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The influence of different enzymatic preparations and skin contact time on aromatic profile of wines produced from autochthonous grape varieties Krstač and Žižak

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Abstract: This study aimed to show aromatic profile of wines produced from two autochthonous grape cultivars Krstač (K) and Žižak (Z). During the wine production two enzymatic preparations (EP) Lallzyme cuvee blanc (CB) and Lallzyme enzymatic preparation β (EB) and different time of skin contact (4 and 8 h) were applied. Aromatic compounds were detected by GC/FID–MS analysis. Significantly higher content of total detected aromatic compounds compared to appropriate controls (168.54 and 161.72 mg L^{-1}) was observed for K EB4h (176.33 mg L^{-1}) and Z CB4h (177.29 mg L^{-1}) wines. Skin contact and usage of EP mostly increased content of 2-phenylethyl and isoamyl alcohols. Wines from both varieties showed higher content of hexanoic and octanoic acids compared to the control. It is interesting to emphasize that content of esters that are responsible for fruity aroma of wine which is important for pleasant taste (isoamyl acetate – banana, ethyl hexanoate – ripe banana, 2-phenylethyl acetate – powerful fruity rose like) were increased in all samples compared to the controls. The highest grades, after sensory evaluation, were obtained for K EB 8h (18.0 out of 20.0) and Z CB 8h (18.2 out of 20.0).

Keywords: aromatic compounds; autochthonous grapevine; GC/FID–MS analysis; must treatment; fruity aroma; sensory.

INTRODUCTION

Wine is rich source of different biologically active compounds. Among them it is possible to highlight polyphenols, which due to their beneficial health effects, are essential in healthy well balanced nutrition.[1] Beside phenolic com-

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pounds it is important to emphasize those that are responsible for aromatic properties of wine. Wine quality mostly depends on aroma of wine.[2] The influence of wine aroma on organoleptic characteristics cannot be neglected too, since it is the key property which leads consumers to choose the wine.[3,4]

Different factors influence wine aromatic complexity, and the most significant are grape variety, pre-fermentative procedures, vinification procedures, fining, stabilization and aging.[5,6] Grape berry skin is one of the richest source of volatile compounds.[7] Prefermentative skin contact and pressure applied during the grape processing are winemaking procedures significantly affecting extraction of aromatic compounds.[8–11] Composition of grape juice and extraction process are responsible for content of aromatic compounds in wine.[12] White wine production process ordinarily includes skin removal to prevent excessive pass of polyphenols into the must which can cause enzymatic oxidative browning of wine. It is important to find balance between white wine skin contact and its removal.[13]

Aromatic compounds can be divided into the two groups, volatile and non-volatile compounds. Volatile compounds present in free form-directly contribute to the aroma, while non-volatile are in bound form.[14] Applying commercial enzymatic preparations which exhibit β-glucosidase activity it is possible to release aglycone from heteroside by cleavage of glycosidic bonds. This can affect the aromatic profile.[4,14] Application of enzymatic preparation especially during the maceration stage can significantly increase content of C_6 alcohols.[15]

White wine quality can be improved by must clarification.[16] It is also possible to improve quality of white wines by decreasing insoluble solids in the juice before fermentation.[12] Clarified musts, used for the production of white wines, can significantly improve organoleptic characteristics with emphasize on aroma.[17]

We have previously studied the influence of enzymatic preparations on the content of phenolic compounds in grape and fruit wines.[18,19] The influence of different enzymatic preparations and skin contact periods on aromatic profile of wines produced from Krstač and Žižak varieties was investigated in this study. For the first time data, regarding content and importance of aromatic compounds from wines produced from these two autochthonous varieties, will be published.

EXPERIMENTAL

Chemicals and plant material

In this study the following chemicals were used: methylene chloride, sodium sulfate anhydrous, methyl alcohol and 4-methyl-1-pentanol. All chemicals were obtained from Sigma–Aldrich, except methylene chloride which was obtained from Merck.

The autochthonous grape varieties Krstač and Žižak were investigated in this study. The grape varieties were grown in a vineyard on Ćemovsko polje in Montenegro. Among the white vine varieties, the most important are Krstač (grown on the “Pista” microlocality) and Žižak variety (grown on the “Bunar 17” microlocality). The training system of grape varieties Krstač and Žižak was a single Guyot, pruned to a mix of canes and spurs. All vines were evenly pruned, leaving one shoot growth on spur with two buds and an arc nine buds long.

Irrigation of the vine was carried out with a drip system. The yield of grapes per vine for the variety Krstač was 1.83 kg, while for Žižak 3.39 kg.

