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TECHNOLOGICAL VALUE OF OSMOTOLERANT YEAST ISOLATED FROM HIGH-SUGAR PEAR JUICES

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Key words: identification of yeast, osmotolerant yeast, wine, secondary products of alcoholic fermentation

Yeast for must fermentation are selected in dependence on amount needed to produce alcohols, initial must extract and expected organoleptic features of wines. Particular kinds of wine yeast differ between each other in optimum fermentation temperature, fermentation abilities and amount of secondary products produced.

The aim of this study was to estimate the technological features of yeast *Saccharomyces bayanus* KK1, which was identified after isolation from high-sugar pear juices with extract 70°Blg.

The yeast ethanol and secondary products production characteristics was performed with the use of GC and HPLC.

On the basis of the estimated technological parameters of identified yeast *Saccharomyces bayanus* KK1, it was stated that they were valuable biological material to be used in winemaking.

INTRODUCTION

Alcoholic fermentation is a combination of complex interactions involving must variety, microbiota and winemaking technology. Obviously, some factors strongly affect alcoholic fermentation, and as a consequence, the quality of wine.

In fermentation practice there are distinguished noble yeast and wild yeast, which are undesirable because of poor fermentation abilities or because of its noisome characteristics *e.g.* sludge blanket formation on the surface, mucus formation and unpleasant odour.

Strains of wine yeasts used in a technological process should make induction and intensive fermentation, provide process correctly, in addition to alcohol they should also produce secondary products with a positive influence on taste and fragrant features, should subside in short time after the fermentation and make quick clarification of wine [Wzorek & Pogorzelski, 1998].

Yeast suitable for must fermentation are selected depending on the amount of alcohols produced, initial extract of must, quantity of contained tannin and expected sensory traits of wine. Particular wine yeast differ between each other not only in the optimum fermentation temperature and fermentation abilities but also in the amount of fermentation secondary products produced, as well as the amount and kind of enzymes produced to environment.

Osmophilic yeast ferment juices with a high content of sugar [Wzorek & Pogorzelski, 1998]. Higher concentrations of sugar lead to an increase in osmotic pressure in cells and stop the growth and motility of yeast, thereby lengthen fermentation significantly and reduce fermentation rate [Wzorek *et al*., 1998].

Growth of yeast must be considered because it may influence the sensory quality of wine [Moreira *et al*., 2005]. Wine contains about 800 different volatile compounds, such as alcohols, ketones, aldehydes, esters. Most of them appear during the fermentation process and their concentrations vary over a wide range [Rodriquez-Bencomo *et al*., 2003]. They are important contributors to the sensory characteristics of wines [Simpson, 1979].

The aim of this work was to estimate the possibility of using yeast isolated from high-sugar pear juices 70°Blg in winemaking.

MATERIAL AND METHODS

Chemical reagents. Folin-Ciocalteu reagent, secondary products standards (Sigma), tests API 20C AUX (BioMerieux), were used. Apple concentrate (70°Blg) was from Z.P.O.W. "Hortex" Skierniewice, dried yeast *Saccharomyces bayanus* BCS 103 (Fould Springer), yeast isolated from high-sugar pear juices (70°Blg) from Spain were used in the experiment.

Preparation of wines. Yeast isolated from high-sugar pear juices 70°Blg were used in the study. A sample of this juice was added to a flask containing sterile nutrient agar and poured into Petri dishes (8 mm in diameter), and incubated at 30°C for 48 h.

After incubation, the yeast was transferred onto sterile

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apple must (15°Blg) for activation and cultivation. After 24 h, the inoculation (grafting) was made on apple must (24°Blg) and after the next 24 h on the next portion of apple must (32°Blg), which finally produced a yeast starter for pitchings. All propagation stages were carried out static at a temperature of 25°C.

Apple must was prepared by diluting an apple concentrate of 70°Blg with water to 9.5°Blg. Two apple pitchings were prepared: 24°Blg and 32°Blg. To achieve wine extract proof of 24°Blg and 32°Blg the original musts were sweetened with saccharose. The pitchings were inoculated with yeast isolated from high-sugar pear juices species in the amount of 10% vol. and appropriate nitrogen source (0.3 g/L $(NH_4)_2HPO_4$). The musts were prepared taking the must consumption factor of 0.6 L/L.

