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ENOLOGICAL PROFILE OF *SACCHAROMYCES CEREVISIAE* YEAST ISOLATED FROM FERMENTING PLUM MASHES

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Background. Śliwowica Łącka is a strong plum brandy (slivovitz) that is produced in a submontane region of Poland by means of spontaneous fermentation of Węgierka plums. The aim of this study was to evaluate enological profile of *S. cerevisiae* indigenous strains isolated from spontaneous plum mash fermentation.
Material and methods. Fourteen strains obtained from three different stages of fermentation (initial, central and final) and characterised by different killer profile were chosen for the analysis. Fermentation assays were performed on the basal synthetic medium with 10% glucose. The fermentation kinetics, basic enological parameters by OIV methods and selected volatile compounds concentration by GC-SPME were analysed.
Results. Analysed strains exhibited different fermentation kinetics, as well as produced diversified amounts of studied volatile compounds. The highest ethanol synthesis (over 40 $g \cdot dm^{-3}$) and fermentation efficiency (over 80%) was found in samples fermented with strains isolated from final stage of fermentation. Cultures from an initial stage were distinguished by higher production of acetaldehyde and acetic acid, and lower of isobutanol, ethanol and ethyl acetate, those originated from central stage showed increased synthesis of ethyl acetate and acetoine, whereas the strains isolated during final stage of fermentation formed more acetaldehyde, acetic acid and fusel alcohols and less esters. Strains that were present throughout the spontaneous fermentation were synthesized average amounts of compounds mentioned above.
Conclusions. High diversity of enological profiles among isolated *S. cerevisiae* strains was determined. The composition of Śliwowica Łącka is strictly dependent on presence and amount of the individual profiles during spontaneous plums fermentation.

Key words: indigenous *Saccharomyces cerevisiae*, spontaneous fermentation, enological profile, plum brandy

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INTRODUCTION

Śliwowica Łącka is a strong plum brandy (slivovitz) that is produced in a submontane region of Poland with specific climatic and soil conditions by means of spontaneous fermentation of Węgierka Zwykła plums. Its originality depends on a high sugar concentration and unique aroma profile of blue plum fruits, as well as diverse microbiota that are present during spontaneous fermentation [Satora and Tuszyński 2008]. The blue plum fruits are colonized mainly by the yeast-like fungi of genus *Aureobasidium* sp. and *Kloeckera apiculata* yeasts which constituted over 80% of fungal microbiota [Tuszyński and Satora 2003]. These microorganisms get through the must during fruits processing and start the fermentation process. The first phase of the fermentation is dominated by the representatives of *Kloeckera apiculata* and *Candida pulcherrima* species, also the growth of *Rhodotorula* and *Aureobasidium* species occurs to a lesser extent. The increase of the yeast microbiota continued during the first four days of fermentation until the maximum of $1.03 \cdot 10^6$ cfu·cm^{-3} is achieved. As the fermentation progresses, the non-*Saccharomyces* species succesively die off, leaving *Saccharomyces cerevisiae* to dominate and complete the fermentation [Satora and Tuszyński 2005].

S. cerevisiae wine strains from natural fermentations have demonstrated the existence of strong polymorphism within this species and it is widely accepted that *S. cerevisiae* strains, producing different amounts of secondary compounds, impart desirable or undesirable characteristics on the flavour and aroma of the alcoholic beverages. It is generally known that *S. cerevisiae* strains exhibit a low variability in the levels of n-propanol, acetaldehyde and ethyl acetate, whereas the other compounds are formed with significant strain variability [Romano et al. 2003].

The aim of this study was to determine the enological profile of different indigenous strains of *S. cerevisiae* isolated from spontaneously fermenting Węgierka Zwykła plum musts.

MATERIAL AND METHODS

Strains and media

The study covered wild *S. cerevisiae* strains isolated from spontaneously fermenting plum mashes from 2005. Cultures were isolated and signed according to the stage of isolation and killer/sensitive profile [Satora et al. 2010]. Stage of isolation of an individual studied strain was shown in the Table 1.

Microorganisms were classified into *S. cerevisiae* species on the basis of vegetative cell and asci morphology, as well as metabolism analysis results obtained using the API 20C AUX system (*Biomerieux*), conventional biochemical assay tests [Barnett et al. 1983] and molecular methods [Pohve Jemec et al. 2001].

All strains were maintained on a slant agar with the YEPD medium (1% of yeast extract, 2% of glucose, 2% of peptone and 2% of agar) at 4°C, and subcultured every three months. All components were from Lab. Conda (Madrid, Spain).

