Glucose, L-Malic Acid and pH Effect on Fermentation Products in Biological Deacidification

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Abstract: Industrial wine yeasts *Saccharomyces cerevisiae* Syrena, an interspecies hybrid (*S. cerevisiae* × *S. bayanus*) HW2-3 and *Schizosaccharomyces pombe* met 3–15 h^+ were examined to determine changes in fermentation profiles in different environmental conditions in YG medium with different concentrations of glucose (2, 6, 40 or 100 g/l), L-malic acid (4, 7 or 11 g/l) and at pH 3.0, 3.5 and 5.0. The results were obtained by HPLC method (organic acids, acetaldehyde, glycerol, diacetyl) and enzymatically (L-malic acid, ethanol). In anaerobic conditions (100 g/l glucose), the optimal parameters for L-malic acid decomposition for *S. cerevisiae* Syrena and the hybrid HW2-3 were 11 g/l L-malic acid and pH 3.0 and 3.5, respectively. *S. pombe* expressed the highest demalication activity at 40 and 100 g/l glucose, 7 g/l L-malic acid and pH 3.0. The fermentation profiles of selected metabolites of yeast were unique for specific industrial strains. These profiles may help in the proper selection of yeast strains to fermentation and make it possible to predict the organoleptic changes in the course of fruit must fermentation.

Keywords: wine yeast; *Saccharomyces cerevisiae*; *Schizosaccharomyces pombe*; L-malic acid; biological deacidification

INTRODUCTION

The excessive acidity of fruit musts is one of the main problems in the wine industry, so biological methods of acidity adjustment are of great interest to wine producers. L-Malic acid is one of the dominant organic acids in musts and it can be both aerobically and anaerobically decomposed by wine yeasts. The ability of *Saccharomyces* yeasts to decompose extracellular L-malic acid differs from insignificant to 45% (Rodriguez & Thornton 1990). In turn, *Schizosaccharomyces pombe* can decompose L-malate completely (Taillandier & Strehaiano 1991), but its application in winemaking is limited due to the production of a specific off-flavour (Taillandier *et al.* 1995). The biological deacidification of musts will enable producing wine with the right sensory properties with a balance between sugar, acids and aroma components (Volschenk *et al.* 2003). L-Malic acid can also promote the growth of lactic acid bacteria causing wine spoilage after bottling (Redzepovic *et al*. 2003). Wine chemical composition is highly dependent on yeast species and environmental conditions. The aim of this study was to determine changes in the fermentation profiles (selected organic acids, acetaldehyde and glycerol) of two wine yeast strains *Saccharomyces* sp. in contrast to fission yeasts *S. pombe*. The impact of glucose and L-malic acid initial concentrations as well as pH on these fermentation products was investigated.

MATERIAL AND METHODS

Microorganisms. Wine yeasts *S. cerevisiae* Syrena and interspecies hybrids *S. cerevisiae* × *S. bayanus* HW2-3 obtained by natural hybridisation are deposited in the Pure Culture Collection at the Institute of Fermentation Technology and Microbiology, Technical University of Lodz LOCK 105. Fission yeast *S. pombe* met 3–15 h^+ was obtained from the Pure Culture Collection at the Institute of Microbiology, Wroclaw University.

Media and fermentations. Fermentations were carried out in YG medium composed of glucose (2, 6, 40 or 100 g/l), L-malic acid (4, 7 or 11 g/l), yeast extract (5 g/l), KH_2PO_4 (5 g/l) and $MgSO_4 \cdot 7H_2O$ (0.8 g/l) at 28°C during 12 hours. Different initial glucose and L-malic acid concentrations at pH

3.0, 3.5, 5.0 in a batch culture in 2.5 l fermentor were studied. The medium was inoculated with precultures of yeasts to a final concentration of 5% (w/v). The precultures were prepared in the YG medium with 4 g/l glucose and without L-malic acid and incubated at 28°C during 24 h before inoculation.

Chemical analysis. L-Malic acid and ethanol were determined enzymatically with specialised kits (Boehringer Mannheim, GmbH Germany). Citric, succinic, lactic and acetic acids, acetaldehyde and glycerol were determined by HPLC method (Frayne 1986).