Winemaking

The grapes of Krstač (K) and Žižak (Z) varieties were harvested manually. Obtained grapes were in a state of full maturity and phytosanitary health 100 % (determined visually). The grapes of both varieties were manually destemmed, crushed, and sulfurized with 10 g $K_2S_2O_5$ per 100 kg mashed grapes. In vinification experiments were used: pure wine yeast culture *Saccharomyces cerevisiae* (ICV D47 Lallemand, Montreal, Canada), enzyme preparation Lallzyme Cuvee blanc (Lallemand, Montreal, Canada) which is mixture of pectinases and enzyme preparation Lallzyme β (Lallemand, Montreal, Canada) which is pectic enzyme complex. All experiments were divided into 5 treatments: Ctrl (control) – without skin contact and addition of enzymatic preparation, CB4h – with addition of enzymatic preparation Lallzyme Cuvee blanc and skin contact 4 h, CB8h – with addition of enzymatic preparation Lallzyme Cuvee blanc and skin contact 8 h, EB4h – with addition of enzymatic preparation Lallzyme β and skin contact 4 h, EB8h – with addition of enzymatic preparation Lallzyme β and skin contact 8 h. The amount of added enzymatic preparations was 2 g per 100 kg mashed grapes, and all skin contacts were performed at 5 °C temperature. After maceration of grape must in all 5 treatments grape juice was separated by static settling for 48 h and then racked. After that, all 5 treatments were inoculated with a pure culture of wine yeast *Saccharomyces cerevisiae* ICV D47 20 g hL^{-1} and left for fermentation. The alcoholic fermentation carried on for approximately 20 days at low temperature 15 °C. All obtained wines were dry (sugar content under 4 g L^{-1}). After sulphiting, racking and refilling vessels wines were prepared for GC–MS analysis.

GC/FID–MS analysis

Sample preparation was conducted by liquid–liquid extraction.[20] Volumes of 25 mL of wine sample and 5 mL of methylene chloride were stirred at 0 °C for 1 h. After one hour of extraction, the mixture was kept for 5 min in ultrasonic bath. Organic phase, which was collected after separation, was treated with sodium sulfate anhydrous to remove water and then filtrated. Subsequently, 0.6 mL of extracted wine sample was used for further GC/FID––MS analysis. Analysis of volatile compounds was conducted by using GC/FID–MS system according the previously described method, with some modifications.[21] The analysis was conducted on gas chromatograph (GC) system Agilent 7890A (Santa Clara, CA, USA). The device was equipped with Agilent 19091N-113 HP-INNOWax fused silica capillary column (30 m×0.32 mm i.d., 0.25 μm film thickness) which was used for separation. Injection was in split mode 3:1 with helium as carrier gas at 1.46 mL min^{-1}, and the injecting volume was 1 μL. The temperature of the GC oven was held at 40 °C for 5 min and then programmed to 220 °C at 10 °C min^{-1}, then held for 4 min at 220 °C. The instrument was equipped with dual detectors: mass selective detector (MSD) 5975C inert XL EI/CI MSD and flame ionization detector (FID) connected by capillary flow technology 2-way splitter with makeup gas. The ion source of the MSD and the transfer line were kept at 230 and 280 °C, respectively. Mass selective detector operated in the positive ion electron impact (EI) mode. Electron impact spectra in scan mode were recorded at 70 eV in mass range from 29 to 300 *m*/*z*. The FID detector was heated to 300 °C.

For quantitative evaluation the internal standard method was applied, with a known amount of 4-methyl-1-pentanol as an internal standard (IS). The (relative) percentages of the identified compounds were computed from the gas chromatography peak areas. The concen-

tration of each volatile was calculated with respect to the IS and presented as relative concentration of each component in analyzed sample.

The identification of the components was based on comparison with the reference spectra (Wiley and NIST databases). The percentages of the identified compounds were computed from the gas chromatography peak areas.

Statistical and sensory analysis

A one-way ANOVA was performed to compare the effect of using skin contact (4 and 8 h, 5 °C) and glycosidase enzyme preparations Lallzyme cuvee blanc and Lallzyme β, on each aromatic compounds separately. Tukey's post-hoc test with significant levels $p < 0.05$, was employed to conduct mean comparisons. The paired samples *T*-test was also applied. Statistical analysis was conducted using SPSS Statistical V20.0 software (IBM, Chicago, IL, USA).

The wine tasting panel, that consisted of three members, conducted sensory analysis of samples according to Bux–Baum method. For wine tasting the highest grade was 20 points.

RESULTS AND DISCUSSION

Effect of skin contact and usage of enzymatic preparation on the content of aromatic compounds in Krstač and Žižak wine

GC/FID–MS analysis of the wines showed that total content of detected aromatic compounds in Krstač samples were in range from 159.3 to 180.0 mg L^{-1}, while in Žižak from 161.7 to 192.5 mg L^{-1}. Statistical analysis, where paired samples *T*-test was applied, showed significant difference in the content of all detected aromatic compounds ($p < 0.05$), between Krstač Ctrl and Krstač EB4 wines (Table I) as well as for Ctrl and CB4 wines produced from Žižak variety (Table II). The concentrations of total detected volatile compounds obtained herein were similar to the literature data.[5,8,10] Wine fermentations of Emir grape cultivar resulted in 162.0 mg L^{-1} of volatiles in control and 187.0 mg L^{-1} of volatiles in skin contact sample.[8] Another study in which Muscat of Bornova wines were analyzed showed that control (158 mg L^{-1}) had lower content of total detected volatile compounds compared to 6 h (168 mg L^{-1}) and 12 h (172 mg L^{-1}) skin contact.[5]

