L-Malic Acid Effect on Organic Acid Profiles and Fermentation By-products in Apple Wines

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Abstract: Industrial wine yeasts *Saccharomyces bayanus* and two interspecies hybrids (*S. cerevisiae* × *S. bayanus*) were checked for their suitability for fermentation of apple musts with different L-malic acid content (4, 7 and 11 g/l). The fermentation profiles including main organic acids, acetaldehyde, diacetyl, glycerol, esters and polyphenols were presented. The results were obtained by HPLC method (organic acids, acetaldehyde, glycerol, diacetyl), GC (esters), colorimetrically (polyphenols) and enzymatically (L-malic acid, ethanol). Although the fermentation profiles of wines were characteristic for specific yeast strains, similarities in organic acid profiles of wines fermented by *S. bayanus* and its hybrid S-779/25 were noted. In all the tested wines L-malic, pyruvic and citric acids were dominant. Statistical analysis of all wine parameters indicates that yeast strains respond individually to different acidities of the fermentation environment. In order to choose the right yeast strain for the fermentation of acidic musts, information about fermentation profiles should be included in the collection certificate of yeast strains.

Keywords: L-malic acid; organic acid profiles; wine yeast; *Saccharomyces bayanus*; interspecies hybrid

INTRODUCTION

The role of yeast in the creation of final chemical composition and sensory properties of wine has been widely discussed (Lambrechts & Pretorius 2000; Fleet 2003; Blanco *et al*. 2008). The quality of wine is also strongly affected by organic acids. One of the main causes of the excessive acidity of wine is L-malic acid. The level of malic acid in grapes varies from 1 g/l to 16 g/l, depending on the climate, region and season (Redzepovic *et al*. 2003). Industrial wine yeasts *Saccharomyces cerevisiae* and *Saccharomyces bayanus* are not usually able to conduct vigorous fermentation of acidic musts. However, as a result of screening a wide range of industrial strains, some yeasts with elevated L-malic acid fermentation ability were selected (Redzepovic *et al*. 2003; Rajkowska & Kunicka 2005). Moreover, interspecies hybrids of *S. cerevisiae* and *S. bayanus* were constructed and checked in a model medium (Rajkowska *et al.* 2005). To assess yeast suitability for acidic must fermentation, it is essential to examine their fermentation profiles in natural apple musts. These fermentation profiles of produced wines are the source of information about the potential application of a particular yeast strain. The aim of the study was to evaluate changes in the fermentation profiles of apple wine fermented by three different yeast strains depending on the initial concentration of L-malic acid in musts. The effect of L-malic acid on organic acids and some fermentation by-products was investigated.

MATERIAL AND METHODS

Microorganisms. *S. bayanus* Y.00779 was originated from the National Collection of Agricultural and Industrial Microorganisms University of Horticulture and Food Science in Budapest. Two interspecies hybrids *S. cerevisiae* × *S. bayanus* HW2-3 and S-779/25 obtained by natural hybridisation are deposited in the Pure Culture Collection Institute of Fermentation Technology and Microbiology, Technical University of Lodz LOCK 105.

Apple musts and fermentations. Fermentations were carried out in 2000 ml apple musts with 190 g/l sucrose; 4, 7 or 11 g/l L-malic acid and incubated at 28°C during 30 days. Young wines were mellowed in storage at 12°C during next 30 days. The musts were inoculated with precultures of yeasts to a final concentration of 5% (w/v). The precultures were prepared in apple must with 4 g/l L-malic acid and incubated at 28°C during 2 days before inoculation.

Chemical analysis. L-Malic acid and ethanol were determined enzymatically with specialised kits (Boehringer Mannheim, GmbH Germany). Pyruvic, tartaric, citric, lactic and acetic acids, acetaldehyde, glycerol and diacetyl were determined by HPLC method (Frayne 1986). Esters were determined by gas chromatography (Soufleros *et al*. 2001) and total polyphenols by the standard colorimetric method (Czyzowska & Pogorzelski 2002). Reducing sugars, total acidity and volatile acidity were determined according to the official analytical methods (PN-90).

Statistical analysis. Results were presented as an arithmetic mean of 6 determinations 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

Table 1 shows the mean value of general parameters' ($n = 6$) of wines fermented by each of the three strains. Only at the lowest initial concentration of L-malic acid (4 g/l) there were no statistically significant differences ($P < 0.05$) in the quantity of ethanol produced. Depending on the strain, the level of ethanol in apple wines was comparable (Soufleros *et al*. 2001; Torres *et al*. 2008) or up to 3.7% lower (Redzepovic *et al*. 2003) than in other wines manufactured from highly acidic musts. The high level of reducing sugars, twice higher than that determined for apple wines studied earlier (Rajkowska & Kunicka 2005), indicates that the must has been fermented incompletely. The total acidity of wines was correlated with the concentration of L-malic acid in the must, which is confirmed in the literature (Redzepovic *et al*. 2003; Rajkowska & Kunicka 2005). The volatile acidity of wines fermented by *S. bayanus* Y.00779 and the hybrid HW2-3 was the highest in the presence of 7 g/l extracellular malic acid. Statistical analysis of the general parameters of the wines indicates an individual response of yeast strains to different acidities of the fermentation

Table 1. General characteristics of apple wines with different L-malic acid initial levels