Statistical analysis. Results were presented as an arithmetic mean of 6 assays and were analysed using a 3-way ANOVA test at a confidence level of $P < 0.05$. Calculations were conducted by means of STATISTICA 5.5. Software.

RESULTS AND DISCUSSION

The fermentation profiles of wine yeast *S. cerevisiae* Syrena, interspecies hybrids *S. cerevisiae* × *S. bayanus* HW2-3 and *S. pombe* were investigated at four different glucose concentrations (2, 6, 40, and 100 g/l), with a presence of L-malic acid at the initial concentration of 7 g/l. *S. cerevisiae* yeast showed the highest demalication activity in the medium with 2 g/l glucose (Table 1). No statistically significant differences ($P < 0.05$) were observed in the concentration of L-malic acid in the cultures of the hybrid HW2-3 in the presence of glucose at 2, 6, and 100 g/l. *S. cerevisiae* yeast has a mixed respiro-fermentative metabolism when the glucose concentration in the growth medium exceeds 1 mM (Verduyn *et al.* 1984). Thus, already at a glucose concentration of 2 g/l glucose repression is observed, while an increase in the share of fermentative metabolism occurs with increased sugar concentrations. In glucose-repressed cells the number of mitochondria decreases (Dejean *et al.* 2000), which negatively affects the malic enzyme located in them and results in decreasing the demalication activity of *Saccharomyces* yeast. The results presented are consistent with studies by Redzepovic *et al.* (2003), where a decrease in the expression of the malic enzyme gene under glucose repression was observed. Differences in the deacidification activity of *S. cerevisiae* Syrena and the interspecies hybrid HW2-3 indicate differences in the regulation of malic acid metabolism between *S. cerevisiae* and *S. bayanus*, which confirms the differentiation of fermentation profiles. Similar results were obtained in studies of *S. bayanus* and *S. paradoxus* yeast (Redzepovic *et al.* 2003). Cultures of *S. pombe* revealed an increased efficiency in L-malic acid decomposition with increased glucose concentrations (Table 1). The malic enzyme of *S. pombe* is dependent on NAD^+ and located in cytosol, and its regulation is different from *Saccharomyces* (Volschenk *et al.* 2003). An increased expression of this enzyme under high glucose concentrations was also observed in other studies (Groenewald & Viljoen-Bloom 2001; Redzepovic *et al.* 2003). The production of glycerol gradually increased with glucose concentration in the hybrid HW2-3 cultures. According to the literature, yeast respond to osmotic stress with higher production of glycerol (Groenewald & Viljoen-Bloom 2001; Volschenk *et al.* 2003), which is consistent with the results of the studies presented. The permanent, low concentration of glycerol in the cultures of *S. cerevisiae* Syrena revealed that this strain was not very sensitive to osmotic stress. The ethanol content depended on the glucose concentration in the medium and no straight correlation between L-malic acid consumption and ethanol production was observed.

In anaerobic conditions (100 g/l glucose), demalication activity increased with the initial concentration of L-malic acid only for S. *cerevisiae* Syrena and the hybrid HW2-3 (Table 1). These results are consistent with previous studies into the activity of *S. cerevisiae* (Delcourt *et al.* 1995; Volschenk *et al.* 2003) and *S. pombe* (Taillandier & Strehaiano 1991).

A gradual increase in the content of succinic acid (statistically significant differences at $P < 0.05$) was observed for *S. cerevisiae* Syrena with increased concentrations of L-malic acid up to 7 g/l and for the hybrid HW2-3 in the studied range of 4–11 g/l. In *Saccharomyces* sp. cells, there are two possible routes for malic acid decomposition. Malate can be transformed into pyruvate via malic enzyme or into fumarate and then succinate via fumarase and fumarate reductase, respectively (Radler 1986). Both the results presented and the literature (Ramon-Portugal *et al.* 1999) confirm that an increase in the malic acid concentration results in the higher production of succinic acid. At the same time, other researchers report differences in the concentration of succinic acid in wines fermented