Table 1. Isolation stages of studied indigenous *S. cerevisiae* strains

Fermentation stage		
Initial	Middle	Final
2S, 3S, 6S		
4S		
	8K, 9S, 10K, 12K, 14K	
	18K	
		22K, 26K
	1S, 5S	

S – sensitive phenotype, K – killer phenotype.

Fermentation assays

To determine the enological profile of strains, a basal synthetic medium with 10% glucose was used. Its composition, similar to that recommended by Wickerham (1951), was reported by Romano et al. [1992]. Fermentation was carried out in 500 cm^3 flasks containing 200 cm^3 of the sterilized synthetic medium, inoculated with 0.5 g DW yeast per 1 dm^3. The samples were incubated statically at 22°C for 14 days, than centrifuged (735·g, 15 min, 5°C) and analysed.

All assays were carried out in triplicate repetitions.

Enological parameters analysis

The ethanol content, total extract, sugar-free extract, reducing sugars and sucrose concentrations were determined using official methods (OIV 2005). pH and titratable acidity (TA) were determined using titrator Mettler DL 25 equipped with a printer (Switzerland). Titratable acidity was calculated from the volume of NaOH used for titration and expressed as $g \cdot dm^{-3}$ of malic acid.

Volatile compounds analysis (GC-SPME)

Two cm^3 of each fermented model solution sample was transferred to a 15 cm^3 amber vial having screw caps (Supelco) with a magnetic stirrer and 1 g of NaCl, which was then spiked with 2 µl of internal standard (4-methyl-2-pentanol; Fluka). The SPME device (Supelco Inc., Bellefonte, PA, USA) coated with PDMS (100 µm) fiber was first conditioned by inserting it into the GC injector port at 250°C during 1 h. For sampling, the fiber was inserted into the headspace under magnetic stirring (300 rpm) for 35 min at 40°C. Subsequently, the SPME device was introduced in the injector port for chromatographic analysis and was remained in the inlet for 2 min. The GC-SPME analysis was performed on a Hewlett Packard 5890 Series II chromatograph system. The tested components were separated on a capillary column HP-INNOVAX (crosslinked polyethylene glycol stationary phase; 30 m × 0.53 mm ID with 1.0 µm film thickness). The detector and injector temperature was 250°C, and the column was heated using the following

temperature program: 35°C for 5 min at an increment 5°C/min to 110°C, then 40°C/min to 220°C and maintaining a constant temperature for 3 min. The carrier gas was helium at a 20.0 $cm^3 \cdot min^{-1}$ flow. Hydrogen flow speed was 33.0 $cm^3 \cdot min^{-1}$, and that of air was 400 $cm^3 \cdot min^{-1}$. The qualitative and quantitative identification of volatile substances (acetaldehyde, ethyl acetate, methanol, propanol, isobutanol, amyl alcohols and acetic acid; Sigma-Aldrich) was based on the comparison of retention times and peak surface area read from sample and standard chromatograms.

All tests were carried out three times.

Statistical analysis

SPSS 13.0 software was applied for statistical results analyses. Statistically significant differences between results ($p < 0.05$) were evaluated using multifactor variance analysis (ANOVA). All samples were compared on the basis of studied volatile compounds contents using principal component analysis (PCA).

RESULTS AND DISCUSSION

High diversity of enological profiles among isolated *S.cerevisiae* yeast strains was determined. Throughout fermentation (14 days), weight loss, associated with the liberation of carbon dioxide, was analysed [Zohre and Erten 2002]. Analysed strains were characterised by diverse kinetics of fermentation. A turbulent stage of fermentation appeared during first 5 days. Satisfactory fermentation rate was found only in samples fermented with cultures isolated during initial stage of spontaneous fermentation (1S, 2S, 4S) and some others like 8K, 18K and 22K, where a final 4% weight loss was reached (Fig. 1). The process with other strains run significantly slower. Some of strains isolated during central stage of spontaneous fermentation (9S, 10K and 12K) were characterised by linear fermentation curves, and the final weight loss ranged from 2 to 3%. The earlier research conducted in the wineries showed that wild strains are characterised by weaker fermentation kinetics and ethanol production compared to industrial ones [Ubeda and Briones 2000, Cocolin et al. 2004].

It is supposed that strains with lower fermentation rate showed higher oxygen metabolism. The 9S culture that fermented the slowest among analysed strains, produced the highest amounts of biomass – 0.049 $g \cdot g^{-1}$ glucose, whereas in other samples its level was much lower – from 0.020 to 0.036 $g \cdot g^{-1}$ glucose (Table 2).