Our findings are in line with the literature data which emphasized higher amounts of volatile compounds in skin contact wine compared to control (wine produced without skin contact).[10] After skin contact of 7 h at 15 °C Muscat of Alexandria wines were significantly enriched with aromatic compounds.[9] During the alcoholic fermentation compounds such as esters and alcohols are generated, and thus volatiles considerably increase in wine *vs.* juice.[8] Applied enzymatic preparation glycosidase during the maceration, cleave glycosidic bonds and so increase content of free form of different compounds potentially responsible for wine aroma.[6,22]

To the contrary, in Bical wines, produced after enzymatic preparation treatment, significant increase of total volatile compounds was not observed.[23]

TABLE I. The concentrations of aromatic compounds (mg L^{-1}) in Krstač wines using skin contact (4 and 8 h, 5 °C) and glycosidase enzyme preparations Lallzyme cuvee blanc and Lallzyme β with results of the one-way ANOVA along with the Tukey *post-hoc*; values are mean (n = 3) followed by different lowercase letters (a, b, c) indicate significant differences between treatments at the 5 % level; t = trace

Compounds	Sample					F	p
	K Ctrl	K CB4h	K CB8h	K EB4h[a]	K EB8h		
1-Hexanol	0.57 a	0.54 a	0.39 b	0.58 a	0.48 ab	12.4	0.001
Isobutyl alcohol	2.45 b	3.62 a	4.03 a	3.88 a	3.97 a	29.3	0.000
Isoamyl alcohol	91.19 bc	94.42 ab	99.14 a	94.32 abc	87.61 c	8.8	0.003
4-Methyl-1-pentanol	8.13	8.13	8.13	8.13	8.13		
3-Ethoxy-1-propanol	t	t	t	t	t		
2,3-Butanediol	t	t	0.12	0.14	0.10	1.7	0.259
3-(Methylthio)-1-propanol	0.22 b	0.22 b	0.25 ab	0.27 a	0.21 b	6.5	0.008
2-Phenylethyl alcohol	50.69 a	41.94 b	48.82 a	50.38 a	40.83 b	11.4	0.001
Total alcohols	153.25	148.87	160.87	157.69	141.33		
Hexanoic acid	1.98 b	2.52 ab	2.76 a	2.73 a	3.08 a	6.5	0.008
Octanoic acid	4.20 c	4.73 bc	5.56 a	5.34 ab	5.65 a	15.4	0.000
Decanoic acid	0.87 a	0.58 b	0.59 b	0.64 b	0.50 b	19.2	0.000
Isobutyric acid	t	0.24	t	0.25	0.26	0.2	0.863
9-Decenoic acid	1.30 a	0.56 b	0.51 b	0.64 b	t	8.7	0.007
Total acids	8.35	8.63	9.42	9.59	9.49		
Ethyl butyrate	0.89 c	1.12 b	1.35 a	1.22 ab	1.21 ab	20.7	0.000
Ethyl hexanoate	0.19 c	0.31 b	0.30 b	0.30 b	0.41 a	26.8	0.000
Ethyl (*S*)-(–) lactate	0.98 a	0.68 bc	0.89 ab	0.74 bc	0.64 c	9.7	0.002
Ethyl octanoate	t	0.16	0.20	0.18	0.25	2.1	0.178
Ethyl decanoate	t	0.19	0.17	t	t	1.7	0.267
Diethyl succinate	0.68 b	1.01 ab	1.07 a	1.07 a	1.00 ab	4.5	0.024
Ethyl 9-decenoate	t	t	t	t	t		
Ethyl 4-hydroxybutanoate	t	t	t	t	t		
Diethyl hydroxybutanedioate	t	t	t	t	t		
Ethyl ester 4-ethoxy benzoic acid	t	0.18	0.22	t	t	0.6	0.482
Ethyl hydrogen succinate	2.20 b	3.12 a	3.40 a	3.18 a	3.01 a	9.1	0.002
Isoamyl acetate	0.27 b	0.61 a	0.69 a	0.66 a	0.60 a	6.1	0.009
Hexyl acetate	t	t	t	t	t		
1,3-Propanediol diacetate	0.14 c	0.15 c	0.19 ab	0.21 a	0.17 bc	19.7	0.000
2-Phenylethyl acetate	0.16 b	0.25 a	0.30 a	0.28 a	0.26 a	8.9	0.002
γ-Butyrolactone	1.44 a	0.78 c	0.93 bc	1.21 ab	0.96 bc	12.3	0.001
Total ethyl esters, acetates and lactones	6.94	8.56	9.72	9.05	8.51		
Total aromatic compounds	168.54	166.06	180.01	176.33	159.33		

[a]Statistically significant difference (p<0.05) in the content of all detected aromatic compounds compared to K Ctrl

Alcohols

Among the alcohols, in Krstač and Žižak wines, the most predominant were higher alcohols, such as isoamyl alcohol, phenyl ethyl alcohol and isobutyl alco-

TABLE II. The concentrations of aromatic compounds (in mg L^{-1}) Žižak wines using skin contact (4 and 8 h, 5 °C) and glycosidase enzyme preparations Lallzyme cuvee blanc and Lallzyme β with results of the one-way ANOVA along with the Tukey *post-hoc*; values are mean (n = 3) followed by different lowercase letters (a, b, c) indicate significant differences between treatments at the 5 % level