A control sample were pitchings inoculated with dried yeast *Saccharomyces cerevisiae* in the amount of 0.3 g/L (rehydration of yeast: temperature 30–35°C, sugar 5%, 15 min) and appropriate nitrogen source (0.3 g/L $(NH_4)_2HPO_4$).

Fermentation of the samples was carried out at a temperature of 25°C, in 3 L glass bottles filled with 2 L of pitching. After the fermentation, the young wines were racked, and then filtered through Filtrox AF 70 filtration plates. After the filtration, the wines were stored for aging.

Calculations and presentation of results. The fermentation experiments were repeated three times using two parallel samples of the same kind in each. A GC and HPLC analysis of each sample was repeated twice. All chromatograms are mean values. The results were analysed statistically, and the mean value, and standard deviation were calculated.

Chemical composition of experimental wines. Proximate chemical analysis (alcohol content, total extract, total acidity, volatile acidity, sugar) and sensory evaluation were carried out according to the Polish Standard [PN-90/A-79120]. Polyphenols were determined with the Folin-Ciocalteu method using gallic acid as a standard [Sejder & Datunašvili, 1972].

GC of secondary products of wine. Methods of determination were developed on the basis of a paper by Coghe *et al*. [2005]. An analytical polyethylene glycol (PEG) capillary column (L=60 m, ID=0.32 mm, 0.5 μm particles) – INNOWAX was used in the study. Headspace conditions: transfer line 70°C, loop 65°C, event times: oven 50°C, injection 0.50 min, loop equilibration 0.05 min, loop fill 0.15 min, pressurization 0.13 min, Vial EQ 30 min. GC conditions: oven 40°C (2 min), 2°C/min to 60°C (3 min), 2°C/min to 100°C, 15°C/min to 200°C, flow 1 mL/min, inlet temp. 200°C, detector temp. 250°C, splitless 40 mL/min for 0.45 min.

Identification of secondary products of fermentation was achieved by comparing their retention time values with those of standards.

HPLC of secondary products of wine. Analyses of glycerol, lactic acid, acetic acid, succinic acid were carried out by HPLC, using an RI detector. An analytical AMINEX HPX 87 H column (L=300 mm, ID=7.8 mm, 5 μm particles) was used, protected with a guard cartridge of the same packing. The elution solvent used was 0.0005n sulphuric acid. The flow rate was 0.6 mL/min and run time 30 min. The run was performed at 60°C. After filtration through a syringe filter Millipore 0.22 μm, a 20 μL sample of wine was injected onto the column.

Concentrations of secondary products of fermentation were then calculated from integrated peak areas of the samples and the calibration curve of glycerol, lactic acid, acetic acid, succinic acid standards.

Identification of yeast. In order to identify yeast, which were isolated from high-sugar pear juices 70°Blg, their morphological and physiological features were studied according to methods recommended by Lodder [1984] and Barnett [1984, 1998]. The estimation of yeast ability to assimilation of selected carbon compounds was carried out using API 20C AUX tests. Morphology of yeast and their ability to form spores, mycelium and pseudomycelium were defined. In the study, determinations were also carried out for the capacity of yeast to assimilate and ferment xylose, arabinose, glucose, galactose, saccharose, maltose, celobiose, trehalose, melibiose, lactose, raffinose, melesytose, soluble starch, α-metyl-D--glucoside, and to assimilate ethanol, glycerol, inositol, sorbitol, xylitol, adonitol and potassium nitrate. Prototrophy of yeast to vitamins, the growth on 50 and 60% (w/w) glucose medium and ability to produce enzyme urease.

RESULTS AND DISCUSSION

The genesis of this research were existing tendencies to enhance wine fermentations. There is a need for yeast features research, also their environment and their affiliation to a given species. Van der Walt defined osmophilic yeast as species able to grow well on 50% (w/w) glucose medium, but not able to grow on medium with 60% content of this sugar. In 1978, Phaff *et al*. [cited after Tilbury, 1980] proposed that

TABLE 1. Utilization of carbon compounds by the isolated yeast.