Direct relationship between fermentation rate and sugars consumption (66-97%) was also detected (Table 2). The lower level was represented by 9S strain that showed weakest fermentation kinetics and the upper – by 2S culture that fermented the quickest. The yeasts isolated from initial stage of fermentation consumed the highest amounts of sugars, however it did not affect the ethanol concentration in the samples.

The highest ethanol synthesis (over 40 $g \cdot dm^{-3}$) and fermentation efficiency (over 80%) was found in samples fermented with strains isolated from final stage of fermentation. Other cultures produced significantly lower amounts of ethanol which did not exceed 39 $g \cdot dm^{-3}$. Ethanol tolerance is one of the most important factors that determined the presence of individual yeast strains during fermentation. Cultures which are less resistant generally occur in the initial stages of the fermentation, whereas ethanol resistant

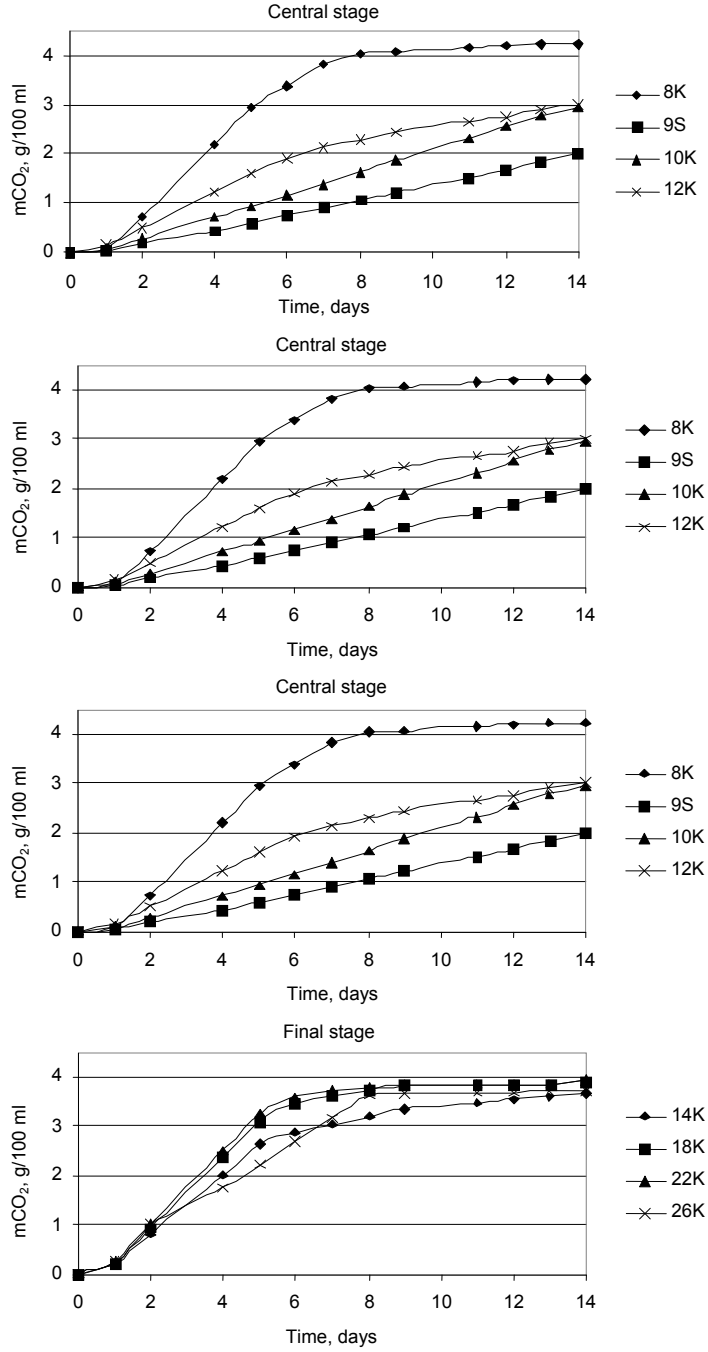

Fig. 1. The fermentation kinetics of model solutions fermented by indigenous *S. cerevisiae* strains isolated from initial, central and final stage of plum mashes spontaneous fermentation

Table 2. The chemical composition of model solutions fermented by indigenous *S. cerevisiae* strain isolated from plum mashes spotaneous fermentation