Compound	Sample					*F*	*p*
	Z Ctrl	Z CB4h[a]	Z CB8h	Z EB4h	Z EB8h		
1-Hexanol	0.62 a	0.53 ab	0.39 b	0.68 a	0.73 a	8.49	0.003
Isobutyl alcohol	5.00 c	5.58 b	6.15 a	5.20 bc	5.58 b	20.77	0.000
Isoamyl alcohol	102.17 b	102.82 b	115.21 a	96.51 b	112.81 a	23.50	0.000
4-Methyl-1-pentanol	8.13	8.13	8.13	8.13	8.13		
3-Ethoxy-1-propanol	t	t	t	t	t		
2,3 Butanediol	0.20	0.36	0.33	0.30	t	0.92	0.474
3-(Methylthio)-1-propanol	0.24 ab	0.15 b	0.25 ab	0.26 ab	0.29 a	4.71	0.041
2-Phenylethyl alcohol	31.37 b	37.46 ab	41.58 a	41.09 ab	40.41 ab	3.79	0.040
Total alcohols	147.73	155.03	172.04	152.17	167.94		
Hexanoic acid	1.94 b	3.54 a	3.12 a	3.40 a	2.92 a	15.91	0.000
Octanoic acid	3.53 b	6.97 a	6.21 a	6.74 a	5.59 a	3.74	0.021
Decanoic acid	0.57	0.86	0.83	1.07	1.06	2.59	0.102
Isobutyric acid	0.34 ab	0.31 bc	0.38 a	0.36 ab	0.26 c	17.41	0.000
9-Decenoic acid	0.25 b	1.07 a	0.40 b	0.98 a	0.95 a	6.23	0.009
Total acids	6.63	12.75	11.23	12.55	10.78		
Ethyl butyrate	1.40 b	1.96 a	1.43 b	1.97 a	1.61 ab	11.34	0.001
Ethyl hexanoate	0.41 c	0.51 a	0.44 bc	0.50 ab	0.45 c	11.53	0.001
Ethyl (*S*)-(–) lactate	0.26 ab	0.15 b	0.19 ab	0.23 ab	0.32 a	3.62	0.045
Ethyl octanoate	0.19 b	0.25 a	0.22 ab	0.22 ab	0.26 ab	4.85	0.020
Ethyl decanoate	t	0.20	t	t	0.12	1.13	0.348
Diethyl succinate	0.45 b	0.84 a	0.96 a	0.85 a	0.74 a	10.11	0.002
Ethyl 9-decenoate	t	t	t	t	t		
Ethyl 4-hydroxybutanoate	1.21 c	1.43 b	1.30 bc	1.69 a	1.41 b	33.62	0.000
Diethyl hydroxybutanedioate	0.10 bc	0.08 bc	0.06 c	0.11 b	0.21 a	26.29	0.000
Ethyl ester 4-ethoxy benzoic acid	t	t	t	t	t		
Ethyl hydrogen succinate	1.63 a	2.29 a	2.33 a	2.23 a	0.54 b	4.18	0.030
Isoamyl acetate	0.69	0.71	1.13	0.79	0.83	2.72	0.091
Hexyl acetate	t	t	t	t	t		
1,3-Propanediol diacetate	0.23 ab	0.28 a	0.23 ab	0.28 a	0.23 b	3.53	0.048
2-Phenylethyl acetate	0.13 b	0.18 b	0.29 a	0.21 ab	0.17 b	6.85	0.006
γ-Butyrolactone	0.65	0.63	0.64	0.74	0.82	3.30	0.057
Total ethyl esters, acetates and lactones	7.36	9.51	9.22	9.83	7.71		
Total aromatic compounds	161.72	177.29	192.49	174.55	186.43		

[a]Statistically significant difference (p<0.05) in the content of all detected aromatic compounds compared to Z Ctrl

hol. The total detected alcohol content in K Ctrl was 153.3 mg L^{-1} (Table I), while in Z Ctrl 147.7 mg L^{-1} (Table II). It is important to highlight that literature data indicated higher alcohols as a major constituents of Muscat of Bornova

wines.[5] Almost 84 % of total free volatiles in wines were higher alcohols which indicated them as the most prominent compounds.[10]

The content of phenyl ethyl alcohol in wines produced from cultivar Krstač was from 40.8 to 50.7 mg L^{-1} (Table I) while in cultivar Žižak from 31.4 to 41.6 mg L^{-1} (Table II). Statistical analysis, where one-way ANOVA with the Tukey test was carried out, showed a statistically significant difference in the content of 2-phenylethyl alcohol ($p<0.05$) between Žižak Ctrl and Z CB8h, and Krstač Ctrl compared to K CB4h and K EB8h.

