Influence of different nutrition conditions on main volatiles of wine yeasts

KATARÍNA FURDÍKOVÁ – JOZEF ŠEVCECH – KATARÍNA ĎURČANSKÁ – HELENA HRONSKÁ – FEDOR MALÍK

Summary
Volatile acids, higher alcohols and esters produced during fermentation are the main components of secondary aroma of wine. Their production relates to predisposition of the yeast strain, physical conditions of fermentation and chemical composition of the fermented medium including presence of necessary vitamins. In particular autochthonous yeasts influence the volatile profile by their own way. We selected 12 autochthonous yeasts from our collection of yeasts, and analysed their impact on main volatile organic compounds profile of defined fermentation media. Each *Saccharomyces cerevisiae* strain had its own demands on nutrition and its metabolism directly or indirectly affected the chemical composition of wine. Even if the concentration of assimilable nitrogen was sufficient, lack of vitamins led to reduction of the production of fusel alcohols (2-butanol, 2,3-butanediol and isoamyl alcohol). In contrary, production of isobutanol did not require external source of vitamins.

Keywords
yeast; wine; *Saccharomyces cerevisiae*; secondary aroma

Majority of substances providing nutrition for the yeasts during fermentation originates from grapes and their profile depends on soil composition, fertilizing and vine treating, climate conditions in vintage, stage of ripeness of grape berries and processing technology. In most cases, grape must provides sufficient saccharides, utilizable nitrogen, inorganic compounds and growth factors to perform the fermentation without problems. Musts with eventual deficiency of important nutritive substances can be improved by adding saccharose or condensed grape must (chaptalization), or supplements containing inorganic ammonium salts and vitamins (thiamine). In order to complete the alcoholic fermentation properly to the end, yeasts *Saccharomyces cerevisiae* need the concentration of assimilable nitrogen of at least 140 $mg \cdot l^{-1}$ [1]. Demand of nitrogen increases with an increasing concentration of saccharides in must [2].

The proper function of whole enzymatic apparatus and yeast cell development require cooperation of growth factors. Different yeast species and strains have variable needs of vitamins [3]. Noble yeasts *S. cerevisiae* are able to synthesize many vitamins, however, the critical growth factors are thiamine (vitamin B_1) and biotin (vitamin H) [4].

Thiamine pyrophosphate is a coenzyme of pyruvate decarboxylase (EC 4.1.1.1), which catalyses decarboxylation of pyruvate to acetaldehyde [5]. Even if *S. cerevisiae* can synthesize small amounts of thiamine [6], the absence of vitamin B_1 in must causes termination of fermentation, growth of yeasts and also dramatic structural changes of sensory profile of wine. Lack of thiamine in grapes is induced by filamentous fungi attacking the grape (insufficiency of vitamin B_1 is typical for grapes attacked by *Botrytis cinerea)*, or by wild apiculates and non-saccharomyces yeasts during spontaneous or insufficiently regulated fermentation. Species *Kloeckera apiculata, Candida stellata* or *Metschnikowia pulcherrima* are also dependent on thiamine and during first stages of spontaneous fermentation are predominant [7]. Uncontrolled growth of wild yeasts in must during first hours of grape must fermentation can cause depletion of vitamins and may result in problems with subse-

Katarína Furdíková, Jozef Ševcech, Katarína Ďurčanská, Helena Hronská, Fedor Malík, Department of Biochemical Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia.

Correspondence author:
Katarína Furdíková, katarina.furdikova@stuba.sk

Tab. 1. Sensory properties of the main wine volatiles.

	Regular concentration in wine [mg·l^{-1}]	*S. cerevisiae* production [mg·l^{-1}]	Sensory threshold [mg·l^{-1}]		Description of smell	
Acetic acid	> 150	150–61 400	600		Vinegar	[14]
Acetaldehyde	30–80	5–6405	1–62*	[9]	Nut/Over-ripe apple	[15]
Ethyl acetate	5–60	5–687	12–630	[10, 11]	Pineapple/Nail polish	[14, 16]
2-Butanol	20–660	9–670	50	[10]	Sweet apricot	[16]
Isobutanol	9–6174	15–6237	75–6100	[10, 12]	Whiskey fusel	[16]
Isoamyl alcohol	6–6490	6–6385	50–660	[10, 12]	Pear/Pungent	[14, 16]
2,3-Butanediol	80–6170	6–6186	600	[13]	Fruity, creamy, buttery	[14]

* – free acetaldehyde.

quent *S. cerevisiae* growth and metabolism.

Three types of differently intensive aromas determine the resulting smell of wine. Primary aroma, which is a typical aroma of the vine variety, derives from grapes and includes terpenoids and, predominantly, noble volatile sulphur compounds. Secondary aroma originates in the activity of yeasts and bacteria during alcoholic and malolactic fermentation. Tertiary aroma arises during maturation of wine in oak barrels or bottles.

Secondary aroma of wine consists of many different substances. Most of these aroma compounds belong to volatile acids, higher alcohols, esters and aldehydes. Their concentration in wine is directly influenced by the yeast strain as well as by the nutrition and fermentation temperature. Each *S. cerevisiae* strain has its own enzymatic equipment, different demand of nutritive compounds and different ability to produce miscellaneous minor substances, which can directly or indirectly influence sensory character of wine [8]. Aromatic characteristics (description of smell, sensory threshold) of various volatile compounds are different and their concentration in wine varies (Tab. 1).

MATERIALS AND METHODS

Fermentation media

Generally, two types of fermentation media have been used – rich in nutrition yeast extract-dextrose (YD) medium composed of 210 g·l^{-1} glucose and 10 g·l^{-1} yeast extract (Merck, Darmstadt, Germany), and medium without a source of vitamins prepared according to the recipe of Yeast Nitrogen Base medium without vitamins, amino acids and cofactors (Difco Laboratories, Detroit, Michigan, USA) – ($(NH_4)_2SO_4$ 5 g·l^{-1}, KH_2PO_4 1 g·l^{-1}, $MgSO_4.7H_2O$ 0.5 g·l^{-1}, NaCl 0.1 g·l^{-1}, $CaCl_2$ 0.1 g·l^{-1}, glucose 210 g·l^{-1}, distilled water).

