BIO Web of Conferences **9**, 02002 (2017) DOI: 10.1051/bioconf/20170902002
40th World Congress of Vine and Wine

Study of the effectiveness of a strain of *Saccharomyces cerevisiae* selected for the production of wines with higher acidity and lower alcoholic strength

Olga Pascual[1], Pere Pons-Mercadé[1], Jordi Gombau[1], Anne Ortiz-Julien[2], José M. Heras[2], Francesca Fort[1], Joan Miquel Canals[1], and Fernando Zamora[1]

[1] Departamento de Bioquímica y Biotecnología, Facultad de Enología de Tarragona, Universidad Rovira i Virgili, C/ Marcel.lí Domingo, 1, 43007 Tarragona, España
[2] Lallemand Bio S.L. C/ Galileu 303, 1^{a}planta, 08028-Barcelona, España

Abstract. Grapes of Tempranillo, Garnacha Tinta and Merlot at very high maturity level were used for red microvinifications using a conventional *Saccharomyces cerevisiae* strain (Lalvin EC 1118®) and new *S. cerevisiae* strain generated using adaptive evolution-based strategies (IONYS^{TM}WF). All microvinifications were performed by triplicate and at low (16°C) and at high (27°C) temperatures. The results show that all the wines fermented with IONYS^{TM}WF, independently of the fermentation temperature and grape cultivar have significant lower ethanol content (average 0.60 %), higher glycerol content (average of 5.6 g/L), higher titratable acidity (average of 1.3 g of tartaric acid/L) and lower pH (average of 0.1 units) than their corresponding controls. It seems therefore that IONYS^{TM}WF strain can be a useful tool to mitigate the excess of ethanol and the lack of acidity that unfortunately many wines present nowadays. Moreover, the high glycerol production can also be an interesting contribution inasmuch as this compound increases mouthfeel and smooth astringency.

1. Introduction

It is a doubtless fact that in recent years the alcoholic strength of wines has increased significantly [1], probably due to climate change and also because winemakers are searching full grape maturity [2,3]. Since high alcohol levels have certain drawbacks, the wine industry is interested in developing techniques to reduce the alcohol content. In this regard, a wide variety of strategies have been proposed with this purpose [3,4], among which we would highlight the selection of yeasts with lower sugar/ethanol transformation ratio [5,6].

According to the stoichiometry of the alcoholic fermentation pathway, 15.45 g/L of sugars are theoretically required to obtain 1% vol. Alcohol [4]. However, besides ethanol yeasts also produce many other compounds such as glycerol, higher alcohols, esters, succinic acid, diacetyl, acetoin, 2,3-butanediol, etc. Moreover, some of the sugars are used by yeasts to increase their biomass, and a percentage of ethanol is evaporated in a greater or lesser extent depending on the CO_2 release rate, the temperature and the dimensions of the tank. Moreover, some winemaking operations such as pumping over can also favor its evaporation [7,8].

It is consequently difficult to know what the real sugar/ethanol transformation ratio (TR) is during alcoholic fermentation. For this reason, the term "Potential Alcohol Content" is used to predict the ethanol content of a wine from the sugar content of the grape juice. OIV considers an average TR of 16.83 g/L. However, winemakers usually use a TR of 16.00 g/L for white wines and 17.00 g/L for red wines. The difference is mainly because white wines are generally fermented at low temperatures and without aeration whereas red wines are fermented at high temperatures and with some operations involving aeration [4,7].

Recently scientists from INRA of Montpellier (France) in collaboration with Lallemand have generated a new S. cerevisiae strain using adaptive evolution-based strategies (100% natural; non-GMO) [9]. Making the yeasts grow continuously and repeatedly under hyperosmotic medium they have generated a S. cerevisiae strain that produces appreciably less alcohol and more glycerol and acidity than usual strains. This yeast is nowadays commercialized with the name IONYS^{TM}WF (International Patent N° WO2015/11411).

The aim of this study was to determine if this strain can really reduce the ethanol content improve wine acidity under real winemaking conditions.

2. Materials and methods

Grapes of Tempranillo, Garnacha tinta and Merlot at very high maturity level were destemmed, crushed and sulphited (30 mg/L). Microvinifications were carried out by triplicate at two fermentation temperatures (16 and 27 °C) using submerged cap procedure [10]. Control musts were inoculated with the commercial strain Lalvin EC1118®and the experimental ones with IONYS^{TM}WF (both yeasts from Lallemand Inc.). After 15 days of

Table 1. General parameters of Tempranillo wines.

