UDC 663.2:577.171.54:663.2
ISSN 1330-9862

scientific note

(FTB-1160)

Effect of Protectants on the Fermentation Performance of Wine Yeasts Subjected to Osmotic Stress

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Received: June 28, 2002
Accepted: April 24, 2003

Summary

During alcoholic fermentation of must from dried grapes, yeasts are subjected to very high sugar concentrations, besides other environmental stresses, and they modify their metabolic behaviour giving low ethanol yield and abnormally high acetic acid production. To investigate the protective effect of catechin, inositol, and SO_2 on wine yeasts, three thermotolerant strains of *Saccharomyces cerevisiae*, selected for wine making of must from dried grapes, and three strains of *Saccharomyces* selected for the production of wine, were inoculated in a sample of must at very high osmotic strength. A significant ($p<0.01$ or $p<0.05$) relationship between the addition of 100 mg/L of catechin, inositol or SO_2 to the grape must and the change in the metabolic behaviour of the yeasts was observed. Compared to the control and depending on strain and protectant, the fermentation rate after 3 days increased up to 55 %, the ethanol content of the wines increased up to 16 %, the unitary succinic acid production increased up to 55 %, the unitary acetic acid production decreased up to 53 %, and the unitary glycerol production decreased up to 69 %. So by adding catechin, inositol or SO_2 to the grape must it is possible to minimise the abnormal fermentation performance that wine yeasts exhibit in wine making of must from dried grapes.

Key words: metabolism, stress, wine yeast

Introduction

In wine production, alcoholic fermentation is an essential step that is usually performed by yeasts belonging to the species *S. cerevisiae*; their ability to carry out the wine making process is largely influenced by their response to stress conditions that affect them throughout the process (*1*). Yeasts have developed different mechanisms in order to resist this adverse situation; a better understanding of these mechanisms could open the possibility to improve the fermentation process (*2*). The responses involve aspects of cell sensing, signal transduction, transcriptional and posttranslational control, protein-targeting to organelles, accumulation of protectants, and activity of repair functions; the efficiency of these processes in a given yeast strain determines its robustness (*3*). Therefore, wine yeasts are affected by stresses under anaerobic conditions, resulting in the increase of ethanol concentration and in the limitation of essential nutrients during alcoholic fermentation. In fermentation of must from dried grapes, yeasts are subjected, in addition, to osmotic stress induced by high sugar concentration. In this case yeasts modify their metabolic behaviour and, probably as a defence mechanism, give an abnormally high production of acetic acid (*4*).

Finding the metabolites able to minimise this reaction to osmotic stress by wine yeasts appears interesting. It has recently been reported that inositol, catechin

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and SO_2 induce positive effects on wine yeasts under concomitant thermal and osmotic stress (*5*).

The present research aims to minimise the metabolic consequences of the stress caused to wine yeast during alcoholic fermentation of must at very high osmotic strength. Thus, it was decided to investigate the effect of inositol, catechin and SO_2 on wine making performance of six wine yeasts under osmotic stress.

Material and Methods

Three thermotolerant yeast strains of *S. cerevisiae*, TT51, TT77, and TT141, selected for wine making of musts from dried grapes (*6*) and three strains of *Saccharomyces*, the laboratory strain *S. cerevisiae* 220 from the DIPROVAL collection, Coviolo (RE), Italy, and two commercial wine yeast strains, *S. cerevisiae* VL1 from Lallemand and *S. bayanus* from Fermichamp (abbreviated as Ferm), were employed. Thermotolerant yeasts were chosen according to the demonstration of relationships between thermotolerance and osmotolerance described in literature (*7*,*8*).

The sample of must from dried grapes utilised had the following characteristics: cultivar *Greco Bianco*, pH= 3.14, titratable acidity 5.76 g/L, sugar content adjusted to over 400 g/L by glucose monohydrate. The grape must was filtered through gauze, clarified at 4 °C for 24 h, and divided into four lots. To three lots the three protectants, (±)catechin produced by Sigma, inositol, and SO_2 as potassium metabisulphite, were added to give a final concentration of 100 mg/L (*5*). The fourth lot was used as control and no protectant was added. Each lot of grape must was divided into aliquots of 100 mL, dispensed into 180 mL flasks, and immediately inoculated in duplicate with 5 mL of 48-h precultures.

Fermentations were performed at 15 °C, and the weight loss caused by CO_2 production was determined. The mass (in g) of CO_2 produced was used to express strain fermentation rate after three days. After 90 days the samples were refrigerated at 4 °C for 48 h and then analysed. Titratable acidity (expressed in mass concentration of tartaric acid – γ) and ethanol content, expressed as % (volume fraction), were determined using standard methods (*9*). Acetic acid, succinic acid and glycerol were tested with specific Boehringer kits on diluted samples. Unitary production of these three parameters was expressed as g/100 mL of ethanol. All parameters were elaborated by ANOVA analysis.

