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Influence of *Saccharomyces cerevisiae* Strain on the Profile of Volatile Organic Compounds of Blossom Honey Mead

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Abstract

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The influence of yeast strain on the volatile profile of meads fermented from blossom honey using three different *Saccharomyces cerevisiae* var. *bayanus* strains was evaluated. Meads were analysed by methods of basic chemical analysis and gas chromatography after 15 days of fermentation. Individual yeast strains produced various metabolites in different concentrations under the same fermentation conditions which significantly influenced the final secondary aroma of mead. Higher concentrations of acetaldehyde and 1-propanol, associated with *S. cerevisiae* var. *bayanus* MM-R2 considerably distinguished this strain from the others, whereas the difference between strains *S. cerevisiae* var. *bayanus* FM-R-Fix1 and MT-R1B was characterised by the production of ethyl hexanoate, ethyl octanoate (FM-R-Fix1), and isobutyl alcohol (MT-R1B).

Keywords: honeywine; yeast; sensory profile

Mead is a traditional alcoholic beverage containing 8–18% (v/v) of ethanol produced during the fermentation of diluted honey by yeasts *Saccharomyces cerevisiae* var. *bayanus* (Šmogrovičová *et al.* 2012; Pereira *et al.* 2013).

The fermentation of mead is a time-consuming process that often takes several weeks while the maturation time varies from 9 months to 2 years (Šmogrovičová *et al.* 2012). Several studies of mead production and its optimization have been performed in the last years. Mendes-Ferreira *et al.* (2010) optimised honey-must preparation for mead production by supplementing the honey-must with potassium tartrate, malic acid and diammonium phosphate and were able to reduce the fermentation time to 11 days, while Pereira *et al.* (2009) achieved fermentations within 8 days.

The sensory profile of mead is influenced by the dilution ratio of honey and water, the type of honey, yeast strain used for the fermentation and temperature of the fermentation process. Also, mead may be flavoured with various spices such as hop, nutmeg, cinnamon or fruit juice, depending upon local traditions (Šmogrovičová *et al.* 2012).

Alcoholic beverages contain a wide range of aromatic compounds which are present in miscellaneous concentrations. The most of volatile compounds of every alcoholic beverage are produced during fermentation (Sun *et al.* 2011). The main compounds which take part in forming the aroma of alcoholic beverages are higher alcohols, esters, volatile acids, and aldehydes (Procopio *et al.* 2013). Yeasts have a significant effect on the sensory characteristics of grape wine, fruit wine as well as honey wine. Therefore the selection of a proper yeast strain is also critical for the development of the desired mead style.

The aim of this work was to compare the production of volatile organic compounds by three different yeast strains of *Saccharomyces cerevisiae* var. *bayanus* in meads prepared by the fermentation of diluted blossom honey.

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MATERIAL AND METHODS

Honey. Blossom honey based on sunflower, field flower and thistle nectar originating from the Žitný ostrov locality in Slovakia was used.

Yeast strain. Three autochthonous yeast strains isolated from grapes of Mueller Thurgau, Moravian Muscat, and Blaufrankisch were used. The strains were identified as *S. cerevisiae* var. *bayanus* using the diagnostic keys (Furdíková *et al.* 2014) verified by PCR, characterised in terms of their oenological properties, and became part of the collection of microorganisms of the Faculty of Chemical and Food Technology (Slovak University of Technology, Bratislava, Slovakia). Axenic cultures of *S. cerevisiae* var. *bayanus* MT-R1B, MM-R2, and FM-R-Fix1 used in experiments are characterised by high osmotic tolerance and ethanol tolerance, and good sedimentation characteristics.

Processing of honey must and fermentation. Honey was diluted with water (at a ratio of weights 1 : 4) and boiled at 65°C for 15 min to precipitate proteins. After cooling the must was enriched with 0.3 g/l of Vitamon Combi® (Erbslöh, Geisenheim, Germany) containing diammonium phosphate and vitamin B_1. Honey must contained 154.2 mg/l and 175.2 mg/l before and after the addition of Vitamon Combi®, respectively. The prepared honey must was filled into 1 l glass flasks, and inoculated by a yeast strain in form of liquid starter (the starting concentration of biomass 10^6 cells in 1 ml) in 3 parallels. Inocula were prepared from a yeast strain culture grown aerobically for 24 h in a liquid medium (20 g/l glucose, 10 g/l yeast extract; 100 ml) in a 500 ml flask, on an orbital shaker at 2 Hz, 28°C. After cultivation, the concentration of 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. The fermentation was executed statically for 15 days at a temperature of 24°C. Young mead was separated from rough yeast sediments and underwent the basic chemical analysis.