Strain	Sugars assimilation $g \cdot dm^{-3}$	Biomass $g \cdot dm^{-3}$	Biomass yield $g \cdot g^{-1}$ glucose	Ethanol $g \cdot dm^{-3}$	Ethanol yield $g \cdot g^{-1}$glucose	Fermentation efficiency %	Titratable acidity $g \cdot dm^{-3}$
1S	95.0 (±5.0)	1.86^{a} (±0.07)	0.020	42.7 (±0.8)	0.449	87.9	2.1^{abc} (±0.1)
2S	97.5 (±2.5)	2.17^{a} (±0.08)	0.022	21.9 (±1.2)	0.225	44.0	2.4^{ad} (±0.2)
3S	96.7 (±5.8)	2.66^{ab} (±0.87)	0.028	39.1 (±2.0)	0.404	79.1	1.9^{bc} (±0.1)
4S	90.8 (±2.9)	2.36^{a} (±0.15)	0.026	32.3 (±9.6)	0.356	69.7	2.6^{de} (±0.5)
5S	87.5 (±2.5)	1.99^{a} (±0.24)	0.023	36.8 (±10.9)	0.421	82.3	2.5^{ad} (±0.3)
6S	90.0 (±2.5)	2.45^{a} (±0.55)	0.027	20.8 (±1.1)	0.231	45.2	2.9^{e} (±0.3)
8K	93.3 (±5.2)	2.11^{a} (±0.16)	0.023	24.8 (±7.6)	0.266	51.9	2.1^{abc} (±0.3)
9S	73.8 (±18.9)	3.59^{b} (±1.17)	0.049	31.8 (±7.7)	0.432	84.4	2.2^{ab} (±0.3)
10K	78.3 (±10.4)	2.78^{ab} (±0.19)	0.036	37.8 (±0.8)	0.483	94.5	1.9^{bc} (±0.1)
12K	88.3 (±8.8)	2.10^{a} (±0.37)	0.024	44.5 (±9.8)	0.504	98.5	1.7^{c} (±0.2)
14K	93.3 (±2.9)	2.03^{a} (±0.80)	0.022	40.0 (±1.5)	0.429	83.9	1.9^{bc} (±0.1)
18K	93.3 (±1.4)	1.83^{a} (±0.09)	0.020	43.8 (±2.5)	0.469	91.8	1.9^{bc} (±0.1)
22K	95.0 (±4.3)	2.45^{a} (±0.82)	0.026	48.0 (±0.4)	0.505	98.8	1.8^{bc} (±0.1)
26K	90.0 (±5.0)	1.96^{a} (±0.11)	0.022	43.6 (±7.6)	0.485	94.9	1.8^{bc} (±0.1)
Sig.	ns	**	–	ns	–	–	***

*, **, *** – display the significance at 5, 1 and 0.1% respectively, by least significant difference. Values not sharing the same superscript letter within the horizontal line are different according to the Duncan test ($p < 0.05$).

ns – not significant.

strains predominate during final stages [Satora and Tuszyński 2005]. Ethanol yield of *S. cerevisiae* species ranged from 0.45-0.51 $g \cdot dm^{-3}$ and is strongly connected with fermentation medium composition [Thuesombat et al. 2007]. Lower values of this parameter found during our experiments could be associated with composition of used basal medium. Probably studied strains, especially those isolated from initial stage of fermentation, needed higher concentration of nutrients then this in used medium.

Chemical characteristics of fermented model solutions was presented in Table 3. One-way analysis of variance (ANOVA) showed statistically significant differences in average content of all analysed volatile compounds (significance coefficience $p < 0.05$).

Table 3. Volatile profile of model solutions fermented by indigenous *S. cerevisiae* strain isolated from plum mashes spotaneous fermentation