Parameter	Inoculated yeast strain		
	Y.00779	HW2-3	S-779/25
L-Malic acid initial concentration 4 g/l			
Ethanol (% v/v)	9.7^{b}	9.8^{b}	9.8^{b}
Reducing sugars (g/l)	49.48^{e}	43.03^{d}	42.00^{d}
Total acidity* (g/l)	6.68^{b}	6.45^{a}	6.45^{a}
Volatile acidity** (g/l)	0.31^{b}	0.30^{b}	0.20^{a}
L-Malic acid initial concentration 7 g/l			
Ethanol (% v/v)	9.5^{a}	9.8^{b}	11.6^{c}
Reducing sugars (g/l)	31.04^{b}	13.52^{a}	32.79^{b}
Total acidity* (g/l)	10.37^{c}	10.83^{d}	10.16^{c}
Volatile acidity** (g/l)	0.63^{e}	0.68^{e}	0.44^{c}
L-Malic acid initial concentration 11 g/l			
Ethanol (% v/v)	10.3^{b}	9.5^{a}	9.3^{a}
Reducing sugars (g/l)	34.45^{b}	37.78^{c}	38.06^{c}
Total acidity* (g/l)	13.15^{f}	$12.89^{e,f}$	12.44^{e}
Volatile acidity** (g/l)	0.56^{d}	0.52^{d}	0.54^{d}

Different letters in the same line indicate significant differences ($P < 0.05$); *as citric acid, **as acetic acid

Table 2. Concentration of organic acids (g/l) and selected fermentation by-products (g/l) in apple wines with different L-malic acid initial levels

Compound (g/l)	Inoculated yeast strain		
	Y.00779	HW2-3	S-779/25
L-Malic acid initial concentration 4 g/l			
L-Malic acid	4.85^{a}	5.75^{b}	5.45^{b}
Pyruvic acid	2.74^{b}	3.39^{c}	3.53^{c}
Tartaric acid	0.59^{b}	0.73^{c}	0.74^{c}
Citric acid	1.06^{b}	1.24^{c}	1.21^{c}
Lactic acid	0.47^{b}	0.35^{a}	0.40^{a}
Acetic acid	0.10^{a}	0.31^{c}	0.13^{a}
Acetaldehyde	nd	0.14^{b}	0.07^{a}
Diacetyl	nd	nd	nd
Glycerol	4.15^{d}	3.83^{c}	$3.99^{c,d}$
Esters (mg/l)	109^{b}	88^{a}	176^{d}
Total polyphenols (mg/l)	678^{c}	646^{b}	665^{c}
L-Malic acid initial concentration 7 g/l			
L-Malic acid	6.18^{c}	6.77^{d}	5.78^{b}
Pyruvic acid	3.02^{b}	2.93^{b}	4.02^{d}
Tartaric acid	0.67^{c}	0.66^{c}	0.72^{c}
Citric acid	1.12^{b}	1.09^{b}	1.12^{b}
Lactic acid	0.68^{c}	0.48^{b}	0.41^{a}
Acetic acid	0.50^{d}	0.39^{c}	0.26^{b}
Acetaldehyde	0.27^{c}	nd	0.06^{a}
Diacetyl	0.03^{a}	nd	0.05^{a}
Glycerol	4.70^{e}	3.60^{c}	4.05^{d}
Esters (mg/l)	173^{d}	204^{e}	144^{c}
Total polyphenols (mg/l)	631^{b}	656^{c}	527^{a}
L-Malic acid initial concentration 11 g/l			
L-Malic acid	9.28^{f}	5.59^{b}	8.03^{e}
Pyruvic acid	2.88^{b}	1.93^{a}	2.93^{b}
Tartaric acid	0.69^{c}	0.41^{a}	0.68^{c}
Citric acid	1.28^{c}	0.64^{a}	1.14^{b}
Lactic acid	0.51^{b}	0.50^{b}	0.51^{b}
Acetic acid	0.29^{c}	nd	0.24^{b}
Acetaldehyde	nd	nd	0.04^{a}
Diacetyl	nd	nd	nd
Glycerol	3.81^{c}	2.36^{a}	3.36^{b}
Esters (mg/l)	123^{b}	120^{b}	75^{a}
Total polyphenols (mg/l)	699^{d}	634^{b}	706^{d}

nd – not detected; different letters in the same line indicate significant differences ($P < 0.05$)

environment. Similar results were obtained for grape wines, where the behaviour of yeasts strains was not significantly influenced by the chemical composition of musts (Torres *et al*. 2008).

Even though the fermentation profiles of wines were particular to specific yeast strains, similarities in organic acid profiles of wines fermented by *S. bayanus* Y.00779 and its hybrid S-779/25 were noted (Table 2). In all the tested wines L-malic, pyruvic and citric acids were dominant. Higher concentrations of citric and lactic acids, relative to grape wines (Torres *et al*. 2008) and the varied levels of pyruvic acid indicate metabolic differences of the yeast strains. The fermentation profile of *Saccharomyces* sp. yeast metabolising L-malic acid with high efficiency is produced as a result of both ethanolic and malo-ethanolic fermentation (Volschenk *et al.* 2003). The presence of diacetyl and acetaldehyde was detected only in a few wines but if, their levels were higher as compared to grape wines (Petravić Tominac *et al.* 2008). Glycerol levels were the lowest in wines with the highest values of L-malic acid initial content, but still comparable with those for grape wines (Soufleros *et al*. 2001; Torres *et al.* 2008). The concentrations of esters determined in the wines were varied: comparable or even twice higher than in grape wines (Petravić Tominac *et al.* 2008). Total polyphenols content was even 5–6 times higher than in white grape wines (Torres *et al*. 2008).

The organoleptic features of wines were assessed by a trained panel of six persons and 20-points scale was applied (data not presented). According to this examination, wines with 4 g/l L-malic acid initial concentrations in musts were of the highest value (17.8–18.7 points).

Statistical analysis of wine parameters indicates an individual response of yeast strains to various acidities of the fermentation environment. Due to the high differentiation of yeast fermentation profiles, the individual properties of strains should be taken into account in choosing yeast for fermentation of various environments. On the basis of our results, it is justified to include information on the fermentation profile into the yeast strain collection certificate.

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