Table 1. Concentrations of selected yeast metabolites (g/l) in medium with different initial levels of glucose[1], L-malic acid[2] and pH[3]

	Inoculated yeast strain											
	S. cerevisiae Syrena				Interspecies hybrid HW2-3				*S. pombe*			
Glucose[1] (g/l)	2	6	40	100	2	6	40	100	2	6	40	100
L-Malic acid	4.94^{c}	6.25^{e}	6.25^{e}	5.79^{d}	5.72^{d}	5.77^{d}	6.88^{f}	5.68^{d}	2.40^{b}	0.90^{a}	nd	nd
Citric acid	2.92^{b,c}	2.71^{b}	2.69^{b}	2.80^{b}	3.00^{c}	3.02^{c}	3.15^{c}	3.30^{c}	2.98^{b,c}	2.87^{b}	3.11^{c}	3.40^{c}
Succinic acid	0.11^{a}	0.16^{a}	0.24^{b}	0.54^{d}	0.16^{a}	0.23^{b}	0.49^{c}	0.63^{e}	0.17^{a}	0.21^{b}	0.65^{e}	0.65^{e}
Lactic acid	0.01^{a}	0.02^{a}	0.10^{b}	nd	nd	0.05^{a}	0.01^{a}	0.06^{a,b}	0.06^{a,b}	0.10^{b}	0.19^{c}	0.22^{c,d}
Acetic acid	0.01^{a}	0.06^{a,b}	0.02^{a}	0.25^{d}	nd	0.17^{c}	0.31^{e}	0.51^{f}	0.01^{a}	0.07^{b}	0.08^{b}	0.18^{c}
Acetaldehyde	nd	0.03^{a}	0.30^{d}	0.43^{f}	0.04^{a}	0.02^{a}	0.24^{d}	0.35^{e}	0.04^{a}	nd	0.01^{a}	0.07^{b}
Glycerol	0.01^{a}	0.03^{a}	0.11^{a,b}	0.16^{b}	nd	0.02^{a}	0.94^{d}	2.27^{e}	0.02^{a}	0.08^{a}	0.97^{d}	1.14^{e}
Ethanol	0.03^{a}	4.77^{d}	12.76^{f}	19.36^{h}	nd	0.40^{b}	15.80^{g}	24.59^{i}	1.74^{c}	5.76^{d}	8.50^{e}	11.09^{f}
L-Malic acid[2] (g/l)	0	4	7	11	0	4	7	11	0	4	7	11
L-Malic acid	0.13^{a}	3.06^{b}	5.79^{d}	7.61^{f}	0.23^{a}	3.18^{b}	5.68^{d}	8.31^{g}	0.02^{a}	0.10^{a}	nd	3.86^{c}
Citric acid	2.19^{a}	2.98^{b,c}	2.80^{b}	2.76^{b}	3.85^{d}	3.20^{c}	3.30^{c}	3.43^{c}	3.39^{c}	3.93^{d}	3.40^{c}	2.92^{b,c}
Succinic acid	0.43^{c}	0.52^{d}	0.64^{e}	0.69^{e}	0.22^{b}	0.36^{c}	0.63^{e}	0.77^{f}	0.54^{d}	0.69^{e}	0.65^{e}	0.31^{b}
Lactic acid	nd	0.04^{a}	nd	nd	0.11^{b}	0.03^{a}	0.06^{a,b}	0.07^{b}	0.15^{c}	0.03^{a}	0.22^{c,d}	0.06^{a,b}
Acetic acid	0.48^{f}	0.06^{a,b}	0.25^{d}	0.22^{d}	0.76^{g}	0.24^{d}	0.51^{f}	0.52^{f}	0.13^{b,c}	0.21^{d}	0.18^{c}	0.10^{b}
Acetaldehyde	0.13^{b,c}	0.08^{b}	0.43^{f}	0.48^{f}	0.09^{b}	0.25^{d}	0.35^{a}	0.40^{e}	0.03^{a}	0.04^{a}	0.07^{b}	0.06^{a,b}
Glycerol	1.04^{d}	2.71^{h}	0.16^{b}	1.60^{f}	1.67^{f}	1.41^{e}	2.27^{g}	2.30^{g}	0.83^{b}	1.46^{e}	1.14^{e}	0.44^{c}
Ethanol	36.23^{j}	16.39^{g}	19.36^{h}	17.62^{g,h}	16.07^{g}	16.19^{g}	24.59^{i}	20.75^{h}	10.42^{f}	15.96^{g}	11.09^{f}	7.85^{e}
pH[3]	3.0	3.5	5.0		3.0	3.5	5.0		3.0	3.5	5.0	
L-Malic acid	5.79^{d}	6.53^{f}	6.82^{f}		5.68^{d}	5.29^{c}	12.68^{h}		nd	1.39^{b}	4.79^{c}	
Citric acid	2.80^{b}	3.21^{c}	3.22^{c}		3.30	3.07^{c}	3.12^{c}		3.40^{c}	3.90^{d}	3.02^{c}	
Succinic acid	0.54^{d}	0.35^{c}	0.60^{e}		0.63^{e}	0.59^{d}	0.42^{c}		0.65^{e}	0.68^{e}	0.60^{e}	
Lactic acid	nd	0.02^{a}	nd		0.06^{a,b}	0.07^{b}	0.01^{a}		0.22^{c,d}	0.04^{a}	0.27^{d}	
Acetic acid	0.25^{d}	0.29^{d}	0.63^{g}		0.51^{f}	0.33^{e}	1.07^{h}		0.18^{c}	0.82^{g}	1.71^{i}	
Acetaldehyde	0.43^{f}	0.27^{d}	0.43^{f}		0.35^{e}	0.26^{d}	0.37^{e}		0.07^{b}	0.27^{d}	0.24^{d}	
Glycerol	0.16^{a,b}	1.75^{f}	2.97^{h}		2.27^{g}	1.60^{f}	2.25^{g}		1.14^{e}	1.40^{e}	0.93^{d}	
Ethanol	19.36^{h}	19.34^{h}	14.58^{g}		24.59^{i}	16.60^{g}	22.75^{i}		11.09^{f}	11.63^{f}	9.09^{e,f}	