Strain	Acetaldehyde	Acetoine	Carbonyl compounds	Ethyl acetate	Esters	Methanol	Propanol	Isobutanol	Amyl alcohols	Total fusels	Acetic acid
	mg·dm^{-3}										
1S	34.3^{abc} (±4.8)	25.7^{a} (±0.5)	190.9^{a} (±16.2)	106.0^{abc} (±3.3)	293.3 (±59.3)	23.5^{a} (±0.5)	27.3^{ab} (±1.1)	48.5^{abc} (±7.9)	72.3^{a} (±11.1)	148.1^{ab} (±20.1)	88.0^{a} (±38.6)
2S	82.1^{de} (±22.3)	10.6^{b} (±0.6)	182.2^{a} (±8.4)	45.7^{d} (±6.0)	246.4 (±63.5)	22.3^{a} (±0.2)	27.5^{ab} (±0.6)	22.7^{de} (±2.0)	57.9^{abcd} (±7.3)	108.1^{c} (±10.0)	312.0^{bcde} (±108.0)
3S	81.3^{de} (±31.4)	11.7^{bc} (±0.5)	88.1^{b} (±17.6)	41.9^{d} (±8.5)	152.5 (±31.3)	22.3^{a} (±0.2)	27.0^{abc} (±1.4)	33.3^{ade} (±7.0)	54.3^{abcd} (±4.1)	114.6^{cd} (±12.5)	460.0^{f} (±34.6)
4S	73.5^{def} (±17.7)	10.8^{b} (±0.3)	82.2^{bc} (±20.9)	44.9^{d} (±1.5)	129.1 (±15.7)	22.3^{a} (±0.2)	27.7^{a} (±0.7)	33.1^{ade} (±5.7)	60.4^{abc} (±11.4)	121.2^{acd} (±17.9)	424.0^{bf} (±61.6)
5S	39.5^{abg} (±7.6)	15.0^{cd} (±1.8)	94.0^{b} (±10.6)	85.5^{abd} (±16.9)	88.0 (±13.6)	22.7^{a} (±0.1)	27.8^{a} (±1.0)	36.2^{abde} (±4.7)	66.8^{ab} (±5.0)	130.8^{abcd} (±10.7)	208.0^{acdg} (±55.4)
6S	58.4^{adfg} (±6.9)	13.4^{bcd} (±3.0)	70.5^{bc} (±5.0)	70.8^{ad} (±16.9)	93.9 (±10.5)	22.6^{a} (±0.1)	26.7^{abc} (±2.9)	19.4^{d} (±9.2)	61.3^{abc} (±24.5)	107.4^{c} (±36.6)	192.0^{acg} (±20.8)
8K	53.2^{afg} (±10.4)	12.5^{bcd} (±2.7)	70.5^{bc} (±14.8)	142.1^{c} (±60.3)	149.6 (±44.3)	22.7^{a} (±0.1)	27.5^{ab} (±0.9)	46.4^{abc} (±14.9)	72.4^{a} (±8.9)	146.3^{abd} (±24.7)	308.0^{bcde} (±144.7)
9S	24.0^{bc} (±0.9)	21.1^{e} (±0.0)	58.7^{c} (±14.9)	211.2^{e} (±0.8)	240.5 (±83.2)	0.0^{b} (±0.0)	26.7^{abc} (±0.0)	38.0^{abce} (±0.1)	41.4^{d} (±0.1)	106.1^{c} (±0.2)	120.0^{a} (±36.0)
10K	13.5^{c} (±9.7)	15.1^{d} (±1.3)	29.4^{d} (±3.0)	142.4^{c} (±36.8)	164.3 (±53.8)	18.0^{c} (±7.1)	28.5^{a} (±1.8)	38.2^{abce} (±12.1)	46.2^{cd} (±5.8)	112.9^{c} (±19.6)	172.0^{ag} (±72.3)
12K	62.3^{dfg} (±0.6)	12.6^{bcd} (±0.9)	70.5^{bc} (±15.2)	120.0^{bc} (±51.2)	187.7 (±64.4)	13.2^{a} (±0.3)	27.4^{ab} (±1.2)	46.2^{abc} (±1.6)	56.0^{abcd} (±11.9)	129.6^{abcd} (±14.7)	260.0^{cdeg} (±18.3)
14K	49.5^{e} (±13.0)	9.7^{bcd} (±0.3)	129.2^{b} (±10.2)	61.7^{c} (±13.8)	123.2 (±17.0)	22.6^{a} (±0.3)	25.3^{c} (±1.0)	48.3^{f} (±13.5)	49.5^{abcd} (±4.4)	123.1^{b} (±18.8)	328.0^{bcdeg} (±90.9)
18K	89.6^{abfg} (±4.5)	11.9^{b} (±3.8)	94.0^{e} (±21.9)	134.8^{ad} (±16.8)	140.8 (±17.0)	22.4^{a} (±0.0)	24.7^{bc} (±0.4)	78.7^{abc} (±14.4)	55.1^{bcd} (±3.8)	158.5^{acd} (±18.5)	292.0^{bde} (±79.9)
22K	55.1^{afg} (±9.8)	9.9^{b} (±0.2)	135.1^{e} (±10.2)	45.2^{d} (±9.1)	82.1 (±22.8)	22.6^{a} (±0.2)	24.9^{c} (±0.3)	54.5^{c} (±10.5)	56.5^{abcd} (±1.9)	135.9^{abcd} (±12.7)	368.0^{bef} (±6.9)
26K	51.5^{afg} (±2.3)	11.9^{bcd} (±0.7)	164.5^{a} (±22.9)	46.6^{d} (±3.9)	58.7 (±8.2)	22.2^{a} (±0.1)	25.0^{c} (±0.6)	52.4^{bc} (±8.2)	53.7^{bcd} (±1.9)	131.1^{abcd} (±10.6)	360.0^{bef} (±60.0)
Sig.	***	***	***	***	ns	***	**	***	*	**	***