Inoculation and conditions of fermentation

Experiment was carried out in 400 ml of media at a temperature of 21 °C. All fermentations started with a concentration of the yeast biomass of 10^6 cells per liter. Inocula were prepared from a yeast strain culture grown aerobically for 24 h in a liquid medium (20 g·l^{-1} glucose, 10 g·l^{-1} yeast extract; 100 ml) in a 500 ml flask, on an orbital shaker at 2 Hz, 28 °C. After cultivation, concentration of the yeast biomass was determined by counting in a Bürker chamber. The calculated volume of biomass was removed and centrifuged (10 min, 1370 ×*g*). Separated biomass was washed with distilled water, centrifuged again and finally added to the fermentation media.

In an experiment, 12 autochthonous *S. cerevisiae* strains were used: four strains *S. cerevisiae* var. *bayanus* (FM-PS1A, FM-PS1B, RB-NA1, MT-PF1B), four strains *S. cerevisiae* var. *cerevisiae* (FM-VVR, RR-KP1, RR-KP3, RR-KP4, D-KP1) and four strains *S. cerevisiae* var. *capensis* (RB-NA2, FM-PF1, FM-PF2, RB-NA3). All strains were previously isolated from natural sources (vine, grapes), identified, characterized and stored in a collection of yeasts in oenological laboratory of Faculty of Chemical and Food Technology (Slovak University of Technology, Bratislava, Slovakia).

S. cerevisiae var. *cerevisiae*, *S. cerevisiae* var. *bayanus* and *S. cerevisiae* var. *capensis* are taxonomically similar being described within one species of *S. cerevisiae* [17]. Differences between these physiological races are predominantly in capability to ferment of different saccharides (*S. cerevisiae* var. *cerevisiae*: galactose, glucose, maltose, raffinose; *S. cerevisiae* var. *bayanus*: glucose, maltose, raffinose; *S. cerevisiae* var. *capensis*: glucose, raffinose) [3]. *S. cerevisiae* var. *capensis* together with *S. cerevisiae* var. *cerevisiae*, *S. cerevisiae* var. *bayanus* and *S. cerevisiae* var. *chevalieri* occur in biofilms formed on the surface of sherry wine during

biological aging and belong to wine flor yeasts [18, 19]. They are components of the natural microflora of vine and spontaneously fermented grape musts, and can be used as pure cultures in various winemaking technologies [20].

Analytical methods

After fermentation, samples of fermented media were analysed. Basic technological parameters as well as basic volatile profiles were measured. Concentrations of reducing saccharides were analysed by methods of International Organization of Vine and Wine (OIV) [21]. Profiles of volatiles were determined by gas chromatography (GC) after simple distillation of samples. A volume of 20 ml of distilled water was added to 50 ml of the fermented medium and the mixture was distilled. After distillation of 45 ml, volume of the distillate was replenished with distilled water to 50 ml in a volumetric flask. Subsequently, samples were analysed using the gas chromatograph 6890N (Agilent Technologies, Santa Clara, California, USA) equipped with autosampler and flame ionization detector (FID). The column was DB FFAP (60 m × 250 μm × 0.25 μm; Agilent Technologies) and the mobile phase was H_2 at a flow rate of 3.2 ml·min^{-1}. Injection was done using a split injector (1:50) heated to 250 °C. Temperature of FID was 250 °C. Solutions of standards in water were used for calibration and quantification purposes. Following standards were used: ethanol (Sigma-Aldrich, Steinheim, Germany, ≥ 99.8%), acetic acid (Sigma-Aldrich, ≥ 99.7%), acetaldehyde (Merck, ≥ 99.5%), ethyl acetate (Sigma-Aldrich, ≥ 99.8%), 2-butanol (Sigma-Aldrich, 99.5%), isobutanol (Sigma-Aldrich, ≥ 99.0%), isoamyl alcohol (Sigma-Aldrich, ≥ 98.0%) and 2,3-butanediol (Sigma-Aldrich, ≥ 98.0%). 2-Hexanol (Sigma-Aldrich, 99%) was used as the internal standard. Calibration solutions underwent the same distillation procedure as samples of fermented media.

Yield coefficient

Yield coefficient of each determined product of fermentation was calculated based on the following formulae:

$$Y_P = \frac{dm_P}{dt} \Big/ \frac{dm_S}{dt} \quad (1)$$

where Y_P is yield of product, dm_P/dt is derivation of the product weight in time, and dm_S/dt is derivation of the substrate weight in time.

$$Y_P = \frac{c_P V}{c_{S0} V_0 - c_S V} \quad (2)$$

where c_P and V are concentration and volume of the product, respectively, V and V_0 are volumes of substrate in the beginning and in the end of fermentation, respectively, and c_{S0} and c_S are concentrations of substrate in the beginning and in the end of fermentation, respectively.

Time of the determination of substrate (S, glucose) and products (P) was the same but the volume of the media decreased during fermentation as a result of leakage of carbon dioxide.

Statistical analysis

All results are described as mean values of samples measured in triplicate. Standard deviations were calculated using MS Excel from the software package MS Office 2007 (Microsoft, Redmond, Washington, USA).

RESULTS AND DISCUSSION

Measurement of main analytical parameters and volatile substances produced the following results. Tab. 2 presents profiles of secondary aromas produced by 12 *S. cerevisiae* strains during fermentation of media with an ideal concentration of nutritive components (glucose, amino nitrogen and growth factors) and in media without the source of vitamins (–VIT). Tab. 3 presents yield coefficients of volatiles to exactly compare the metabolism of *S. cerevisiae* at different conditions of fermentation.