nd – not detected; all values are an arithmetic mean of 6 assays; different letters in the same line indicate significant differences ($P < 0.05$); [1]L-malic acid 7 g/l, pH 3.0; [2]glucose 100 g/l, pH 3.0; [3]glucose 100 g/l, L-malic acid 7 g/l

by different strains (Heerde & Radler 1978; Redzepovic *et al*. 2003), which is also confirmed by the results presented in this paper.

The changes in pH affected L-malic acid consumption by the tested yeast. Optimal pH for the metabolism of this acid by *S. cerevisiae* Syrena and *S. pombe* was 3.0, and 3.5 for hybrid HW2-3. It is known that L-malic acid enters *S. cerevisiae* cells by simple diffusion and the optimal pH range for this process is 3.0–3.5 (Delcourt *et al.* 1995; Volschenk *et al.* 2003), so the presented findings are consistent with these data. The observed statistically significant changes ($P < 0.05$) in glycerol formation by *S. cerevisiae* Syrena and the hybrid HW2-3 were the result of even a slight pH shift from 3.0 to 3.5. Vigorous glycerol production is usually the effect of adverse environmental conditions during acidic must fermentation (Soufleros *et al*. 2001); therefore, its concentration in young wines may be elevated. The tested strains are sensitive to pH changes, which is also reflected in the patterns of their fermentation profiles.

In anaerobic conditions (100 g/l glucose), optimal parameters for L-malic acid decomposition for *S. cerevisiae* Syrena and the hybrid HW2-3 were 11 g/l L-malic acid and pH 3.0 and 3.5, respectively. *S. pombe* expressed the highest demalication activity at 40 and 100 g/l glucose, 7 g/l L-malic acid and pH 3.0. The results presented showed that the fermentation profiles of selected metabolites of yeast are unique for specific industrial strains used for acidic must fermentation. These profiles may help in selecting the right yeast strain for fermentation and make it possible to predict the organoleptic changes in the course of fruit must fermentation.

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