*, **, *** – display the significance at 5, 1 and 0.1% respectively, by least significant difference. Values not sharing the same superscript letter within the horizontal line are different according to the Duncan test ($p < 0.05$).

ns – not significant.

Analysed strains formed higher amounts of carbonyl compounds and majority of them constituted acetaldehyde (over 90% in case of 3S and 18K strains). The most of these compounds were produced by yeast cultures isolated from the initial and final stages of spontaneous fermentation. It should be mentioned 1S and 2S cultures which synthesized 190.9 and 182.2 $mg \cdot dm^{-3}$ of carbonyl compounds, respectively, that was even several times higher than in other samples. The characteristic feature of strains isolated from middle stage of fermentation was low production of discussed group of compounds that ranged from 29 up to 70 $mg \cdot dm^{-3}$. This tendency can prove the thesis made by Czupryński et al. [2004] that affirmed that the highest amount of aldehydes and ketones are synthesized at the beginning of fermentation.

The concentration of acetaldehyde showed statistically significant differences between studied strains. The highest level of this compound was found in the model solutions fermented by 18 K culture (89.6 $mg \cdot dm^{-3}$) and constituted over 95% of total carbonyl compounds produced by this strain. The same variability of acetaldehyde concentration were determined during analysis of grape musts fermentation by different strains of *S. cerevisiae* [Regodon Mateos et al. 2006], however obtained in our studies results were much lower that might be connected with lesser content of sugars in model solutions.

The level of acetoine that is also a carbonyl compound, was similar in all samples and its average value amounted to 13.4 $mg \cdot dm^{-3}$. Similar results were obtained by Romano et al. [1992] which analysed over 100 different *S. cerevisiae* strains and found that yeast of this species generally produced low amount of acetoine (about 12 $mg \cdot dm^{-3}$), mainly at the early stage of fermentation.

Esters has a significant effect on the organoleptic characteristics of alcoholic beverages, it may contribute a pleasant, fruity fragrance to the general aroma [Satora and Tuszyński 2008, Satora et al. 2008]. The samples fermented using *S. cerevisiae* strains originated from initial stages of spontaneous fermentation (1S and 2S) were distinguished by the highest esters concentration that exceeded 240 $mg \cdot dm^{-3}$. Simultaneously ethyl acetate predominated among esters and constituted generally over 30% of these compounds. Only 2S and 3S cultures isolated at the beginning of fermentation produced less ethyl acetate and more other ester compounds. It could be connected with higher synthesis of other esters like methyl acetate, isoamyl acetate, ethyl butyrate, hexyl acetate, 2-phenylethyl acetate and others which might be formed by *S. cerevisiae* yeasts during fermentation [Rojas et al. 2003]. During spontaneous fermentation of plum mashes were present also strains which produced almost solely ethyl acetate (over 85% of total esters). They occurred throughout fermentation however predominated during central stage of the process. The weakest producers of esters were isolated from final stage of fermentation that might mean that this group of compounds is formed mainly at the beginning of fermentation.

Acetic acid dominates among the organic acids detected in alcoholic beverages (75-85%). The amount of acetic acid produced is associated with sugar and nitrogen compound concentration, pH value, used yeast strain and temperature during fermentation. The content of this compound in wines increases with sugar concentrations above about 20% (w/v), below pH 3.2 and at pH values more neutral than pH 4 [Satora et al. 2008]. The level of acetic acid in analysed samples showed statistically significant differences. The largest amounts of this compound were produced by the cultures isolated from an initial and final stage of fermentation. Relatively high concentration of acetic acid were found in solutions fermented by 3S (460 $mg \cdot dm^{-3}$) and 4S (424 $mg \cdot dm^{-3}$)

strains. It was shown that samples which contained more ethyl acetale were characterised by lower amount of acetic acid. It should be supposed that it was connected with an activity of yeast esterases which used up volatile acids for appropriate esters production [Antonelli et al. 1999].