Compounds	Utilization by yeast strain
Adonitol	Non-assimilation
Arabinose	Non-assimilation
Cellobiose	Non-assimilation
Ethanol	Assimilation
Galactose	Assimilation and fermentation
Glucose	Assimilation and fermentation
Glycerol	Non-assimilation
Inositol	Non-assimilation
Lactose	Non-assimilation
Maltose	Assimilation and fermentation
Melibiose	Non-assimilation
Raffinose	Assimilation and fermentation
Saccharose	Assimilation and fermentation
Soluble starch	Non-assimilation
Sorbitol	Non-assimilation
Trehalose	Non-assimilation
Xylitol	Non-assimilation
Xylose	Non-assimilation
α-Methyl-D-glucoside	Non- assimilation

yeast capable to grow on 40–70% (w/w) glucose medium should be named "sugar-tolerant yeasts".

In order to define yeast affiliation to a given species their identification was carried out. The yeast cells examined are of the oval shape. The cells occurred as a single or double. The yeast isolated were observed to sporulate, forming from 2 to 4 spores. The ability to produce mycelium and pseudomycelium was not revealed.

The yeast isolated had the ability to assimilate and ferment glucose, galactose, saccharose, maltose and raffinose. The ability to ethanol assimilation was registered, but not the assimilation of: xylose, arabinose, galactose, cellobiose, trehalose, melibiose, lactose, glycerol, soluble starch, inositol, sorbitol, xylitol, adonitol and α-methyl-D-glucoside (Table 1).

The yeast isolated did not assimilate nitrates. The yeast growth on mineral medium without vitamins was observed. It follows that these microorganisms are prototrophs in relation to vitamins. According to van der Walt, the growth of yeast on 50% (w/w) glucose medium was registered [Tilbury, 1980]. The examined yeast can be numbered among osmotolerant ones. Neither growth of yeast on medium with 60% sugar content nor their ability to produce enzyme urease were observed in the study (Table 2).

The presented research proved that yeast isolated from high-sugar pear juices belong to species *Saccharomyces bayanus*, which were named KK1.

The fermentation process of apple must, which was monitored by a decrease in extract content, revealed faster completion in wines received as a result of fermentation led by dried yeasts *Saccharomyces bayanus*, in contrary to wines fermented with the use of yeasts *Saccharomyces bayanus* KK1, which fermented about 10 days longer (Figure 1). However, the yeast isolated were able to produce higher alcohol proof of 16% vol. The isolated yeasts produced high levels of foam and resided on the vessel wall. In a 3 L glass bottle, the foam level ranged from 3 to 4 cm in the main stage of fermentation.

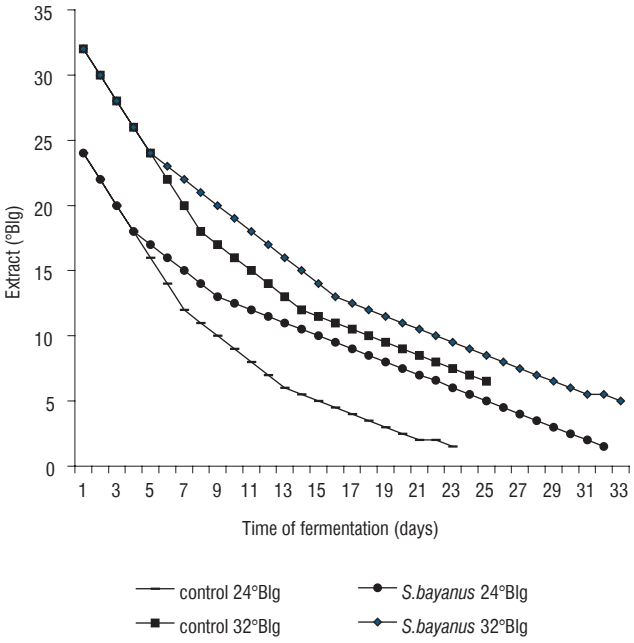

FIGURE 1. Kinetics of the fermentation process.

After the fermentation, taking into account sugars remained in the pitchings and alcohol produced, it was found that dry wines were obtained for pitching 24°Blg and sweet wines for pitching 32°Blg.