Ethanol fermentation

Production of ethanol in *S. cerevisiae* yeasts depends on the presence of thiamine in media. Insufficiency of vitamins causes a decrease in ethanol production and a decrease in yield coefficient in all tested *S. cerevisiae* strains. Concentration of ethanol in media fermented by different yeast strains was very individual. The most considerable diminution of ethanol production was determined in *S. cerevisiae* var. *bayanus* MT-PF1B (95.7%). Average decrease of ethanol during fermentation of media without vitamins, compared to YD media, was in *S. cerevisiae* var. *bayanus* 77.7%, in *S. cerevisiae* var. *cerevisiae* 36.7%, and in *S. cerevisiae* var. *capensis* 85.7%. Among the tested yeasts, two strains were able to produce relevant concentrations of ethanol in the absence of vitamins. Strains *S. cerevisiae* var. *cerevisiae* RR-KP1 and RR-KP4 produced only 23% less ethanol in the absence of vitamins, compared to YD medium. Low need of vitamins for the proper ethanol fermentation decreases the costs of nutrition both in wine-making and also in the production of fuel ethanol. Thus, the demand for vitamins is not only

Tab. 2. Concentration of volatiles in media with or without vitamins after fermentation with different *S. cerevisiae* varieties and strains.

S. cerevisiae			Glucose [g·l^{-1}]	Ethanol [g·l^{-1}]	Acetic acid [g·l^{-1}]	Acetaldehyde [mg·l^{-1}]	Ethyl acetate [mg·l^{-1}]	2-Butanol [mg·l^{-1}]	Isobutanol [mg·l^{-1}]	Isoamyl alcohol [mg·l^{-1}]	2,3-Butanediol [mg·l^{-1}]
var. *bayanus*	FM-PS1A	YD	0.31 ± 0.01	98.80 ± 4.77	0.81 ± 0.04	73.2 ± 3.5	14.9 ± 0.6	40.2 ± 1.8	22.6 ± 1.0	72.9 ± 3.3	97.4 ± 4.4
		–VIT	131.00 ± 4.82	30.03 ± 1.45	0.19 ± 0.01	139.8 ± 6.6	0.0 ± 0.0	11.9 ± 0.5	27.7 ± 1.3	14.0 ± 0.6	15.7 ± 0.7
	FM-PS1B	YD	0.31 ± 0.01	98.50 ± 4.76	0.81 ± 0.04	36.9 ± 1.7	19.2 ± 0.8	36.5 ± 1.6	29.3 ± 1.3	96.5 ± 4.4	29.0 ± 1.3
		–VIT	123.21 ± 4.53	37.62 ± 1.74	0.47 ± 0.02	29.7 ± 1.4	5.0 ± 0.2	16.1 ± 0.7	50.7 ± 2.3	24.1 ± 1.1	24.4 ± 1.1
	MT-PF1B	YD	0.39 ± 0.01	100.90 ± 4.87	0.66 ± 0.03	25.5 ± 1.2	11.7 ± 0.5	38.5 ± 1.7	44.3 ± 2.0	78.1 ± 3.5	61.7 ± 2.8
		–VIT	133.53 ± 4.91	4.31 ± 0.18	0.28 ± 0.01	167.2 ± 7.9	0.0 ± 0.0	9.1 ± 0.4	31.6 ± 1.4	10.0 ± 0.5	6.4 ± 0.3
	RB-NA1	YD	0.14 ± 0.01	98.11 ± 4.74	0.82 ± 0.04	356.3 ± 16.9	17.6 ± 0.7	42.1 ± 1.9	20.2 ± 0.9	64.9 ± 2.9	71.1 ± 3.2
		–VIT	128.18 ± 4.72	15.84 ± 0.77	0.30 ± 0.01	251.7 ± 11.9	0.0 ± 0.0	11.8 ± 0.5	37.9 ± 1.7	12.5 ± 0.6	17.2 ± 0.8
var. *cerevisiae*	RR-KP1	YD	0.32 ± 0.01	103.78 ± 5.01	0.52 ± 0.02	25.5 ± 1.2	0.0 ± 0.0	26.7 ± 1.2	57.8 ± 2.6	89.6 ± 4.0	124.6 ± 5.6
		–VIT	10.00 ± 0.37	79.81 ± 3.85	0.92 ± 0.04	136.8 ± 6.5	17.4 ± 0.7	24.7 ± 1.1	237.1 ± 10.7	90.5 ± 4.1	42.5 ± 1.9
	RR-KP3	YD	0.11 ± 0.00	106.64 ± 5.15	0.51 ± 0.02	48.1 ± 2.3	21.7 ± 0.9	30.6 ± 1.4	51.9 ± 2.3	103.4 ± 4.7	96.4 ± 4.4
		–VIT	39.24 ± 1.44	52.82 ± 2.55	0.60 ± 0.03	128.5 ± 6.1	22.4 ± 0.9	20.3 ± 0.9	233.7 ± 10.6	59.3 ± 2.7	19.0 ± 0.9
	RR-KP4	YD	0.53 ± 0.02	95.34 ± 4.48	0.43 ± 0.02	23.8 ± 1.1	23.5 ± 0.9	33.2 ± 1.5	69.7 ± 3.2	146.6 ± 6.6	46.2 ± 2.1
		–VIT	15.92 ± 0.59	73.56 ± 3.55	0.94 ± 0.04	158.9 ± 7.5	31.6 ± 1.2	21.7 ± 1.0	249.8 ± 11.3	80.4 ± 3.6	36.4 ± 1.6
	D-KP1	YD	0.82 ± 0.03	100.32 ± 4.85	0.42 ± 0.02	96.2 ± 4.6	22.7 ± 0.9	42.8 ± 1.9	91.2 ± 4.1	160.2 ± 7.2	63.0 ± 2.8
		–VIT	68.81 ± 2.53	49.83 ± 2.41	0.72 ± 0.03	282.5 ± 13.4	22.5 ± 0.9	18.7 ± 0.8	279.5 ± 12.6	59.3 ± 2.7	17.2 ± 0.8
var. *capensis*	FM-PF1	YD	0.22 ± 0.01	97.40 ± 4.70	0.95 ± 0.04	92.7 ± 4.4	9.8 ± 0.4	44.6 ± 2.0	50.0 ± 2.3	120.6 ± 5.5	113.3 ± 5.1
		–VIT	162.31 ± 5.97	15.52 ± 0.66	0.24 ± 0.01	22.9 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	26.5 ± 1.2	11.6 ± 0.5	4.7 ± 0.2
	FM-PF2	YD	0.41 ± 0.02	96.70 ± 4.67	0.81 ± 0.04	24.7 ± 1.2	20.1 ± 0.8	45.1 ± 2.0	22.7 ± 1.0	74.2 ± 3.4	81.0 ± 3.7
		–VIT	133.52 ± 4.91	11.71 ± 0.48	0.38 ± 0.02	175.2 ± 8.3	0.0 ± 0.0	11.8 ± 0.5	54.6 ± 2.5	15.3 ± 0.7	29.9 ± 1.4
	RB-NA2	YD	0.16 ± 0.01	94.09 ± 4.54	0.91 ± 0.04	394.1 ± 18.6	11.3 ± 0.4	42.2 ± 1.9	50.0 ± 2.3	79.2 ± 3.6	78.1 ± 3.5
		–VIT	121.21 ± 4.46	17.17 ± 0.83	0.32 ± 0.01	232.9 ± 11.0	0.0 ± 0.0	9.8 ± 0.4	40.7 ± 1.8	9.2 ± 0.4	8.9 ± 0.4
	RB-NA3	YD	0.13 ± 0.00	101.89 ± 4.11	1.11 ± 0.05	55.9 ± 2.6	19.0 ± 0.7	43.6 ± 2.0	31.1 ± 1.4	68.2 ± 3.1	140.6 ± 6.4
		–VIT	132.12 ± 4.86	11.09 ± 0.54	0.41 ± 0.02	170.6 ± 8.1	0.0 ± 0.0	8.8 ± 0.4	32.5 ± 1.5	7.9 ± 0.4	0.0 ± 0.0