Higher alcohols dominate the group of volatile compounds in alcoholic beverages and have a significant effect on their sensory characteristics and quality. Among these compounds, isobutanol and amyl alcohols prevailed in studied samples. The 18K, 22K and 26K strains isolated at final stage of fermentation, produced relatively high amounts of these compounds, 78.7, 54.5, 52.4 $mg \cdot dm^{-3}$ of isobutanol and 55.1, 56.5, 53.7 $mg \cdot dm^{-3}$ of amyl alcohols, respectively. According to Romano et al. [2003] isobutanol and amyl alcohols are the volatile compounds which could be used for diversification of individual strains of *S. cerevisiae*. In this matter, isobutanol and amyl alcohols ratio is also very important. In case of strains originated from central and final stage of fermentation, it is nearly or higher than 1. Cultures isolated in the initial stage of fermentation synthesized over two times more of amyl alcohols then isobutanol. Other important fusel alcohol – propanol was detected in analysed samples on the similar level (25.0-27.7 $mg \cdot dm^{-3}$). Isobutanol and amyl alcohols are synthesized mainly from corresponding amino acids (valine, leucine and isoleucine), other amino acids and sugars, while propanol is produced only from threonine and sugars [Vidrih and Hribar 1999].

Principal Component Analysis (PCA) showed three groups of isolated *S. cerevisiae* strains (Fig. 2) that correspond to the stages of isolation during fermentation. Cultures from an initial stage were characterized by higher production of propanol, acetaldehyde and acetic acid, and lower of isobutanol, ethanol and ethyl acetate, those originated from central stage were distinguished by increased synthesis of ethyl acetate and acetoine, whereas the strains isolated during final stage of fermentation formed more acetaldehyde, acetic acid and fusel alcohols and less esters and propanol. 1S and 5S strains that were present throughout the spontaneous fermentation were placed outside the groups mentioned above.

CONCLUSIONS

1. Tested indigenous *Saccharomyces cerevisiae* strains are characterised by strong polymorphism.
2. Analysed strains exhibit diversified fermentation kinetics as well as produce different amounts of studied volatile compounds. All cultures synthesize similar level of acetoine and propanol.
3. Cultures from an initial stage are distinguished by higher production of acetaldehyde and acetic acid, and lower of isobutanol, ethanol and ethyl acetate. Those originated from central stage show increased synthesis of ethyl acetate and acetoine, whereas the strains isolated during final stage of fermentation form more acetaldehyde, acetic acid and fusel alcohols and less esters and propanol. Strains that were present throughout the spontaneous fermentation synthesize average amounts of compounds mentioned above.