Study of Albariño wines, in which malolactic fermentation was not conducted, content of phenyl ethyl alcohol increased up to 20 mg L^{-1}.[2] Literature data suggested contribution of phenyl ethyl alcohol to pleasant wine aroma which reminds to rose.[5,12]

Our findings are supported by the literature data which indicated that skin contact and/or usage of enzymatic preparations, which possess glycosidase activity, generate higher content of free form of phenyl ethyl alcohol.[15] Application of enzymatic preparation in experiment with Bical wines did not significantly increase content of aromatic alcohols.[23] Wines produced in vinification with skin contact showed significantly higher content of phenyl ethanol and other fusel alcohols.[12] Muscat of Alexandria wines in which skin contact was applied significantly increased total level of alcohol.[9]

Prolonged maceration time and usage of enzymatic preparation Lallzyme β, in the wines produced from Krstač variety, decreased alcohol content. Generation of higher alcohols have been decreased with longer skin contact. It is important to point out that higher alcohol formation is mainly conducted by Ehrlich mechanism. Decrease of higher alcohols can be explained by the fact that Ehrlich mechanism blockage is result of higher levels of nitrogenous substances in vinifications.[6,24]

Concentration of 2,3-butanediol in Krstač wines were from 0.10 to 0.14 mg L^{-1} (Table I) while in Žižak wines were from 0.20 to 0.36 mg L^{-1} (Table II), respectively. There was no statistically significant difference between treatments as regards the content of 2,3-butanediol ($p>0.05$) in Krstač and Žižak wines. Krstač and Žižak wines produced by addition of various enzyme preparations (cuvee blanc, β-enzyme) and skin contact of 8 h had statistically significant higher content of isoamyl and isobutyl alcohols ($p<0.05$), as compared to the control wines (except K EB8h for isoamyl alcohol). The study, in which were analyzed Muscat of Bornova wines, reported almost similar results as in our study.[5]

Compounds with 6 carbon atoms can be formed from fatty acids in grapes during the pre-fermentative stage.[15] Significant increase of C_6 alcohols is a result of skin-contact process which ensures more fatty acids and lipoxygenase enzyme during fermentation.[8,12,15] Those compounds are responsible for herbaceous and leafy notes which are unfavourable for wine quality.[5,8] The content of 1-hexanol

was from 0.39 to 0.58 mg L^{-1} for Krstač wines, while for Žižak it ranged from 0.39 to 0.73 mg L^{-1}, respectively. Skin contact (8 h) and the use of Lallzyme enzyme β preparation, resulted in the higher content of 1-hexanol in Žižak wine (p<0.05), as compared to Z CB8h. The highest content of total detected alcohols, for both used grape cultivars, was observed when vinification was conducted by maceration, during the 8 hours, with enzymatic preparation Lallzyme cuvee.

Acids

Krstač wines obtained after applied skin contact (4 and 8 h) and usage of enzymatic preparation glycosidase showed higher content of hexanoic and octanoic acids (Table I). Wines obtained from cultivar Žižak, by the same vinification procedure, were enriched with hexanoic, octanoic and decanoic acids (Table II). Based on the Tukey test results, a statistically significant differences in the content of hexanoic and octanoic acids (p<0.05) between control (K Ctrl, Z Ctrl) and all other Krstač and Žižak skin contact wines (excluding K CB4h for hexanoic and octanoic acid) were found.

Our findings are in line with literature data which emphasized average content of 6 (3.7 mg L^{-1}), 8 (3.3 mg L^{-1}) and 10 (0.8 mg L^{-1}) carbon atoms fatty acids.[2] Another study reported almost similar values for content of 6 (3.3 mg L^{-1}), 8 (3.9 mg L^{-1}) and 10 (1.2 mg L^{-1}) carbon atoms fatty acids.[25] Skin contact Emir and Muscat of Bornova wines increased content of fatty acids.[5,8] Albariño wines produced with usage of enzymatic preparation during maceration showed almost double concentration of hexanoic and octanoic acids compared to other samples.[15]

Ethyl esters, acetates and lactones

Esters are important compounds which are responsible for fruity aroma (ethyl butanoate – pineapple, isoamyl acetate – banana, ethyl hexanoate – ripe banana, 2-phenyl ethyl acetate – powerful fruity, rose like).[5,12] It is important to emphasize that highest content of ethyl butyrate, ethyl hexanoate, diethyl succinate and ethyl hydrogen succinate was observed in wines produced from both grape cultivars. Significant concentrations of ethyl octanoate and ethyl 4-hydroxybutanoate were observed in wines produced from Žižak.

The literature data in which were studied other grape varieties reported similar findings related to esters.[5,6,9] The data related to ethyl esters and acetates content for Assyrtiko wines showed that skin contact has not significantly increased content of those compounds.[12]

A one-way Anova revealed that the use of maceration (4 and 8 h) and enzyme preparations led to a statistically significant increase in the content of ethyl butyrate, ethyl hexanoate, ethyl hydrogen succinate, isoamyl acetate and 2-phenylethyl acetate and a decrease in concentration of ethyl lactate and γ-butyrolactone (p<0.05) in all Krstač wines (except K CB8h for ethyl lactate and K EB4h for γ-butyrolactone). Literature data suggested that skin contact increase

content of ethyl hexanoate,[5,9] ethyl butanoate, 2-phenylethyl acetate,[9] ethyl octanoate, diethyl succinate and isoamyl acetate.[5] Our results are in agreement with findings which highlighted decrease of ethyl lactate[6] and γ-butyrolactone.[5,10]