Mead analysis. Basic oenological parameters as well as detailed volatile profile of all meads were determined. Total acids expressed as the tartaric acid were determined by acid-base titration with 0.1 mol/l NaOH using phenolphthalein. Concentration of reducing sugars was analysed by DNS method, concentration of alcohol and extract pycnometrically. The concentration of proteins was determined by the Bradford method (Bradford 1976).

Gas chromatography

Sample extraction and gas chromatography. The samples were prepared for the analysis as previously described (Šmogrovičová *et al.* 2012). Volatile compounds were analysed by the solid-phase microcolumn extraction (SPMCE) method using a GC 8000 Top Series, CE Instruments (Rodano, Milan, Italy) equipped with a modified inlet (Hrivňák *et al.* 2010). The chromatographic elution was temperature programmed as follows: isothermal at 40°C (1 min), then increased at a rate of 5°C/min to 220°C and held for 10 minutes. The temperature of the inlet chamber was 230°C and helium was used as a carrier gas. The trapped aroma compounds were desorbed at 10 kPa by heating the microcolumn to 230°C for 1 minute. After desorption, the carrier gas pressure was increased to 60 kPa and the temperature program was started. During the analysis, the microcolumn remained in the GC inlet.

Data processing and calibration. A computer program (Class-VP.2, SP1; Shimadzu, Duisburg, Germany) was used for data acquisition. For a quantitative analysis, five-point calibration curves were measured over the range of appropriate concentrations in 13% (v/v) ethanol. Calibration solutions underwent the same extraction procedure as wine samples.

Statistical analysis. All values in this work are the averages of the results obtained in triplicate, while the data variation was less than 5% of RSD (percentage relative standard deviation).

The experimental values were evaluated by the statistical dispersion method ANOVA (Analysis of Variance among Groups) by the program STATGRAPHICS plus for Windows 3.0 and for each parallel group of results the *P*-factor value was calculated. Principal component analysis (PCA) was performed using Statistica® software (version 10, Statsoft, Inc.).

RESULTS AND DISCUSSION

Meads fermented from blossom honey by three different strains of *S. cerevisiae* var. *bayanus* were analysed after 15 days of fermentation by methods of basic chemical analysis and gas chromatography to evaluate the influence of the yeast strain on the profile of volatile organic compounds (VOC) in mead. The results of the basic analysis of meads are shown in Table 1. ANOVA

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Table 1. Basic parameters of meads fermented by different strains *Saccharomyces cerevisiae* var. *bayanus*

Yeast strain	MT-R1B	MM-R2	FM-R-Fix1
Ethanol (% v/v)	13.60 ± 0.68	17.47 ± 0.87	15.46 ± 0.77
Y_{EtOH} (g/g)	0.42 ± 0.02	0.56 ± 0.03	0.48 ± 0.02
Proteins (mg/l)	43.51 ± 2.18*	43.51 ± 2.18*	64.10 ± 3.21
Total acids (g/l)	3.35 ± 0.17	2.68 ± 0.13	2.90 ± 0.14
Red. sugars (g/l)	25.47 ± 1.27*	34.95 ± 1.75	25.94 ± 1.30*
Extract (g/l)	89.70 ± 4.48	75.80 ± 3.79	68.70 ± 3.44
pH (–)	3.40 ± 0.17	3.48 ± 0.17	3.54 ± 0.18

Honey must dilution 1 : 4; reducing sugars t_0 = 282.4 g/l; total acids t_0 = 0.92 g/l; extract t_0 = 296.3 g/l; proteins t_0 = 154.2 mg/l, pH t_0 = 5.69; the data are mean values of triplicate samples; $P < 0.001$; same characters in the same column (*) correspond to statistically not significant difference ($P > 0.05$)

performed for the basic analysis of mead samples showed significant differences ($P < 0.001$) for all descriptors except the means of protein concentration in meads fermented by strains MT-R1B and MM-R2 and concentration of reducing sugars in meads fermented by strains MT-R1B and FM-R-Fix1 which were evaluated as not significantly different ($P > 0.05$).

