologna.

ZENELAJ, R. (2004) “Studimi i majave me rëndësi enologjike. Izolimi i shtameve nga kultivarë autoktonë të rrushit, karakteristikat, Identifikimi dhe seleksionimi i tyre për përdorim në fermentimin e drejtuar të verërave dhe pijeve alkoolike” (Study of important oenological yeast. Isolation of strains from indigenous grape cultivars, characteristics, identification and selection of them for directed fermentation of wines and spirits). Raport shkencor 2004.