Trace amounts of hexyl acetate, ethyl 9-decenoate, and 3-ethoxy-1-propanol are present in all wines. Skin contact caused an increase in hexyl acetate content in Albillo wines[6] and a decrease in 3-ethoxy-1-propanol concentration in Muscat of Bornova wines.[5] Addition of glycosidase enzyme preparations resulted in 3--ethoxy-1-propanol content rise in wines.[23] The highest effect on content of 3--ethoxy-1-propanol was exerted by a yeast strain used during the alcoholic fermentation.[26] C_6-alcohols and C_6-aldehydes are precursors to hexyl acetate.[27] In the literature, based on study of Chardonnay wines, concentrations of hexyl acetate vary between 0.020 and 0.068 mg L^{-1},[5,6] ethyl 9-decenoate 0.020 mg L^{-1},[28] and 3-ethoxy-1-propanol 0.099 mg L^{-1},[5] which is in line with our research.

Skin contact (4 h) and the use of cuvee blanc enzyme preparation resulted in the higher content of ethyl octanoate in Žižak wine (p<0.05) as compared to Žižak Ctrl.

Diethyl hydroxybutanedioate (diethyl malate) and ethyl 4-hydroxybutanoate are present in Žižak wines, and their trace amounts are found in Krstač wines. By using the Tukey *post-hoc* test, the highest statistically significant difference in the content of ethyl 4-hydroxybutanoate (p<0.05) between Z CB4h and Z EB4h was established. Vinification with skin contact of 8 h and the use of Lallzyme β enzyme preparation increased content of diethyl hydroxybutanedioate (p<0.05), as compared to Z CB8h and Z EB4h wines. The concentration is considerably higher in skin contact wine (Z EB8h) than in the control wine, which is in line with the data found in the literature.[9]

The precursor of diethyl hydroxybutanedioate is malic acid.[29] The correlation between diethyl hydroxybutanedioate and its precursor indicates potential reason for the different content of this compound in Žižak and Krstač varieties.

Higher ethyl 4-hydroxybutanoate content in Žižak wines, as compared to Krstač wines, may be interpreted as a result of glutamic acid higher concentration in Žižak grape juice. Ethyl 4-hydroxybutanoate is produced from glutamic acid through 4-hydroxybutanoic acid.[26,30]

Žižak wines produced by addition of Lallzyme β enzyme preparation and skin contact of 4 h had statistically significant higher content of 1,3-propanediol diacetate (p < 0.05), as compared to wine which was produced with the same enzyme preparation and prolonged skin contact time (8 h).

Content of isoamyl acetate was higher in Z Ctrl wine (0.69 mg L^{-1}) compared to K Ctrl wine (0.27 mg L^{-1}). Obtained results are in line with the literature data which reported similar findings.[25] It is interesting to emphasize that higher concentration of isoamyl acetate could contribute to “banana” nuance aroma of wines.[15]

Concentration of 2-phenylethyl acetate in Krstač wines was from 0.16 to 0.30 mg L^{-1} while in Žižak from 0.13 to 0.29 mg L^{-1}. Similar content was observed in Muscat of Alexandria wine[9] while the study of Loureira wines reported 0.26 to 0.30 mg L^{-1}.[25] Diethyl succinate content in Krstač wines was in the range from 0.68 to 1.07 mg L^{-1} while in Žižak from 0.45 to 0.96 mg L^{-1}. Our findings are in accordance with the literature data related to content of diethyl succinate.[2] Only one lactone detected in this study, γ-butyrolactone, had higher concentration in Krstač wines compared to Žižak.

Sensory evaluation of wines produced from Krstač and Žižak varieties

Wine samples produced from Krstač variety after skin contact (4 and 8 h, 5 °C) and usage of enzymatic preparations (Lallzyme Cuvee blanc and Lallzyme enzymatic preparation β) have had bright yellow colour and were without any difference in colour between different vinifications. Aroma intensity increased from K Ctrl wine to K EB8h wine which was the most intense. The taste of wine from different vinification showed remarkable difference. Wine obtained after vinification in which skin contact was applied during the 8 h and Lallzyme enzymatic preparation β had the soft taste and long-lasting aroma. Vinification with skin contact during the 8 h and Lallzyme Cuvee blanc influenced the production of wine without bitterness and hardness, while control showed the most intense sharp and bitter taste. Krstač wine samples, obtained after skin contact during the 8 h, have had higher grades after sensory evaluation compared to 4 h skin contact. The lowest grades, after sensory evaluation, were recorded for control vinification (without skin contact and enzymatic preparations, Fig. 1).

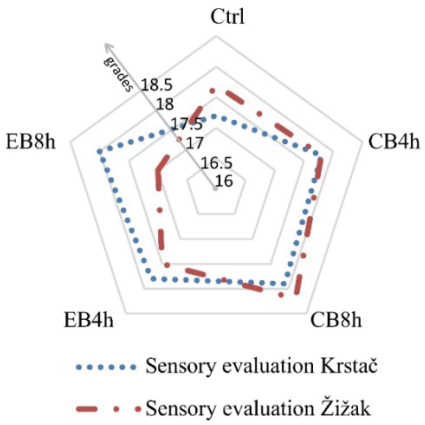

Fig. 1. Sensory evaluation Krstač and Žižak wines.