The influence of yeast strains on the volatile aromatic composition of mead is presented in Table 2. The total of 14 fermentative aroma compounds which contribute to the sensorial qualities of alcoholic beverages were identified and quantified in all samples. To evaluate the contribution of a certain chemical compound to the aroma of mead, the odour activity values (OAV; the ratio between the concentration of individual compound in a sample and the threshold concentration of this compound) were calculated. Only those compounds whose OAV was greater than 1 were considered to cause a significant contribution to the mead aroma. The OAV, odour descriptors and odour thresholds are shown in Table 3.

Fusel alcohols and esters are the main groups of aroma compounds produced during fermentation. The most predominant higher alcohols in the studied meads were isobutyl alcohol with a characteristic mild, sweet-musty flavour, and isopentyl alcohol with a rather bitter fruity flavour. Isoamyl alcohol was determined in each sample above its threshold concentration. However, in mead fermented by the yeast strain MM-R2 of *S. cerevisiae* var. *bayanus* only

Table 2. Volatile organic compounds concentration of meads fermented by different strains *S. cerevisiae* var. *bayanus*

	MT-R1B	MM-R2	FM-R-Fix1
Acetaldehyde	5.63 ± 0.28*	105.92 ± 5.30	11.23 ± 0.56*
1-Propanol	3.16 ± 0.16*	121.47 ± 6.08	4.44 ± 0.22*
Isobutyl alcohol	76.60 ± 3.83	27.98 ± 1.40	55.55 ± 2.78
1-Butanol	0.17 ± 0.01	0.09 ± 0.00	nd
Isopentyl alcohol	78.33 ± 3.92*+	45.49 ± 2.27+#	73.96 ± 3.70*#
1-Octanol	0.12 ± 0.01*	0.14 ± 0.01*	0.16 ± 0.01*
2-Phenylethanol	13.46 ± 0.67*	25.80 ± 1.29*+	50.41 ± 2.52+
Ethyl acetate	29.04 ± 1.45	55.22 ± 2.76	36.19 ± 1.81
Ethyl butyrate	nd	0.41 ± 0.02	0.19 ± 0.01
Ethyl hexanoate	nd	nd	0.13 ± 0.01
Ethyl octanoate	0.06 ± 0.00*	0.04 ± 0.00	0.07 ± 0.00*
Isobuthyl acetate	nd	nd	nd
2-Phenethyl acetate	nd	nd	0.20 ± 0.01
Octanoic acid	1.07 ± 0.05*	2.16 ± 0.11*	0.51 ± 0.03

The results are shown as the mean values and their standard deviations in mg/l; nd – not determined; the data are mean values of triplicate samples; $P < 0.05$; same character in the same row (*, +, #) correspond to statistically not significant difference ($P > 0.05$)

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Table 3. Odour activity values (OAV) of volatile organic compounds of meads fermented by different strains *S. cerevisiae* var. *bayanus*

Compounds	MT-R1B	MM-R2	FM-R-Fix1	Odour descriptor	Odour threshold (μg/l)
Acetaldehyde	**11.3**	**211.8**	**22.5**	fresh, green leaves	500[a]
1-Propanol	0.0	0.1	0.0	alcohol, pungent	830 000[b]
Isobutyl alcohol	**1.9**	0.7	**1.4**	alcohol, wine, solvent	40 000[a,b]
1-Butanol	0.6	0.3	0.0	banana, fusel	300[c]
Isoamyl alcohol	**2.6**	**1.5**	**2.5**	fruity, nail polish	30 000[a,b,c]
1-Octanol	**1.0**	**1.2**	**1.3**	jasmine, lemon, wax, green, citrus, coconut	120[d,e]
2-Phenylethanol	**1.3**	**2.6**	**5.0**	honey, rose, spicy	10 000[b,d,f]
Ethyl acetate	**2.4**	**4.6**	**3.0**	solvent, pineapple, balsamic	12 000[a,b,d,f]
Ethyl butyrate	0.0	**20.6**	**9.4**	fruity, sweet	20[a,d]
Ethyl hexanoate	0.0	0.0	**9.5**	fruity, green apple, banana	14[b,d,f]
Ethyl octanoate	**11.7**	**7.4**	**14.1**	fruity, sweet, roses, honey	5[a,b,d,f]
Isobuthyl acetate	0.0	0.0	0.0	sweet, fruity	350[c]
2-Phenethyl acetate	0.0	0.0	0.8	flowery, sweet, honey, fruity, rose	250[a,b,d,f]
Octanoic acid	**2.1**	**4.3**	**1.0**	oily, rancid, sweet, cheese	500[a,b,d,f]