Results and Discussion

Table 1 reports the values of fermentation rate of the six wine yeasts utilised after three days. When protectants were added to the grape must, three strains did not exhibit significant effects; on the contrary, strains TT77, 220 and Ferm showed significant increase after the addition of catechin and inositol, and less significant after the addition of SO_2. Examining the significant ($p< 0.05$) values, the increase of fermentation rate varied, compared to the control, from 7 % (strain Ferm) to 55 % (strain TT77) when catechin was added, from 14 % (strain 220) to 38 % (strain TT77) when inositol was added, and from 0 % (strain Ferm) to 34 % (strain 220) when SO_2 was added.

Table 1. Fermentation rate of the six strains of wine yeasts after 3 days expressed as $\gamma(CO_2)/(g/100\ mL)$ for control samples and for the samples with the addition of 100 mg/L of catechin, inositol and SO_2, respectively

Strains	Control	+ Catechin	+ Inositol	+ SO_2
TT51	0.80	1.11	0.89	0.68
TT77	0.58^{c}	0.90^{a}	0.80^{ab}	0.71^{b}
TT141	0.87	1.18	1.13	0.84
220	0.85^{b}	0.97^{ab}	0.97^{ab}	1.14^{a}
VL1	0.97	1.03	1.05	1.04
Ferm	0.69^{b}	0.74^{ab}	0.83^{a}	0.69^{b}

^{a}, ^{b}, ^{c}: $p < 0.05$

Table 2 reports the values of titratable acidity of the wines produced by the six wine yeasts. Apart from strains TT141 and Ferm, which produced wines with notably increased titratable acidity when protectants were added, the other strains did not show any notable effects.

Table 2. Titratable acidity of the wines produced by the six strains of wine yeasts expressed as γ(tartaric acid)/(g/L) for control samples and for the samples with the addition of 100 mg/L of catechin, inositol and SO_2, respectively

Strains	Control	+ Catechin	+ Inositol	+ SO_2
TT51	6.15^{b}	6.26^{a}	6.24^{a}	6.24^{a}
TT77	6.97^{b}	7.01^{b}	7.01^{b}	7.20^{a}
TT141	7.22^{d}	7.84^{a}	7.59^{b}	7.46^{c}
220	6.97^{c}	7.05^{b}	6.97^{c}	7.24^{a}
VL1	7.39	7.39	7.39	7.39
Ferm	6.67^{b}	6.81^{a}	6.90^{a}	6.86^{a}

^{a}, ^{b}, ^{c}, ^{d}: $p < 0.05$

Table 3 reports the values of ethanol content of the wines produced by the six wine yeasts. All the protectants induced a significant ($p<0.01$ or $p<0.05$) increase in ethanol yield, above all for thermotolerant strains. The

Table 3. Ethanol content of the wines produced by the six strains of wine yeasts expressed as φ(ethanol)/% for control samples and for the samples with the addition of 100 mg/L of catechin, inositol and SO_2, respectively

Strains	Control	+ Catechin	+ Inositol	+ SO_2
TT51	11.50^{c}	12.80^{a}	13.00^{a}	12.20^{b}
TT77	11.60^{C}	13.00^{A}	13.00^{A}	12.50^{B}
TT141	14.70^{C}	17.00^{A}	17.10^{A}	16.20^{B}
220	15.40^{B}	16.00^{A}	16.50^{A}	16.00^{A}
VL1	16.00^{B}	17.30^{A}	17.50^{A}	17.30^{A}
Ferm	17.00^{b}	18.00^{a}	18.50^{a}	18.00^{a}

^{a}, ^{b}, ^{c}: $p < 0.05$; ^{A}, ^{B}, ^{C}: $p < 0.01$

increase in ethanol yield varied, compared to the control, from 4 % (strain 220) to 16 % (strain TT141) when catechin was added, from 7 % (strain 220) to 16 % (strain TT141) when inositol was added, and from 4 % (strain 220) to 10 % (strain TT141) when SO_2 was added. According to another report (*10*), there seems to be an overlap between osmotolerance and ethanol endurance in *S. cerevisiae*.

Table 4 reports the unitary acetic acid production of the wines produced by the six wine yeasts. All the protectants induced a very significant ($p<0.01$) decrease in the unitary acetic acid production. The decrease varied, compared to the control, from 18 % (strain Ferm) to 35 % (strain TT51) when catechin was added, from 28 % (strains TT77 and TT141) to 53 % (strain Ferm) when inositol was added, and from 17 % (strain TT77) to 28 % (strain VL1) when SO_2 was added. Therefore, it seems confirmed that it is possible to minimise the abnormal production of acetic acid by wine yeasts under stressful conditions using protectants as catechin, inositol, or SO_2 (*5*), which is very important in wine making.