Loss of polyphenols content during the fermentation process was induced mainly by their adsorption on the surface of yeast cells and their reaction with the proteins of yeast cell. Assuming that polyphenols content transfer from must to pitchings was similar, we can suppose that the isolated yeast did not prove particular attributes to absorb polyphenol components, and that differences were related with the amount of cell-bound biomass (Table 3).

TABLE 2. Selected physiological features of isolated yeast.

Yeast strain	Assimilation of nitrates	Growth on medium without vitamins	Growth on glucose medium		Formation of urease
			50%	60%	
KK1	-	+	+	-	-

TABLE 3. Chemical and sensory analysis of apple wines.

	Type of the probe of wine			
	S. bayanus pitching 24°Blg	control pitching 24°Blg	*S. bayanus* pitching 32°Blg	control pitching 32°Blg
Alcohol (% vol.)	14.50 ± 0.25[a]	14.30 ± 0.35	15.8 ± 0.25	13.7 ± 0.30
Total extract (g/L)	30.28 ± 5.25	32.34 ± 4.34	107.05 ± 5.28	126.67 ± 4.67
Total sugars (g glucose/L)	4.56 ± 4.04	3.10 ± 2.31	83.63 ± 3.94	97.36 ± 2.89
Reducing sugars (g glucose/L)	4.08 ± 1.27	2.40 ± 2.52	78.25 ± 1.75	92.26 ± 3.01
Saccharose (g/L)	0.45 ± 0.65	0.66 ± 0.73	5.11 ± 0.36	4.84 ± 0.83
Sugar-free extract (g/L)	25.75 ± 5.98	29.28 ± 4.94	23.69 ± 6.03	29.57 ± 5.35
Total acidity (g apple acid/L)	5.66 ± 0.22	5.19 ± 0.16	5.12 ± 0.32	5.13 ± 0.36
Volatile acidity (g acetic acid/L)	0.14 ± 0.02	0.38 ± 0.04	0.49 ± 0.04	0.89 ± 0.05
Polyphenols (mg gallic acid/L)	341 ± 28.93	356 ± 25.92	361 ± 27.53	392 ± 32.04
Sensory evaluation (point)	15.9 (db)	16.12 (db)	16.88 (db)	17.02 (db)

db – classified to a group of good wines in a sensory test

The HPLC method enables simultaneous analysis of glycerol, succinic acid, lactic acid and acetic acid content giving recurrent effects. In the wines obtained there were proved differences in glycerol and lactic acid contents. The concentration of glycerol was higher in control wines, in contrary to wines obtained using the isolated yeast (Table 4). In no case did glycerol production exceed 5.2 g/L, which is the threshold taste level of sweetness [Clemete-Jimenez *et al*., 2004].

Glycerol is a secondary product of fermentation that has a slightly sweet taste leaving a smoothness impression on the palate [Noble & Bursick, 1984].

Wines fermented by the isolated yeast *Saccharomyces bayanus* KK1 were characterised by high levels of lactic acid content, which is a secondary product of alcoholic fermentation. Lactic acid may be produced by the yeast in the amount of about 5 g/L. Wines obtained from pitching 24°Blg were found to contain 7.11g/L of lactic acid (Table 4).

During the winemaking process there was observed a steady low growth of volatile acidity – mainly high acetic acid content. This acid is a secondary product of alcoholic fermentation and the increase of acetic acid to 0.9 g/L for white wines and to 1.2 g/L for red wines is considered as normal. Usually after high-sugar pitching fermentation volatile acids level is much higher than normally. In wines fermented with the use of yeast isolated from pear juice, a lower content of acetic acid was reported in contrary to control wines (Table 4).

Secondary products of alcoholic fermentation are important contributors to the sensory characteristics of wines, in particular higher alcohols have a significant influence on the taste and character of wine. Below 300 mg/L higher alcohols contribute positively to wine quality, while excessive amounts (higher than 400 mg/L) may deteriorate its quality [Soufleros & Bertrand, 1979; Rapp & Versini, 1991; Lambrechts & Pretorius, 2000].

It was found that the content of higher alcohols was lower if the pitchings were enriched in nonorganic nitric medium. Furthermore, in red wines higher alcohols content is generally much higher than the average value determined in white wines. Higher alcohols in experimental wines do not exceed the level of 300 mg/L (Table 5).