Concentration is expressed as mean ± standard deviation. YD – medium with yeast extract, –VIT – medium without a source of vitamins.

Tab. 3. Yield coefficients of volatiles in media with or without vitamins produced by different *S. cerevisiae* varieties and strains.

S. cerevisiae			Ethanol [g·kg^{-1}]	Acetic acid [g·kg^{-1}]	Acetaldehyde [g·kg^{-1}]	Ethyl acetate [g·kg^{-1}]	2-Butanol [g·kg^{-1}]	Isobutanol [g·kg^{-1}]	Isoamyl alcohol [g·kg^{-1}]	2,3-Butanediol [g·kg^{-1}]
var. *bayanus*	FM-PS1A	YD	466 ± 23	3.824 ± 0.185	0.346 ± 0.017	0.070 ± 0.003	0.190 ± 0.009	0.107 ± 0.005	0.344 ± 0.017	0.460 ± 0.022
		–VIT	377 ± 18	2.386 ± 0.115	1.756 ± 0.085	0.000 ± 0.000	0.149 ± 0.007	0.348 ± 0.017	0.176 ± 0.009	0.197 ± 0.010
	FM-PS1B	YD	465 ± 22	3.824 ± 0.185	0.174 ± 0.008	0.091 ± 0.004	0.172 ± 0.008	0.138 ± 0.007	0.456 ± 0.022	0.137 ± 0.007
		–VIT	429 ± 21	5.363 ± 0.259	0.339 ± 0.016	0.057 ± 0.003	0.184 ± 0.009	0.578 ± 0.028	0.275± 0.013	0.278 ± 0.013
	MT-PF1B	YD	477 ± 23	3.117 ± 0.151	0.120 ± 0.006	0.083 ± 0.004	0.199 ± 0.010	0.095 ± 0.005	0.307 ± 0.015	0.336 ± 0.016
		–VIT	55 ± 3	3.610 ± 0.174	2.156 ± 0.104	0.000 ± 0.000	0.152 ± 0.007	0.489 ± 0.024	0.161 ± 0.008	0.222 ± 0.011
	RB-NA1	YD	463 ± 22	3.868 ± 0.187	1.681 ± 0.081	0.055 ± 0.003	0.182 ± 0.009	0.209 ± 0.010	0.368 ± 0.018	0.291 ± 0.014
		–VIT	184 ± 9	3.477 ± 0.168	2.917 ± 0.141	0.000 ± 0.000	0.105 ± 0.005	0.366 ± 0.018	0.116 ± 0.006	0.075 ± 0.004
var. *cerevisiae*	RR-KP1	YD	490 ± 24	2.455 ± 0.119	0.120 ± 0.006	0.000 ± 0.000	0.126 ± 0.006	0.273 ± 0.013	0.423 ± 0.020	0.588 ± 0.028
		–VIT	396 ± 19	4.462 ± 0.216	0.678 ± 0.033	0.086 ± 0.004	0.122 ± 0.006	1.176 ± 0.057	0.449 ± 0.022	0.211 ± 0.010
	RR-KP3	YD	503 ± 24	2.406 ± 0.116	0.227 ± 0.011	0.102 ± 0.005	0.144 ± 0.007	0.245 ± 0.012	0.488 ± 0.024	0.455 ± 0.022
		–VIT	307 ± 15	3.491 ± 0.169	0.748 ± 0.036	0.130 ± 0.006	0.118 ± 0.006	1.360 ± 0.066	0.345 ± 0.017	0.111 ± 0.005
	RR-KP4	YD	451 ± 22	2.032 ± 0.098	0.113 ± 0.005	0.111 ± 0.005	0.157 ± 0.008	0.330 ± 0.016	0.693 ± 0.033	0.219 ± 0.011
		–VIT	376 ± 18	4.602 ± 0.222	0.812 ± 0.039	0.162 ± 0.008	0.111 ± 0.005	1.277 ± 0.062	0.411 ± 0.020	0.186 ± 0.009
	D-KP1	YD	475 ± 23	1.988 ± 0.096	0.456 ± 0.022	0.107 ± 0.005	0.203 ± 0.010	0.432 ± 0.021	0.758 ± 0.037	0.298 ± 0.014
		–VIT	350 ± 17	4.921 ± 0.238	1.986 ± 0.096	0.158 ± 0.008	0.131 ± 0.006	1.965 ± 0.095	0.417 ± 0.020	0.121 ± 0.006
var. *capensis*	FM-PF1	YD	460 ± 22	4.483 ± 0.217	0.437 ± 0.021	0.046 ± 0.002	0.210 ± 0.010	0.236 ± 0.011	0.569 ± 0.027	0.535 ± 0.026
		–VIT	305 ± 15	4.715 ± 0.228	0.450 ± 0.022	0.000 ± 0.000	0.000 ± 0.000	0.521 ± 0.025	0.228 ± 0.011	0.092 ± 0.004
	FM-PF2	YD	457 ± 22	3.826 ± 0.185	0.117 ± 0.006	0.095 ± 0.005	0.213 ± 0.010	0.107 ± 0.005	0.350 ± 0.017	0.383 ± 0.018
		–VIT	147 ± 7	4.768 ± 0.230	2.198 ± 0.106	0.000 ± 0.000	0.148 ± 0.007	0.685 ± 0.033	0.192 ± 0.009	0.375 ± 0.018
	RB-NA2	YD	444 ± 21	4.293 ± 0.207	1.859 ± 0.090	0.054 ± 0.003	0.199 ± 0.010	0.236 ± 0.011	0.374 ± 0.018	0.368 ± 0.018
		–VIT	187 ± 9	3.261 ± 0.158	2.532 ± 0.122	0.000 ± 0.000	0.107 ± 0.005	0.443 ± 0.021	0.101 ± 0.005	0.097 ± 0.005
	RB-NA3	YD	481 ± 23	5.235 ± 0.253	0.264 ± 0.013	0.090 ± 0.004	0.206 ± 0.010	0.147 ± 0.007	0.322 ± 0.016	0.663 ± 0.032
		–VIT	139 ± 7	4.999 ± 0.241	2.132 ± 0.103	0.000 ± 0.000	0.111 ± 0.005	0.406 ± 0.020	0.098 ± 0.005	0.000 ± 0.000