Žižak variety wines produced after skin contact (4 and 8 h, 5 °C) and usage of enzymatic preparations (Lallzyme Cuvee blanc and Lallzyme enzymatic preparation β) have had pale gold colour almost same intensity in all samples. All samples have had fruity aroma. Aroma increased in samples in which was skin

contact prolonged. The most intense aroma was in Z CB8h sample, while lowest intensity was observed for Z Ctrl. The taste of Z CB8h wine characterized as the most fruity and the softest. Žižak wines obtained after vinification, in which was applied Lallzyme enzymatic preparation β, have had softer taste without bitterness and astringency. Generally, the best taste characteristics showed Z CB8h wine (Fig. 1). Observing all samples from both varieties the highest grades, after sensory evaluation, were obtained after 8 h skin contact K EB8h (18.0 out of 20.0) and Z CB8h (18.2 out of 20.0, Fig. 1).

CONCLUSION

The wines produced with skin contact showed mostly higher content of 2-phenylethyl, isoamyl and other fusel alcohols. Krstač wines obtained after applied skin contact (4 and 8 h) and usage of enzymatic preparation showed higher content of hexanoic and octanoic acids. Significant concentrations of ethyl octanoate and ethyl 4-hydroxybutanoate were observed in wines produced from Žižak. Skin contact (4 and 8 h) and usage of enzymatic preparation increased content of ethyl butyrate, ethyl hexanoate, ethyl octanoate, diethyl succinate, isoamyl acetate and 2-phenylethyl acetate in wines produced from both cultivars. Observing all samples from both varieties the highest grades, after sensory evaluation, were obtained after 8 h skin contact K EB8h and Z CB8h.

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ИЗВОД

УТИЦАЈ РАЗЛИЧИТИХ ЕНЗИМСКИХ ТРЕТМАНА И ВРЕМЕНА КОНТАКТА ПОКОЖИЦЕ НА АРОМАТСКЕ ПРОФИЛЕ ВИНА ПРОИЗВЕДЕНИХ ОД АУТОХТОНИХ СОРТИ ГРОЖЂА КРСТАЧ И ЖИЖАК

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Ова студија је имала за циљ да прикаже профиле ароматичних једињења вина произведених од аутохтоних сорти грожђа Крстач (K) и Жижак (Z). Током производње вина од обе сорте коришћени су ензимски препарати (EP): Lallzyme cuvee blanc (CB), Lallzyme enzymatic preparation β (EB) и различито време контакта покожице (4 и 8 h). Ароматична једињења су анализирана GC/FID–MS техником. За вина K EB4h (176,33 mg L^{-1}) и Z CB4h (177,29 mg L^{-1}) уочава се значајно већи садржај укупних ароматичних једи-

њења у поређењу са одговарајућим контролним винима (168,54 and 161,72 mg L^{-1}). Продужење времена контакта покожице и употреба EP углавном повећава садржај 2-фенилетил- и изоамил-алкохола. Вина обе сорте су показала већи садржај хексанске и октанске киселине у односу на контролна вина. Занимљиво је поменути да је у свим узорцима повећан садржај естара који су одговорни за воћну арому вина, која је заслужна за пријатан укус (изоамил-ацетат – банана, етил-хексаноат – зрела банана, 2-фенилетил-ацетат – јак воћни мирис руже), у поређењу са контролним винима. Највише оцене, након сензорног оцењивања, добијене су за K EB 8h (18,0 од максималних 20,0) и Z CB 8h (18,2 од максималних 20,0).