The esters, which are produced during wine fermentation from volatile and non-volatile acids and higher alcohols, play an important role in the formation of sensory traits of wine.

The volatile esters are an important element of wine bouquet, whereas the non-volatile esters are constituents of compounds which affect taste features. Their formation in most cases is catalyzed by yeast enzymes from the esterase group [Clemete-Jimenez *et al*., 2005].

The characteristic fruit flavour of wine is primarily due to a mixture of hexyl acetate, ethyl caproate, ethyl caprylate, isoamyl acetate, and 2-phenylethyl acetate [Falque *et al*., 2001]. In particular, ethyl caprylate is associated with a pear aroma [Lambrechts & Pretorius, 2000].

According to Minarik & Navara [1986], esters occur at the level of 25–300 mg/L in young wines and at higher concentrations in sherries.

Ethyl acetate is the most abundant ester in wines and is

TABLE 4. Secondary products of alcoholic fermentation (in g/L) detected in HPLC analysis.

	Type of the probe of wine			
	S. bayanus pitching 24°Blg	control pitching 24°Blg	*S. bayanus* pitching 32°Blg	control pitching 32°Blg
Succinic acid	0.55 ± 0.01[a]	0.75 ± 0.02	0.33 ± 0.01	1.01 ± 0.03
Lactic acid	7.11 ± 0.15	0.08 ± 0.01	4.76 ± 0.09	trace amounts
Glycerol	4.65 ± 0.18	6.47 ± 0.24	5.75 ± 0.17	9.24 ± 0.28
Acetic acid	0.37 ± 0.01	0.62 ± 0.03	0.22 ± 0.01	1.14 ± 0.04

[a] mean ± SD

TABLE 5. Concentration of major volatile compounds (in mg/L) in wines detected in GC analysis.

	Type of the probe of wine			
	S. bayanus pitching 24°Blg	control pitching 24°Blg	*S. bayanus* pitching 32°Blg	control pitching 32°Blg
2-Methyl-1-butanol	10.48 ± 1.03	16.57 ± 2.31	11.91 ± 0.09	19.68 ± 2.04
2-Methyl-1-propanol	74.37 ± 6.39	170.21 ± 10.36	23.78 ± 2.96	165.37 ± 8.34
3-Heptanone	0.41 ± 0.01	0.64 ± 0.01	0.20 ± 0.01	0.74 ± 0.03
3-Methyl-1-butanol	64.39 ± 7.28	118.86 ± 9.42	51.86 ± 4.58	100.26 ± 6.89
Acetaldehyde	48.84 ± 4.93[a]	171.19 ± 13.67	46.58 ± 6.28	132.14 ± 9.37
Ethyl acetate	46.70 ± 3.85	80.61 ± 9.20	25.64 ± 2.95	89.64 ± 7.38
Ethyl butyrate	0.58 ± 0.02	0.77 ± 0.02	0.28 ± 0.01	0.77 ± 0.02
Ethyl caproate	0.24 ± 0.01	0.25 ± 0.01	0.14 ± 0.01	0.24 ± 0.01
Ethyl caprylate	0.38 ± 0.02	0.17 ± 0.01	0.09 ± 0.01	0.16 ± 0.01
Iso-pentyl acetate	1.14 ± 0.03	1.46 ± 0.04	0.14 ± 0.01	2.39 ± 0.06
n-Propanol	23.82 ± 2.71	17.74 ± 2.26	3.33 ± 0.51	40.72 ± 3.59

[a] mean ± SD

produced by the yeast during the alcoholic fermentation and in the acetic bacteria metabolism. High amounts of ethyl acetate can be considered to be a symptom of wine spoilage [Rodriquez-Bencomo *et al*., 2003]. When the content of ethyl acetate exceeds 200 mg/L, the organoleptic characteristics typical of acetic acid appears. Its low contents (50–80 mg/L) contribute favourably to wine quality [Moreira *et al*., 2005]. Ethyl acetate must be present in wines at concentrations below the threshold taste level of 150 mg/L [Rapp & Mandery, 1986], (Table 5).

Aldehydes are secondary products of alcoholic fermentation. In young wines the content of aldehydes should not exceed 75 mg/L [Margalith, 1981]. During alcoholic fermentation acetaldehyde is formed in the highest quantities, followed by propionic aldehyde, isoamyl aldehyde and isovalerian aldehyde.