Concentration is expressed as mean ± standard deviation. YD – medium with yeast extract, –VIT – medium without a source of vitamins.

the diagnostic sign of the species [4, 16] but also an important technological property of a *S. cerevisiae* culture.

Acetic acid

Usually, concentration of volatile acids in wine ranges between 200 mg·l^{-1} and 1000 mg·l^{-1} (10–15% of total acids). Acetic acid originates dominantly from glycolysis and represents 90% of volatile acids in wine [22–24]. Minor volatile acids (propionic acid, butyric acid, capronic acid, caprylic acid and caprinic acid) are products of the metabolism of fatty acids by yeasts and bacteria. Presence of lower fatty acids in wine contributes to negative aromas and to inhibition of yeast growth [25]. Their production depends on must composition and fermentation conditions [26].

Production of acetic acid by different *S. cerevisiae* strains in rich in nutrition media YD was variable and depended on *S. cerevisiae* variety and strain. Four tested *S. cerevisiae* var. *cerevisiae* strains produced only about a half of the concentration of acetic acid compared to *S. cerevisiae* var. *bayanus* or *S. cerevisiae* var. *capensis* strains. These results were opposite to ANTONELLI [27] who detected concentration of acetic acid in a range of 0.11–0.22 mg·l^{-1} for *S. cerevisiae* var. *bayanus* and 0.21–0.72 mg·l^{-1} for *S. cerevisiae* var. *cerevisiae*. Production of acetic acid in media without a source of vitamins depended on *S. cerevisiae* variety and strain (Tab. 2, Tab. 3). All tested *S. cerevisiae* var. *cerevisiae* strains, in media without growth factors, rapidly produced acetic acid (from 17.6% to 109.3%), but all *S. cerevisiae* var. *bayanus* and *S. cerevisiae* var. *capensis* strains lowered its concentration. Despite this, the yield factors of acetic acid for most yeast strains were increased by fermentation without vitamins. This meant that a lack of growth factors changed the metabolism of *S. cerevisiae* and acetic acid was overproduced.

Acetaldehyde

Acetaldehyde represents 90% of all wine aldehydes and plays an important role in creation of wine aroma. It is formed after glycolysis by decarboxylation of pyruvate and belongs to main products of alcoholic fermentation [28]. Concentration of acetaldehyde is different in distinct types of wine. Generally, wines produced by reductive technology contain approximately 30–80 mg·l^{-1}, sherry wines even 300 mg·l^{-1} of acetaldehyde [29]. At low concentrations, acetaldehyde has decent fruity and nut flavour, excessive concentrations evoke over-ripe apples or walnut peels.

As Tab. 2 shows, different *S. cerevisiae* varieties and strains produced diverse amounts of acetaldehyde under the same fermentation conditions, which corresponds to ANTONELLI [27]. Acetaldehyde is typical for wines made by oxidative technology and for oxidized ones, as a result of oxygen access or bacterial contamination [30]. However, acetaldehyde at moderate concentration may be produced by *S. cerevisiae*. For this reason, acetaldehyde production in winemaking should be controlled by the use of selected yeast strains use.

The absence of vitamins caused that most of the yeast strains produced dramatically higher amounts of acetaldehyde, increase in acetaldehyde concentration represented from 90% to 600%. Whereas some strains showed a decrease in acetaldehyde concentration, the increment of its yield factor was observed by every tested yeast strain. It was evident that missing vitamins during fermentation of saccharides shifted the *S. cerevisiae* metabolism towards an increased production of acetaldehyde, giving the wine more oxidized tones.

Ethyl acetate

Esters are considered to be the most important compounds of secondary aroma of wine, but the general opinion that a higher production of esters positively amplifies the aroma of wine is not correct. Optimal concentrations of esters support the fruitiness of wine but their excess can totally destroy the sensory profile of wine. The major esters produced during alcoholic fermentation by yeasts are ethyl acetate and isoamyl acetate [9]. In low concentration, ethyl acetate smells like pineapple, and isoamyl acetate brings the typical pear-like aroma [15]. Concentration of one of these above 30 mg·l^{-1} causes that the wine will smell like nail polish. Production of esters is influenced by many factors including aeration, concentration of fatty acids, concentration of higher alcohols and their precursors. Important role in the production of esters play the species and strain of yeast [31].