PARAMETER	Temperature: 16 ± 1 °C		Temperature: 27 ± 1 °C	
	EC1118	IONYS™WF	EC1118	IONYS™WF
Ethanol (% v/v)	14.1 ± 0.13 **B**	13.58 ± 0.08 **A**	14.22 ± 0.13 **B**	13.72 ± 0.13 **A**
Glycerol (g/L)	6.53 ± 0.35 **A**	10.77 ± 0.42 **B**	6.73 ± 0.06 **A**	11.13 ± 0.46 **B**
Titratable Acidity (g/L)	7.00 ± 0.30 **A**	8.37 ± 0.12 **B**	7.20 ± 0.10 **A**	8.07 ± 0.49 **B**
Volatile Acidity (g/L)	0.15 ± 0.07 **A**	0.17 ± 0.03 **A**	0.17 ± 0.02 **A**	0.18 ± 0.03 **A**
pH	3.67 ± 0.01 **B**	3.56 ± 0.01 **A**	3.75 ± 0.02 **B**	3.67 ± 0.05 **A**
Glucose + Fructose (g/L)	0.17 ± 0.05 **A**	0.20 ± 0.04 **A**	0.30 ± 0.23 **A**	0.37 ± 0.24 **A**

Results are expressed as mean ± standard deviation of three replicates. Different letters indicate a statistical difference ($p < 0.05$).

Table 2. General parameters of Garnacha Tinta wines.

PARAMETER	Temperature: 16 ± 1 °C		Temperature: 27 ± 1 °C	
	EC1118	IONYS™WF	EC1118	IONYS™WF
Ethanol (% v/v)	16.3 ± 0.05 **B**	15.40 ± 0.52 **A**	15.87 ± 0.08 **B**	15.42 ± 0.15 **A**
Glycerol (g/L)	6.43 ± 0.42 **A**	10.93 ± 0.55 **B**	7.13 ± 0.25 **A**	13.73 ± 0.49 **B**
Titratable Acidity (g/L)	5.53 ± 0.12 **A**	6.87 ± 0.29 **B**	5.60 ± 0.10 **A**	7.23 ± 0.23 **B**
Volatile Acidity (g/L)	0.10 ± 0.01 **A**	0.15 ± 0.03 **B**	0.31 ± 0.04 **A**	0.28 ± 0.03 **A**
pH	3.58 ± 0.02 **B**	3.44 ± 0.02 **A**	3.60 ± 0.01 **B**	3.47 ± 0.01 **A**
Glucose + Fructose (g/L)	0.17 ± 0.09 **A**	0.97 ± 0.67 **B**	0.23 ± 0.06 **A**	0.70 ± 0.87 **A**

Results are expressed as mean ± standard deviation of three replicates. Different letters indicate a statistical difference ($p < 0.05$).

Table 3. General parameters of Merlot wines.

PARAMETER	Temperature: 16 ± 1 °C		Temperature: 27 ± 1 °C	
	EC1118	IONYS™WF	EC1118	IONYS™WF
Ethanol (% v/v)	15.5 ± 0.01 **B**	14.83 ± 0.06 **A**	15.38 ± 0.08 **B**	14.73 ± 0.15 **A**
Glycerol (g/L)	6.07 ± 0.12 **A**	13.73 ± 0.12 **B**	6.37 ± 0.21 **A**	12.80 ± 0.42 **B**
Titratable Acidity (g/L)	6.10 ± 0.00 **A**	7.27 ± 0.21 **B**	6.40 ± 0.10 **A**	7.73 ± 0.29 **B**
Volatile Acidity (g/L)	0.27 ± 0.02 **A**	0.32 ± 0.02 **B**	0.30 ± 0.01 **A**	0.32 ± 0.01 **A**
pH	3.34 ± 0.02 **B**	3.25 ± 0.02 **A**	3.39 ± 0.01 **B**	3.33 ± 0.02 **A**
Glucose + Fructose (g/L)	0.77 ± 0.50 **A**	0.83 ± 0.15 **A**	0.43 ± 0.58 **A**	0.35 ± 0.23 **A**

Results are expressed as mean ± standard deviation of three replicates. Different letters indicate a statistical difference ($p < 0.05$).

maceration free run wines were separated from the pomace, sulphited to prevent malolactic fermentation (40 mg/L) and stabilized for 30 days at 4 °C. Wines were analyzed one month later.