The influence of aldehydes on the bouquet of wine must be considered depending on the type of wine. For effervescent wines high levels of aldehydes are not desirable, but in sherries they play an important role in sensory evaluation. A higher content of aldehydes content, *i.e.* 250 mg/L, was found in wines obtained from high-sulphur must [Minarik & Navara, 1986].

Acetaldehyde is an important secondary product of wine fermentation, ranging for 90% of total aldehydes and is formed in pyruvate decarboxylation [Ciani, 1997; Etievant, 1991]. Sulphuric acid may be partially bounded by acetaldehyde, which prevents its reduction to ethanol [Osborne *et al*., 2000]. Acetaldehyde is highly volatile and present in excess imparts an undesirable green, grassy, apple-like aroma [Zoecklein *et al*., 1995], which is usually masked by the addition of SO_2 [Burroughs & Sparks, 1973].

Average values of acetaldehyde range from 40 mg/L for red wine and about 80 mg/L for white wine, to 300 mg/L for sherries [Liu & Pilone, 2000]. In experimental wines fermented by the isolated yeast *Saccharomyces bayanus* KK1 the level of acetaldehyde ranged from 45 mg/L to 50 mg/L and was found appropriate. In the control wines its content was decidedly higher and ranged from 130 mg/L to 170 mg/L (Table 5).

According to Romano *et al*. [1997a,b, 2003], the synthesis of secondary products is an individual and reproducible characteristic of yeasts strain. The isolated yeast *Saccharomyces bayanus* KK1 may be used in the technological winemaking process. They do not produce considerable amounts of secondary products of alcoholic fermentation. Substantially higher levels of higher alcohols, esters, glycerol and acetaldehyde were revealed in the control wines. Ciani & Picciotti [1995] exclude the possibility of using yeasts which produce large amounts of ethyl acetate and acetic acid in winemaking.

The share of yeasts *Saccharomyces bayanus* KK1 during the alcoholic fermentation might be of technological interest, but further studies on these yeasts for the biotechnological applications in winemaking are needed.

CONCLUSIONS

1. Yeast isoalted from high-sugar pear juices belong to species *Saccharomyces bayanus* (KK1).

2. Yeast *Saccharomyces bayanus* (KK1) are able to ferment pitching with about 50% sugar content. In this study, the pitchings analyzed (extract 32°Blg) were freely fermented.

3. Yeast *Saccharomyces bayanus* (KK1) produce high levels of alcohol achieving proof of 16% vol. and produce appropriate amounts of secondary products of alcoholic fermentation.

4. On the basis of the estimated technological parameters of the identified yeast *Saccharomyces bayanus* KK1 it was stated that they are valuable biological material to be used in winemaking.

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WARTOŚĆ TECHNOLOGICZNA OSMOTOLERANCYJNYCH DROŻDŻY WYODRĘBNIONYCH Z WYSOKOSŁODZONYCH SOKÓW GRUSZKOWYCH

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Drożdże do fermentacji moszczów dobierane są w zależności od ilości mającego wytworzyć się alkoholu, początkowego ekstraktu moszczu czy też od oczekiwanych właściwości sensorycznych win. Poszczególne rasy drożdży winiarskich różnią się między sobą optimum temperaturowym fermentacji, uzdolnieniami fermentacyjnymi oraz ilością wytwarzanych produktów ubocznych fermentacji.

Celem przeprowadzonych badań była ocena właściwości technologicznych drożdży, zidentyfikowanych po wyizolowaniu z wysokosłodzonych soków gruszkowych o ekstrakcie 70°Blg, *Saccharomyces bayanus* KK1. Dokonano charakterystyki drożdży pod względem ilości wytwarzanego etanolu oraz produkcji substancji ubocznych wykorzystując metody chromatografii gazowej (GC) oraz wysokosprawnej chromatografii cieczowej (HPLC).

Na podstawie ocenianych parametrów technologicznych zidentyfikowanych drożdży *Saccharomyces bayanus* KK1 stwierdzono, że stanowią one cenny materiał biologiczny do wykorzystania w winiarstwie.