After fermentation with tested yeast strains, concentrations of ethyl acetate determined in YD media with rich source of vitamins were in all cases appropriate and no excessive production of ethyl acetate was observed. Another work [27] showed a higher concentration of ethyl acetate in all *S. cerevisiae* var. *cerevisiae* strains than *S. cerevisiae* var. *bayanus* strains. Presence of vitamins in media influenced the production of ethyl acetate differently with distinct *Saccharomyces* varieties. Fermentation by *S. cerevisiae* var. *bayanus* or *S. cerevisiae* var. *capensis* caused a total decrease in the concentration and in the yield factor in media without vitamins. Under conditions without growth factors, only *S. cerevisiae* var. *cerevisiae* strains produced

approximately the same or a little higher concentration of ethyl acetate, while the yield factor increased in all cases (Tab. 3).

Fusel alcohols

Redundant concentrations of fusel alcohols lead to strong, pungent flavour and taste of beverages. However, optimal concentrations (up to 400 $mg \cdot l^{-1}$) give the wine fruity character [9, 32, 33]. Concentration of total higher alcohols in wine varies between 100–500 $mg \cdot l^{-1}$ and the use of different yeast strains during fermentation leads to diversification of higher alcohol profiles [27, 34–36]. Besides glucose, amino acids are precursors of higher alcohols and their concentration in the medium should proportionally influence the concentration of fusel alcohols in wine [37]. Resulting concentration of these volatiles depends on the concentration of ethanol, fermentation temperature, pH and on the composition of must, aeration, variety of vine, ripeness of grapes and time of maceration [38].

Isoamyl alcohol

The main fusel alcohol in wine (more than 50% of total alcohols) is isoamyl alcohol. Similar to ethyl acetate, low concentrations of isoamyl alcohol support fruity character of wine, excessive concentrations make the wine aroma similar to nail polish. Precursors of isoamyl alcohol are amino acids leucine and valine, but it can be formed also by the metabolism of pyruvate [9]. Depending on *S. cerevisiae* variety and strain, concentrations of isoamyl alcohol in YD media varied. Its production by *S. cerevisiae* var. *bayanus* and *S. cerevisiae* var. *capensis* was similar, whereas *S. cerevisiae* var. *cerevisiae* showed on average 60% increase compared to *S. cerevisiae* var. *bayanus* [27]. In another work [32], isoamyl alcohol production by *S. cerevisiae* var. *bayanus* was by 15% higher than by *S. cerevisiae* var. *cerevisiae*. Absence of vitamins in the medium induced diminution of concentration and also of yield factor of isoamyl alcohol (Tab. 2, Tab. 3). The decrease in isoamyl alcohol produced by *S. cerevisiae* var. *bayanus* and *S. cerevisiae* var. *capensis* was more intensive than by *S. cerevisiae* var. *cerevisiae* (decrease of 80% to 87%, compared to 42%).

Isobutanol

Based on our experiments, production of isobutanol did not require vitamin feed. This was entirely in accordance with results of TER SCHURE et al. [39] and confirmed the knowledge that absence of thiamine in medium has minimal effect on the production of some fusel alcohols. Conversely in most cases, concentration of isobutanol in media without vitamins after fermentation was higher than in media with ideal composition. However, yield coefficient of isobutanol in media without vitamins was dramatically higher than in YD media (Tab. 3) and reflected a shift of the yeast metabolism towards increased production of isobutanol. We observed that also production of this metabolite differed between *S. cerevisiae* varieties. All tested *S. cerevisiae* var. *cerevisiae* strains produced very high amounts of isobutanol under conditions without vitamins. The increase of isobutanol concentration, compared to the media rich in nutritive components, was from 300% to 400% (Tab. 2), and the increase in the yield factor was from 410% to 450%.

2,3-Butanediol

2,3-Butanediol is the most prominent diol in wines. It has a specific creamy aroma but it appears to have a little sensory significance for the wine because its regular concentration in wine is deeply under its sensory threshold (600 $mg \cdot l^{-1}$). In *S. cerevisiae* cells, it is synthesized from pyruvate through α-acetolactate, which is reduced to diacetyl and acetoine [11]. During alcoholic fermentation, vitamin deprivation impacts multiplex lowering of 2,3-butanediole concentration and also of its yield. The decrease of 2,3-butanediol production by *S. cerevisiae* var. *bayanus* and *S. cerevisiae* var. *cerevisiae* was similar and ranged from 16% to 84% (Tab. 3). The biggest decrease of 2,3-butanediol was observed by *S. cerevisiae* var. *capensis* strains (from 63% to 100%). As an extreme, strain *S. cerevisiae* var. *capensis* RB-NA3 produced 140.6 $mg \cdot l^{-1}$ of 2,3-butanediol at ideal nutrition conditions, and absolutely no 2,3-butanediol in the medium without vitamins.

2-Butanol

2-Butanol is presumably formed by direct reduction of a compound whose hydrocarbon chain remains unchanged. A possible precursor for 2-butanol would be 2,3-butanediol. 2-Butanol has a fruity, apricot-like odour and its perception threshold concentration in wine is 50 $mg \cdot l^{-1}$ [40]. The type of yeast strain used for fermentation has a significant effect on the concentration of 2-butanol in the resulting wine. Similar to 2,3-butanediol, 2-butanol is very dependent on the supply of growth factors. Insufficient concentration of vitamins in the fermentation medium lowered both concentration and yield factor of 2-butanol. Our experiments showed that average production of 2-butanol by *S. cerevisiae* var. *cerevisiae* strains was naturally slightly lower than its production

by *S. cerevisiae* var. *bayanus* and *S. cerevisiae* var. *capensis*. The highest production of 2-butanol was observed by *S. cerevisiae* var. *capensis* (Tab. 3). However, after the fermentation without vitamins, these strains showed the most rapid decrease of 2-butanol concentrations (82%, compared to 33% by *S. cerevisiae* var. *cerevisiae*), and also the most rapid decrease of its yield factor.