Table 4. Unitary acetic acid production of the wines produced by the six strains of wine yeasts expressed as

$$\frac{\gamma\,(\text{acetic acid})/(\text{g/L})}{\varphi\,(\text{ethanol})/\%}$$

for control samples and for the samples with the addition of 100 mg/L of catechin, inositol and SO_2, respectively

Strains	Control	+ Catechin	+ Inositol	+ SO_2
TT51	1.17^{A}	0.76^{C}	0.75^{C}	0.88^{B}
TT77	1.68^{A}	1.27^{B}	1.21^{B}	1.39^{AB}
TT141	1.24^{A}	0.94^{B}	0.89^{B}	0.91^{B}
220	0.99^{A}	0.73^{B}	0.68^{B}	0.73^{B}
VL1	1.27^{A}	0.97^{B}	0.72^{D}	0.91^{C}
Ferm	0.89^{A}	0.63^{B}	0.42^{D}	0.55^{C}

$^{A}, ^{B}, ^{C}, ^{D}$: $p < 0.01$

Table 5 reports the unitary succinic acid production of the wines produced by the six wine yeasts. For the thermotolerant strains, all the protectants induced a significant ($p<0.05$) increase in the unitary succinic acid production. The increase varied, compared to the control, from 20 % (strain TT51) to 55 % (strain TT77) when catechin was added, from 21 % (strain TT141) to 42 % (strain TT77) when inositol was added, and from 9 % (strain TT51) to 37 % (strain TT77) when SO_2 was added. Strains 220 and VL1 did not show any notable effects; on the contrary, strain Ferm produced wines with about 100 % increase in the unitary succinic acid production, compared to the control.

Table 6 reports the unitary glycerol production of the wines produced by the six wine yeasts. All the protectants induced a very significant ($p<0.01$) decrease in the unitary glycerol production. The decrease varied, compared to the control, from 20 % (strain 220) to 59 % (strain TT51) when catechin was added, from 30 % (strain Ferm) to 55 % (strain 220) when inositol was added, and from 25 % (strain Ferm) to 69 % (strain 220)

Table 5. Unitary succinic acid production of the wines produced by the six strains of wine yeasts expressed as

$$\frac{\gamma\,(\text{succinic acid})/(\text{g/L})}{\varphi\,(\text{ethanol})/\%}$$

for control samples and for the samples with the addition of 100 mg/L of catechin, inositol and SO_2, respectively

Strains	Control	+ Catechin	+ Inositol	+ SO_2
TT51	0.44^{c}	0.53^{b}	0.57^{a}	0.48^{c}
TT77	0.40^{c}	0.62^{a}	0.57^{b}	0.55^{b}
TT141	0.42^{c}	0.64^{a}	0.51^{b}	0.54^{b}
220	0.65^{b}	0.64^{b}	0.55^{c}	0.72^{a}
VL1	0.43^{B}	0.49^{A}	0.52^{A}	0.49^{A}
Ferm	0.26^{B}	0.50^{A}	0.53^{A}	0.49^{A}

$^{a}, ^{b}, ^{c}$: $p < 0.05$; $^{A}, ^{B}$: $p < 0.01$

Table 6. Unitary glycerol production of the wines produced by the six strains of wine yeasts expressed as

$$\frac{\gamma\,(\text{glycerol acid})/(\text{g/L})}{\varphi\,(\text{ethanol})/\%}$$

for control samples and for the samples with the addition of 100 mg/L of catechin, inositol and SO_2, respectively

Strains	Control	+ Catechin	+ Inositol	+ SO_2
TT51	11.94^{A}	4.90^{C}	7.10^{B}	4.20^{D}
TT77	13.47^{A}	6.79^{C}	8.90^{B}	4.41^{D}
TT141	9.01^{A}	6.59^{B}	4.65^{C}	3.41^{D}
220	7.11^{A}	5.68^{B}	3.21^{C}	2.23^{D}
VL1	8.99^{A}	5.13^{C}	5.40^{B}	4.41^{D}
Ferm	8.74^{A}	5.74^{D}	6.08^{C}	6.55^{B}

$^{A}, ^{B}, ^{C}, ^{D}$: $p < 0.01$

when SO_2 was added. Glycerol is a major fermentation product of *S. cerevisiae* that contributes to the sensory character of wine (*11*); increasing glycerol production is of concern for wine makers in improving the quality of certain wines (*12*). On the other hand, the significance of glycerol as osmoregulatory solute when the external osmotic pressure increases was demonstrated (*13*). Recently *S. cerevisiae* and other yeasts have been tested for glycerol production under osmotic stress; of all the yeasts, *S. cerevisiae* exhibited the highest level of osmotolerance and the highest glycerol yield, 82.3 % of the theoretical glycerol yield (*14*). In *S. cerevisiae*, similar to many organisms, there is a correlation between the increase of metabolites and the osmotic stress tolerance; this *osmotic adjustment* is comparable to the concept typically applied to accumulating metabolites in plants (*15*).