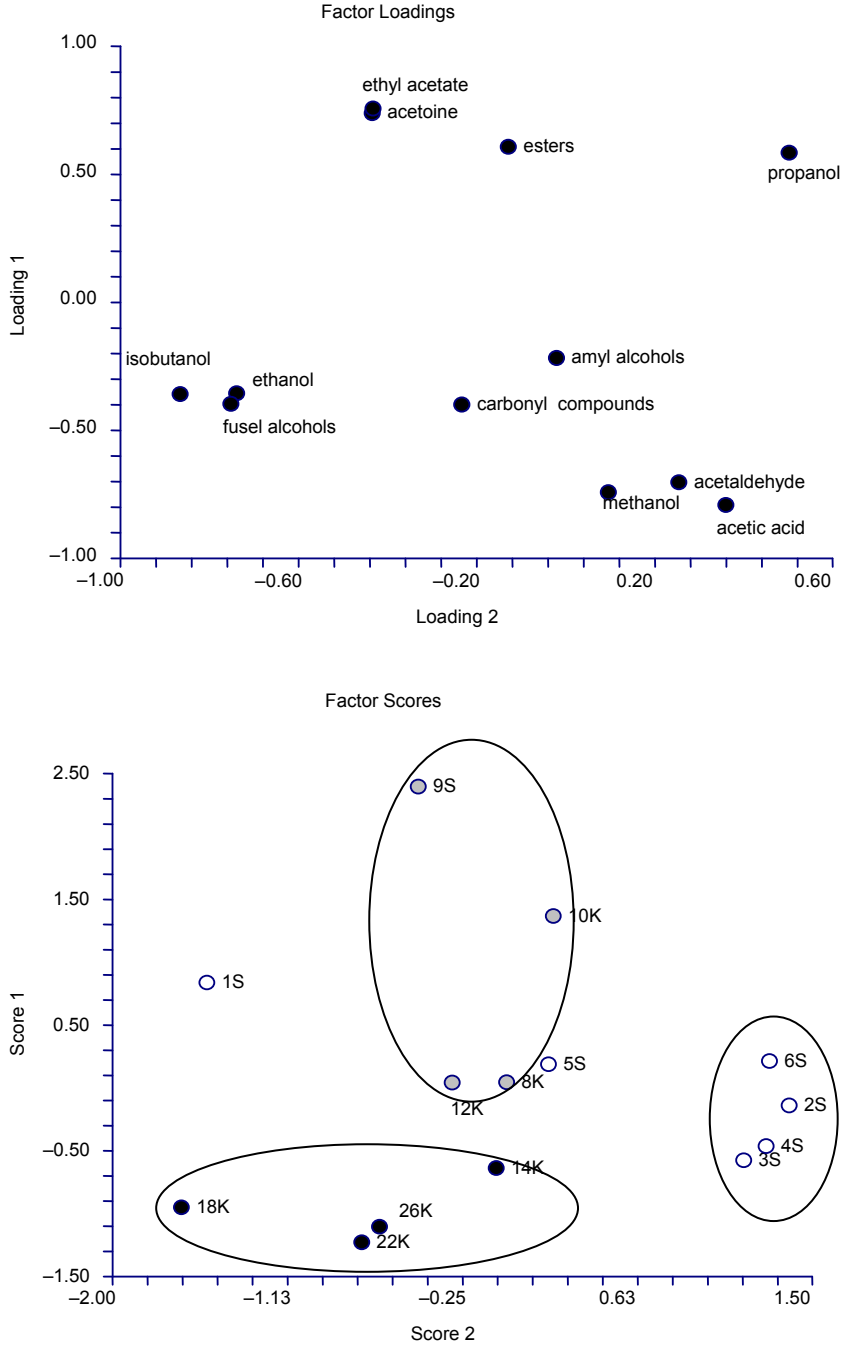

Fig. 2. Two dimensional plots of fermented model solutions composition dependent on 12 variables (analysed volatile compounds)

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PROFIL ENOLOGICZNY DROŻDŻY *SACCHAROMYCES CEREVISIAE* WYIZOLOWANYCH Z FERMENTUJĄCEJ MIAZGI ŚLIWKOWEJ

Wstęp. Śliwowica Łącka jest spirytusem śliwkowym (śliwowicą) wytwarzanym na drodze fermentacji spontanicznej śliwek Węgierek w podgórskim rejonie Polski. Celem badań było określenie profilu enologicznego dzikich szczepów *S. cerevisiae* wyizolowanych z fermentującej spontanicznie miazgi śliwkowej.
Materiał i metodyka. Do analiz wytypowano czternaście szczepów uzyskanych z trzech kolejnych etapów fermentacji (początkowej, środkowej i końcowej), charakteryzujących się różnym profilem killerowym. Próby fermentacyjne przeprowadzono na podłożu syntetycznym zawierającym 10% glukozy. Badano kinetykę procesu fermentacji, podstawowe parametry enologiczne metodami OIV oraz stężenie wybranych związków lotnych z użyciem GC-SPME.
Wyniki. Analizowane szczepy wykazywały zróżnicowaną kinetykę fermentacji, jak również produkowały różne ilości badanych związków lotnych. Najwyższą syntezę etanolu (ponad 40 $g \cdot dm^{-3}$) oraz wydajność fermentacji (ponad 80%) stwierdzono w próbach fermentowanych z udziałem szczepów wyizolowanych w końcowej fazie fermentacji spontanicznej. Kultury pochodzące z początkowej fazy odznaczały się produkcją większych ilości aldehydu i kwasu octowego oraz mniejszych izobutanolu, etanolu i octanu etylu. Szczepy wyizolowane ze środkowego etapu fermentacji wykazywały wzmożoną syntezę octanu etylu i acetoiny, natomiast drożdże uzyskane z finalnej fazy fermentacji spontanicznej tworzyły więcej aldehydu i kwasu octowego oraz alkoholi fuzlowych, a mniej estrów. Szczepy obecne w czasie całego przebiegu fermentacji spontanicznej miazgi śliwkowej syntezowały przeciętne ilości wspomnianych związków.
Wnioski. Wśród badanych szczepów *S. cerevisiae* stwierdzono duże zróżnicowanie profili enologicznych. Ich obecność, a także ilość w czasie fermentacji spontanicznej miazgi śliwkowej bezpośrednio wpływa na skład chemiczny otrzymywanej Śliwowicy Łąckiej.

Słowa kluczowe: dzikie szczepy *Saccharomyces cerevisiae*, fermentacja spontaniczna, profil enologiczny, śliwowica

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