The data were mean values of triplicate samples; [a]Perreira *et al.* (2013); [b]Carrau *et al.* (2008); [c]Barbosa *et al.* (2009); [d]Roldán *et al.* (2011); [e]Hellman and Small (1974); [f]Jiang and Zang (2010)

45.49 mg/l of isoamyl alcohol was produced while the other strains produced 73.96 and 78.33 mg/l of it. 1-Propanol is a higher alcohol with alcoholic, pungent smell, produced during the fermentation process. The concentrations determined in meads fermented by strains MT-R1B and FM-R Fix1 (3.16 and 4.44 mg/l) were in accordance with those of Šmogrovičová *et al.* (2012), but lower than those of Pereira *et al.* (2013). Although the concentration of 1-propranol in mead fermented by the strain MM-R2 was much higher (121.47 mg/l), it was still under its threshold concentration (830 mg/l). No significant differences in concentrations of 1-octanol were observed in meads fermented by various yeast strains. 1-Butanol was detected only in meads fermented by yeast strains MT-R1B (0.17 mg/l) and MM-R2 (0.09 mg/l). 2-Phenylethanol has a characteristic honey and rose-like odour. In our samples it was detected in lower concentrations (from 13.46 to 50.41 mg/l) than in those of Mendes-Ferreira *et al.* (2010), but they were much higher than in commercial meads analysed by Šmogrocičová *et al.* (2012).

Esters are produced during the fermentation process by the metabolism of yeasts. This group of volatile compounds has in general fruity and sweet odour. Similarly like in Mendes-Ferreira *et al.* (2010), Šmogrovičová *et al.* (2012), and Pereira *et al.* (2013), the predominant ester in our mead samples was ethyl acetate in concentrations 2.4- and 4.6-times above its threshold concentration. Ethyl octanoate has a very low threshold concentration (5 μg/l), therefore even at a low concentration it can influence the bouquet of beverages (Pereira *et al.* 2013). A statistically significant difference was determined in the content of octanoic acid between meads fermented by strains MM-R2 (2.16 mg/l) and FM-R-Fix1 (0.51 mg/l). While ethyl butyrate was not detected only in a mead sample fermented by the strain MT-R1B, ethyl hexanoate and 2-phenethyl acetate were not detected even in meads fermented by the strain MM-R2, isobutyl acetate was not detected in any of the meads.

Acetaldehyde is characterised by fresh, nutty, green leaves flavour, causing an unpleasant odour at higher concentrations. In the presence of ethanol acetaldehyde prevents alcohol-induced growth inhibition (Roustan & Sablayrolles 2002). Pereira *et al.* (2014) determined acetaldehyde in mead samples from 5 to 10 mg/l using a high-cell-density fermentation process and after fermentation also with immobilised yeast strains. The concentration of acetaldehyde in our mead samples was comparable except the mead fermented by the yeast strain MM-R2 (105.92 mg/l).