Conclusions

These preliminary results show significant correlations between the addition of protectants and the change in metabolic behaviour of yeasts under osmotic stress. Therefore, by the addition of the mentioned pro-

tectants it seems possible to minimise the abnormal fermentation performance that wine yeasts exhibit in wine making of must with very high sugar content.

Finally, it is important to suggest that the resistance of *S. cerevisiae* to high osmotic stress is enhanced at low temperatures (*16*); therefore, the temperature at which the wine making at very high osmotic strength is performed greatly affects the yeast cell behaviour. This indicates that temperature control could be another suitable means of reducing the abnormal fermentation performance in response to osmotic stress.

Acknowledgement

The research was supported by a grant from the Ministry of Scientific Research and Technology, Research fund RdB (ex 60 %) to A. Caridi.

References

1. P. Carrasco, A. Querol, M. del Olmo, *Arch. Microbiol. 175* (2001) 450–457.
2. C. Ivorra, J. E. Perez–Ortin, M. del Olmo, *Biotechnol. Bioeng. 64* (1999) 698–708.
3. P. V. Attfield, *Nat. Biotechnol. 15* (1997) 1351–1357.
4. A. Caridi, P. Crucitti, D. Ramondino, *Biotechnol. Lett. 21* (1999) 617–620.
5. A. Caridi, *Lett. Appl. Microbiol. 35* (2002) 98–101.
6. A. Caridi, P. Crucitti, D. Ramondino, E. Santagati, P. Audino, *Ind. Bevande, 28* (1999) 247–252.
7. J. G. Lewis, R. P. Learmonth, K. Watson, *Microbiology, 141* (Pt 3) (1995) 687–694.
8. J. G. Lewis, R. P. Learmonth, P. V. Attfield, K. Watson, *J. Ind. Microbiol. Biotechnol. 18* (1997) 30–36.
9. C. S. Ough, M. A. Amerine: *Methods for Analysis of Musts and Wines*, John Wiley and Sons, New York (1988).
10. S. C. Sharma, *FEMS Microbiol. Lett. 152* (1997) 11–15.
11. J. M. Eglinton, A. J. Heinrich, A. P. Pollnitz, P. Langridge, P. A. Henschke, M. de Barros Lopes, *Yeast, 19* (2002) 295–301.
12. F. Remize, J. M. Sablayrolles, S. Dequin, *J. Appl. Microbiol. 88* (2000) 371–378.
13. A. J. Meikle, J. A. Chudek, R. H. Reed, G. M. Gadd, *FEMS Microbiol. Lett. 66* (1991) 163–167.
14. B. Petrovska, E. Winkelhausen, S. Kuzmanova, *Can. J. Microbiol. 45* (1999) 695–699.
15. B. Shen, S. Hohmann, R. G. Jensen, H. J. Bohnert, *Plant Physiol. 121* (1999) 45–52.
16. L. Beney, P. A. Marechal, P. Gervais, *Appl. Microbiol. Biotechnol. 56* (2001) 513–516.

Utjecaj zaštitnih sredstava na tijek fermentacije vinskih kvasaca pod osmotskim stresom

Sažetak

Tijekom alkoholnog vrenja mošta dobivenog od suhoga grožđa, osim drugih stresova iz okoliša, kvasci su izloženi vrlo velikim koncentracijama šećera pa mijenjaju svoj metabolizam proizvodeći malo etanola i neprirodno veliku količinu octene kiseline. Da bi se ispitao zaštitni učinak katehina, inozitola i SO_2 na vinske kvasce, odabrana su tri termotolerantna soja *S. cerevisiae* i tri soja *Saccharomyces* za proizvodnju vina iz mošta dobivenoga od suhoga grožđa. Sojevi su inokulirani u uzorke mošta pri velikom osmotskom tlaku. Opažen je značajan odnos ($p<0,01$ ili $p<0,05$) između dodatka 100 mg/L katehina, inozitola ili SO_2 u mošt i promjene u metabolizmu kvasaca. U usporedbi s kontrolnim uzorkom, a ovisno o soju i zaštitnom faktoru, nakon tri dana povećala se brzina fermentacije do 55 %, porastao je udjel etanola u vinima do 16 %, povećala se proizvodnja jantarne kiseline do 55 %, a octene smanjila do 53 %, te glicerola do 69 %. Dodatkom katehina, inozitola ili SO_2 u mošt može se smanjiti neprirodna fermentacija kvasaca koja se događa pri vrenju mošta iz suhoga grožđa.