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REFERENCES

1. U. Čakar, S. Šobajić, B. Vidović, B. Djordjević, *Progr. Nutr.* **20** (2018) 38 (https://doi.org/10.23751/pn.v20i1.5435)
2. E. Falqué, P. Darriet, E. Fernández, D. Dubourdieu, *Int. J. Food Sci. Technol.* **43** (2008) 464 (https://doi.org/10.1111/j.1365-2621.2006.01474.x)
3. M. J. Gómez-Míguez, M. Gómez-Míguez, I. M. Vicario, F. J. Heredia, *J. Food Eng.* **79** (2007) 758 (https://doi.org/10.1016/j.jfoodeng.2006.02.038)
4. E. Sánchez-Palomo, R. Alonso-Villegas, M. A. González-Viñas, *Food Chem.* **173** (2015) 1195 (https://doi.org/10.1016/j.foodchem.2014.10.148)
5. S. Selli, A. Canbas, T. Cabaroglu, H. Erten, Z. Günata, *Food Chem.* **94** (2006) 319 (https://doi.org/10.1016/j.foodchem.2004.11.019)
6. E. Sánchez-Palomo, M. A. González-Viñas, M. C. Díaz-Maroto, A. Soriano-Pérez, M. S. Pérez-Coello, *Food Chem*. **103** (2007) 631 (https://doi.org/10.1016/j.foodchem.2006.08.033)
7. E. García, J. L Chacón, J. Martínez, P. M. Izquierdo, *Food Sci. Tech. Int*. **9** (2003) 33 (https://doi.org/10.1177/1082013203009001006)
8. T. Cabaroglu, A. Canbas, R. Baumes, C. Bayonove, J. P. Lepoutre, Z. Günata, *J. Food Sci.* **62** (1997) 680 (https://doi.org/10.1111/j.1365-2621.1997.tb15434.x)
9. T. Cabaroglu, A. Canbas, *Acta Aliment. Hung.* **31** (2002) 45 (https://doi.org/10.1556/aalim.31.2002.1.5)
10. S. Selli, A. Canbas, T. Cabaroglu, H. Erten, J. P. Lepoutre, Z. Günata, *Food Control* **17** (2006) 75 (https://doi.org/10.1016/j.foodcont.2004.09.005)
11. D. Kechagia, Y. Paraskevopoulos, E. Symeou, M. Galiotou-Panayotou, Y. Kotseridis, *J. Agric. Food Chem.* **56** (2008) 4555 (https://doi.org/10.1021/jf073550q)
12. M. Maggu, R. Winz, P. A. Kilmartin, M. C. T. Trought, L. Nicolau, *J. Agric. Food Chem.* **55** (2007) 10281 (https://doi.org/10.1021/jf072192o)
13. E. García, J. L. Chacón, J. Martínez, P. M. Izquierdo, *Food Sci. Tech. Int.* **9** (2003) 33 (https://doi.org/10.1177/1082013203009001006)
14. S. C. Diéguez, L. C. Lois, E. F. Gómez, M. L. G. de la Peña, *LWT – Food Sci. Technol.* **36** (2003) 585 (https://doi.org/10.1016/S0023-6438(03)00064-1)
15. L. Armada, E. Fernández, E. Falqué, *LWT – Food Sci. Technol.* **43** (2010) 1517 (https://doi.org/10.1016/j.lwt.2010.06.009)
16. M. Ferrando, C. Güell, F.López, *J. Agric. Food Chem.* **46** (1998) 1523 (https://doi.org/10.1021/jf9703866)

17. V. M. Burin, T. M. Gomes, V. Caliari, J. P. Rosier, M. T. B. Luiz, *Microchem. J.* **122** (2015) 20 (https://doi.org/10.1016/j.microc.2015.03.011)
18. U. Čakar, A. Petrović, M. Janković, B. Pejin, V. Vajs, M. Čakar, B. Djordjević, *Eur. J. Hortic. Sci.* **83** (2018) 49 (https://doi.org/10.17660/eJHS.2018/83.1.7)
19. N. Lisov, A. Petrović, U. Čakar, M. Jadranin, V. Tešević, Lj. Bukarica-Gojković, *Maced. J. Chem. Chem. Eng.* **39** (2020) 185 (http://dx.doi.org/10.20450/mjcce.2020.2060)
20. V. Avram, C. G. Floare, A. Hosu, C. Cimpoiu, C. Măruţoiu, Z. Moldovan, *Anal. Lett.* **48** (2014) 1099 (https://doi.org/10.1080/00032719.2014.974054)
21. S. Veljović, N. Tomić, M. Belović, N. Nikićević, P. Vukosavljević, M. Nikšić, V. Tešević, *Food Technol. Biotechnol.* **57** (2019) 408 (https://doi.org/10.17113/ftb.57.03.19.6106)
22. T. Cabaroglu, S. Selli, A. Canbas, J. P. Lepoutre, Z. Günata, *Enzyme Microb. Tech.* **33** (2003) 581 (https://doi.org/10.1016/S0141-0229(03)00179-0)
23. S. M. Rocha, P. Coutinho, I. Delgadillo, A. D. Cardoso, M. A. Coimbra, *J. Sci. Food Agric.* **85** (2005) 199 (https://doi.org/10.1002/jsfa.1937)
24. A. Rapp, G. Versini, *Developments in food science, influence of nitrogen compounds in grapes on aroma compounds of wines*, San Michele all'Adige, 1995, p. 1659 (https://doi.org/10.1016/S0167-4501(06)80257-8)
25. E. Falqué, E. Fernández, D. Dubourdieu, *J. Agric. Food Chem.* **50** (2002) 538 (https://doi.org/10.1021/jf010631s)
26. A. Antonelli, L. Castellari, C. Zambonelli, A. Carnacini, *J. Agric. Food Chem.* **47** (1999) 1139 (https://doi.org/10.1021/jf9807317)
27. E. G. Dennis, R. A. Keyzers, C. M. Kalua, S. M. Maffei, E. L. Nicholson, P. K. Boss, *J. Agric. Food Chem.* **60** (2012) 2638 (https://doi.org/10.1021/jf2042517)
28. H. Li, Y. S. Tao, H. Wang, L. Zhang, *Eur. Food Res. Technol.* **227** (2008) 287 (http://dx.doi.org/10.1007/s00217-007-0722-9)
29. L. Khvalbota, A. Machyňáková, J. Čuchorová, K. Furdíková, I. Špánik*, J. Food Comp. Anal.* **96** (2021) (https://doi.org/10.1016/j.jfca.2020.103719)
30. L. Dufossé, A. Latrasse, H. E. Spinnler, *Sci. Aliments* **14** (1994) 17 (https://hal.archives-ouvertes.fr/hal-02637614).