As an overview of the results, PCA was used to identify the volatile yeast products that best dis-

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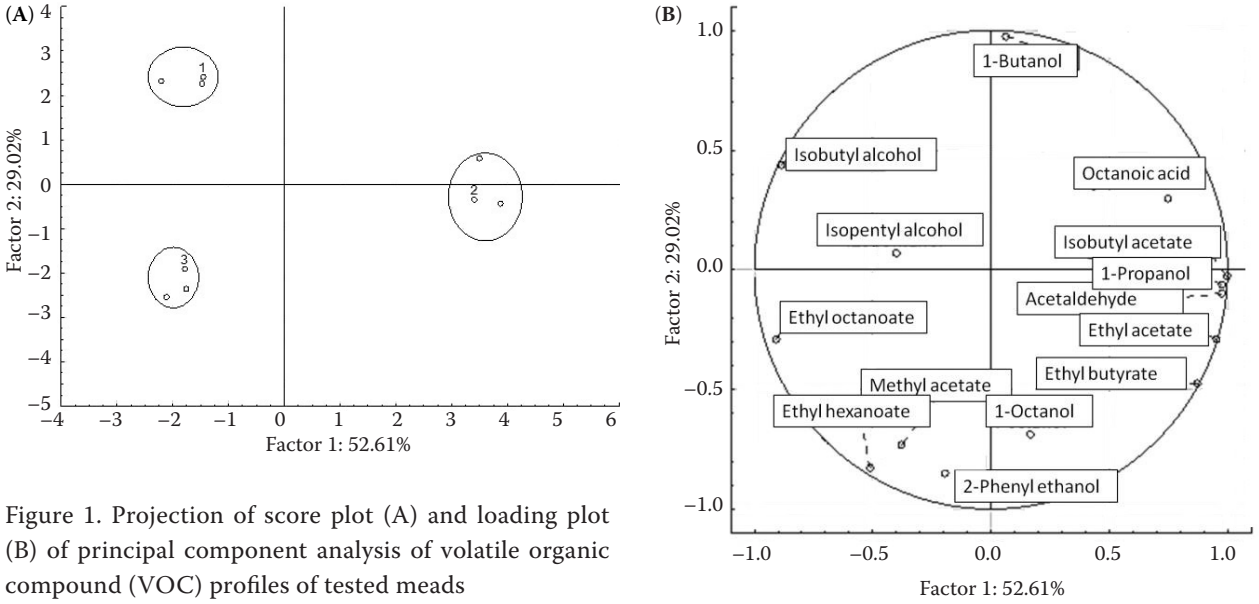

Figure 1. Projection of score plot (A) and loading plot (B) of principal component analysis of volatile organic compound (VOC) profiles of tested meads

criminated between the yeast strains. The principal component plot shown in Figure 1 highlights the relevance of *Saccharomyces cerevisiae* var. *bayanus* strain used in the production of aroma compounds in blossom mead. The correlation matrix was calculated in order to discriminate the variables (yeast strains), thus selecting 14 parameters. The principal component analysis explained 81.63% of total variance. The first principal component (PC1) accounted for 52.61% of the total variation, while PC2 explained 29.02% of the total variation. Samples of meads fermented by strains MT-R1B and FM-R-Fix1 were separated in PC2, while the strain MM-R2 is separated from the others in PC1. Compounds such as octanoic acid, isobutyl acetate, 1-propanol, acetaldehyde, ethyl acetate, and ethyl butyrate were positively correlated with PC1 and separated the strain MM-R2 from the others. Isobutyl alcohol and isopentyl alcohol were negatively correlated with PC1, their production characterised the strain MT-R1B. Ethyl octanoate, methyl acetate, ethyl hexanoate were negatively correlated with PC1 and PC2, and distinguished the strain FM-R-Fix1.

CONCLUSION

Blossom honey meads were fermented by three different *Saccharomyces cerevisiae* var. *bayanus* strains to compare the production of volatile organic compounds (VOC) which are responsible for the secondary aroma of alcoholic beverages. Monitoring of the volatile profile of meads led to the following conclusions. Individual yeast strains produced various metabolites in different concentrations under the same fermentation conditions which significantly influenced the final mead flavour. Higher concentrations of acetaldehyde, 1-propanol, associated with the strain MM-R2, considerably distinguished this strain from the others, whereas the difference between strains FM-R-Fix1 and MT-R1B is characterised by production of ethyl hexanoate, ethyl octanoate (FM-R-Fix1), and isobutyl alcohol (MT-R1B). As a result, the most suitable strain to produce blossom mead was evaluated the strain FM-R-Fix1. This strain was able to produce a balanced profile of monitored VOC and influenced a final aroma of mead in a neutral way.

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