The analytical methods recommended by the OIV were used to determine the ethanol content, pH and volatile acidity (Organisation Internationale de la Vigne et du Vin, 2014). Glycerol and sugars (D-glucose and D-fructose) were measured using enzymatic kits (R-Biopharm AG., Darmstadt, Germany).

All data are expressed as the arithmetic average ± the standard deviation from three replicates. One-factor analysis of variance (ANOVA) and Tukey's test were carried out with SPSS software (SPSS Inc., Chicago, Illinois, USA).

3. Results and discussion

Tables 1, 2 and 3 show the general parameters of the Tempranillo, Garnacha Tinta and Merlot wines.

Results are expressed as mean ± standard deviation of three replicates. Different letters indicate a statistical difference ($p < 0.05$).

Results are expressed as mean ± standard deviation of three replicates. Different letters indicate a statistical difference ($p < 0.05$).

Results are expressed as mean ± standard deviation of three replicates. Different letters indicate a statistical difference ($p < 0.05$).

All fermentations were correctly performed since the Glucose + Fructose concentration were in all the cases below 1 g/L and the volatile acidity below 0.35 g of acetic acid/L.

In general, no important differences were found between the wines fermented at low (16°C) and high (27°C) temperature for a same yeast in any of the studied parameters. The small differences between the ethanol content at low and at high temperature for a same yeast may be related with the fact that all fermentation were performed with submerged cap which would reduce the temperature effect on ethanol evaporation.

In contrast, the ethanol content was significant lower and glycerol concentration significant higher in all the wines fermented with IONYS^{TM}WF strain than in their corresponding controls, independently of the fermentation temperature and grape cultivar. The average difference was of 0.60% for ethanol content and of 5.6 g/L for glycerol concentration. These results clearly indicate that IONYS^{TM}WF strain redirect part of the metabolic flow of the sugars towards the production of glycerol.

In addition, all the wines fermented with IONYS^{TM}WF strain have significant lower pH and significant higher titratable acidities than their corresponding controls. Specifically, the average difference was of o.1 units for pH and of 1.3 g of tartaric acid/L for titratable acidity.

It can be concluded that this new yeast can be a useful tool to mitigate the excess of ethanol and the lack of acidity that unfortunately many wines present nowadays. Moreover, the high glycerol production can also be an interesting contribution inasmuch as this compound increases mouthfeel and smooth astringency.

This study has been funded by CDTI (CIEN-VINySOST2014 project).

References

[1] P. Godden, R. Muhlack, Aust. NZ. Grapegrow. Winemak. **558**, 47–61 (2010)
[2] H.R. Schultz, G.V. Jones, J. Wine Res. **21**, 137–145 (2010)
[3] N. Kontoudakis, M. Esteruelas, F. Fort, J.M. Canals, F. Zamora, Aust. J. Grape Wine Res. **17**, 230–238 (2011)
[4] F. Zamora, Delacoholised Wines and Low-Alcohol Wines. En "Wine Safety, Consumer Preference, and Human Health". Ed. M.V. Moreno-Arribas & B. Polo. Bartolomé Sualdea, Springer. New York, pp 163–182 (2016)
[5] S. Michnick, J.L. Roustan, F. Remiz, P. Barre, S. Dequin, Yeast **13**, 783–793 (1997)
[6] M. Quirós, V. Rojas, R. Gonzalez, P. Morales, Int. J. Food Microbiol. **181**, 85–91 (2014)
[7] M., Gil, N. Kontoudakis, S. Estévez, E. González-Royo, M. Esteruelas, F. Fort, J.M. Canals, F. Zamora, (2013) Non microbiological strategies to reduce alcohol in wines. En: Alcohol Reduction in Wine; Oneoviti International Network. Vigne et

Vin Publications Internationales. Merignac, France, pp. 25–28 (2013)

[8] O. Pascual, E., González-Royo, M. Gil, S. Gómez-Alonso, E. García-Romero, J.M. Canals, I. Hermosín-Gutíerrez, F. Zamora, (2016) J. Agric. Food Chem. **64**, 6555–6566 (2016)

[9] V. Tilloy, A. Ortiz-Julien, S. Dequin, Appl. Environ. Microbiol. **80**, 2623–32 (2014)

[10] T.L. Sampaio, J.A. Kennedy, M.C. Vasconcelos, Am. J. Enol. Vitic. **58**, 534–539 (2007)

[11] Organisation Internationale de la Vigne et du Vin. Methods of analysis of wines and must (2014)