CONCLUSION

Monitoring of the volatile profile of media fermented with different *Saccharomyces cerevisiae* strains under different conditions led to following conclusions. Individual *S. cerevisiae* strains had variable need for nutrition and growth factors. Different *S. cerevisiae* strains tolerated the lack of vitamins differently. Insufficient concentration of vitamins caused a decrease in the conversion of saccharides to ethanol and carbon dioxide, and a rapid increase in the production of acetaldehyde and volatile acids. Even if the concentration of assimilable nitrogen was sufficient, lack of vitamins led to a reduction in the production of fusel alcohols (isoamyl alcohol, 2,3-butanediol and 2-butanol). In contrast, production of isobutanol did not require an external source of vitamins. Each strain of the species and variety had different properties. Production of every metabolite was influenced by the composition of the fermentation medium and by the yeast strain in a characteristic manner. Only an exactly specified profile of the main volatile compounds can be a reliable key to select wine yeasts suitable for winemaking. Production of substances responsible for secondary aroma of wine depends on both the yeast strain and fermentation conditions.

Acknowledgement

This work was supported by the Scientific Grant Agency of Ministry of Education, Science, Research and Sport of Slovak Republic, project VEGA No. 1/0560/14, and by Slovak Research and Development Agency under the contract No. APVV-0488-07.

REFERENCES

1. Vilanova, M. – Ugliano, M. – Varela, C. – Siebert, T. – Pretorius, I. S. – Henschke, P. A:. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. Applied Microbial and Cell Physiology, *77*, 2007, pp. 145–157. DOI: 10.1007/s00253-007-1145-z.
2. Jiranek, V. – Langridge, P. – Henschke, P.: Nitrogen requirement of yeast during fermetnation. In: Williams, P. J.– Davidson, D. M. – Lee, T. H. (Ed.): Proceedings of the Seventh Australian Wine Industry Technical Conference. Adelaide : Australian Wine Research Institute, 1990, pp. 166–171. ISSN 0811-0743.
3. Kreger van Rij, N. J. W.: The yeasts, a taxonomic study. 3rd edition. Amsterdam : Elsevier Science Publishers, 1984. ISBN 0-444-80421-8.
4. Kurtzmann, C. P. – Fell, J. W.: The yeasts, a taxonomic study. 4th edition. Amsterdam : Elsevier Science Publishers, 2000. ISBN 978-0080542690.
5. Carrascosa, A. V. – Munoz, R. – Gonzales, R.: Molecular wine microbiology. 1st edition. Amsterdam : Elsevier Science Publishers, 2011. ISBN 978-0123750211.
6. Li, M. – Petteys, B. J. – McClure, J. M. – Valsakumar, V. – Bekiranov, S. – Frank, E. L. – Smith, J. S.: Thiamine biosynthesis in *Saccharomyces cerevisiae* is regulated by the NAD^+-dependent histone deacetylase Hst1. Molecular Cell Biology, *30*, 2010, pp. 3329–3341. DOI: 10.1128/MCB.01590-09.
7. Ciani, M. – Picciotti, G.: The growth kinetics and fermentation behaviour of some non-*Saccharomyces* yeasts associated with wine-making. Biotechnology Letters, *17*, 1995, pp. 1247–1250. DOI: 10.1007/BF00128395.
8. Romano, P. – Fiore, C. – Paraggio, M. – Caruso, M. – Capece, A.: Function of yeast species and strains in wine flavour. International Journal of Food Microbiology, *86*, 2003, pp. 169–180. DOI: 10.1016/S0168-1605(03)00290-3.
9. Swiegers, J. H. – Bartowsky, E. J. – Henschke, P. A. – Pretorius, I. S.: Yeast and bacterial modulation of wine aroma and flavour: The Australian Journal of Grape and Wine Research, *11*, 2005, pp. 139–173. DOI: 10.1111/j.1755-0238.2005.tb00285.x.
10. Engan, S.: Organoleptic threshold values of some alcohols and esters in beer. Journal of the Institute of Brewing, *78*, 1972, pp. 33–36. DOI: 10.1002/j.2050-0416.1972.tb03425.x.
11. Bartowsky, E. – Henschke, P. A.: The 'buttery' attribute of wine diacetyl desirability, spoilage and beyond. International Journal of Food Microbiology, *96*, 2004, pp. 235–252. DOI: 10.1016/j.ijfoodmicro.2004.05.013.
12. Meilgaard, M. C.: Flavour chemistry of beer. Part 2. Flavour and threshold of 239 aroma volatiles. Technical Quarterly - Master Brewers Association of America, *12*, 1975, pp. 151–173.
13. Berg, H. W. – Filipello, F. – Hinreiner, E. – Webb, E. D.: Evaluation of thresholds and minimum difference concentrations for various constituents of wine. I. Water solutions and pure substances. Food Technology, *9*, 1955, pp. 23–26.
14. Zea, L. – Moyano, L. – Moreno, J. A. – Medina, M.: Aroma series as fingerprints for biological ageing in fino sherry-type wines. Journal of the Science of Food and Agriculture, *87*, 2007, pp. 2319–2326. DOI: 10.1002/jsfa.2992.
15. Samappito, S. – Butkhup, L.: Effect of skin contact treatments on the aroma profile and chemical

components of mulberry (Morus alba Linn.) wines. African Journal of Food Science, *4*, 2010, pp. 52–61.
16. Yarrow, D.: Genus 22. *Saccharomyces* Meyen ex Reess. In: Kreger van Rij, N. J. W. (Ed.): The yeasts, a taxonomic study. 3rd revised and enlarged edition. Amsterdam : Elsevier Science Publishers, 1984, pp. 379–396. ISBN 0-444-80421-8.
17. Fleet, G.: Wine microbiology and biotechnology. Padstow : Harwood Academic Publishers, 1993. ISBN 978-0415278508.
18. Delfini, C. – Formica, J. V.: Wine microbiology: science and technology. 1st edition. Savigliano : L'Artistica Savigliano, 2001. ISBN 978-0824705909.
19. Peinado, R. A. – Mauricio, J. C. – Moreno, J.: Aromatic series in sherry wines with gluconic acid subjected to different biological aging conditions by *Saccharomyces cerevisiae* var. capensis. Food Chemistry, *94*, 2006, pp. 232–239. DOI: 10.1016/j.foodchem.2004.11.010.
20. Martinez, J. – Millan, C. – Ortega, J. M.: Growth of natural flora during the fermentation of inoculated musts from "Pedro Ximenez" grapes. South African Journal of Enology and Viticulture, *10*, 1989, pp. 31–35.
21. Method OIV-MA-AS-311-01A. Reducing substances (Resolution Oeno377/2009). In: Compendium of International Methods of Analysis – OIV. Paris : International Organisation of Vine and Wine, 2009.
22. Fowles, G. W. A.: Acids in grapes and wines: A review. Journal of Wine Research, *3*, 1992, pp. 25–41. DOI: 10.1080/09571269208717912.
23. Henschke, P. A. – Jiranek, V.: Yeasts: Metabolism of nitrogen compounds. In: Fleet, G. H. (Ed.): Wine microbiology and biotechnology. Chur: Harwood Academic Publishers, 1993, pp. 77–163. ISBN 978-0415278508.
24. Radler, F.: Yeast: Metabolism of organic acids. In: Fleet, G. H. (Ed.): Wine microbiology and biotechnology. Chur: Harwood Academic Publishers, 1993, pp. 165–182. ISBN 978-0415278508
25. Bábíková, P. – Baroň, M. – Kumšta, M. – Sotolář, R.: Increasing the efficiency of sulphur dioxide in wine by using of saturated higher fatty acids. Acta Universitatis agriculturae et silviculturae Mendelianae Brunensis : Acta of Mendel University of agriculture and forestry Brno, *1*, 2012, pp. 17–22.
26. Selli, S. – Canbas, A. – Cabaroglu, T. – Erten, H. – Gunata, Z.: Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment. Food Chemistry, *94*, 2006, pp. 319–326. DOI: 10.1016/j.foodchem.2004.11.019.
27. Antonelli, A. – Castellari, L. – Zambonelli, C. – Carnacini, A.: Yeast influence on volatile composition of wines. Jounal of Agricultural and Food Chemistry, *47*, 1999, pp. 1139–1144. DOI: 10.1021/jf9807317.
28. Romano, P.: Taxonomic and ecological diversity of food and beverage yeasts. In: Querol, A. – Fleet, G. H. (Ed.): Yeasts in food and beverages. Berlin: Springer, 2006. ISBN 978-3540283881.
29. Liu, S. Q. – Pilone, G. J.: An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. International Journal of Food Science and Technology, *35*, 2000, pp. 49–61. DOI: 10.1046/j.1365-2621.2000.00341.x.
30. Romano, P. – Suzzi, G. – Turbanti, L. – Polsinelli, M.: Acetaldehyde production in *Saccharomyces cerevisiae* wine yeasts. FEMS Microbiology Letters, *118*, 1994, pp. 213–218. DOI: 10.1016/0378-1097(94)90506-1.
31. Plata, C. – Millán, C. – Mauricio, J. C. – Ortega, J. M.: Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. Food Microbiology, 20, 2003, pp. 217–224. DOI: 10.1016/S0740-0020(02)00101-6.
32. Lambrechts, M. G. – Pretorius, I. S.: Yeast and its importance to wine aroma – a review. South African Journal of Enology and Viticulture, *21*, 2000, pp. 97–129.
33. Rapp, A. – Versini, G.: Influence of nitrogen compounds in grapes on aroma compounds in wine. Proceedings of the International Symposium on Nitrogen in Grapes and Wine. Seattle, Washington : Davis, 1991. ISBN 0-9630711-0-6.
34. Cabrera, M. J. – Morero, J. – Ortega, J. M. – Medina, M.: Formation of ethanol, higher alcohols, esters and terpenes by five strains in musts from Pedro Ximenez grapes in various degrees of ripeness. American Journal of Enology and Viticulture, *39*, 1988, pp. 283–287.
35. Giudici, P. – Romano, P. – Zambonelli, C.: A biometric study of higher alcohol production in *Saccharomyces cerevisiae*. Canadian Journal of Microbiology, *36*, 1990. pp. 61–64.
36. Patel, S. – Shibamoto, T.: Effect of different strains of *Saccharomyces cerevisiae* on production of volatiles in Napa Gamay wine and Petite Sirah wine. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 5649–5653.
37. Bell, S. J. – Henschke, P. A.: Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research, *11*, 2005, pp. 242–295. DOI: 10.1111/j.1755-0238.2005.tb00028.x.
38. Ribéreau-Gayon, P. – Glories, Y. – Maujean, A – Dubourdieu, D.: Handbook of enology. Volume 2: The chemistry of wine stabilization and treatments. 2nd Edition. Chichester : John Wiley & Sons, 2006. ISBN 978-0-470-01037-2.
39. ter Schure, E. G. – Flikweert, M. T. – van Dijken, J. P. – Pronk, J. T. – Verrips, C. T.: Pyruvate decarboxylase catalyzes decarboxylation of branched-chain 2-oxo acids but is not essential for fusel alcohol production by *Saccharomyces cerevisiae.* Applied and Environmental Microbiology, *64*, 1998, pp. 1303–1307.
40. Peinado, R. A. – Moreno, J. – Bueno, J. E – Moreno, J. A. – Mauricio, J. C.: Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chemistry, *84*, 2004, pp. 585–590. DOI: 10.1016/S0308-8146(03)00282-6.

Received 15 December 2013; revised 5 March 2014; accepted 7 March 2014; published online 17 September 2014.