CHANGES IN VOLATILE COMPOSITION OF KRALJEVINA WINES BY CONTROLLED MALOLACTIC FERMENTATION

UTJECAJ KONTOROLIRANE MALOLAKTIČNE FERMENTACIJE NA SASTAV HLAPIVIH SPOJEVA U VINIMA KRALJEVINE

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ABSTRACT

The effect of malolactic fermentation (MLF) on the volatile composition of white wines made from autochtonous grape variety Kraljevina was studied by inoculation with selected lactic acid bacteria. At the end of malolactic fermentation, after the decomposition of the malic acid present in wine the non volatile compounds were analyzed by HPLC, while volatile compounds were analyzed by gas chromatography. All wines were also sensory analyzed. Results showed changes in acetaldehyde, some higher alcohols, ethyl esters, free and bound monoterpenes and some organic acids that contribute to enhance the sensory properties and quality of Kraljevina wines that underwent malolactic fermentation.

Keywords: Kraljevina, malolactic fermentation, volatile compounds

SAŽETAK

Istraživan je utjecaj malolaktične fermentacije (MLF) na sastav hlapivih spojeva u vinima Kraljevine primjenom selekcioniranih mliječno kiselih bakterija. Malolaktična fermentacija te analiza nehlapivih spojeva provedena je na HPLC-u dok su hlapive komponente analizirane pomoću plinske kromatografije. Sva vina također su i senzorno ocijenjena. Rezultati su pokazali promjene u koncentracijama acetaldehida, nekih viših alkohola, etil estera, slobodnih i vezanih monoterpena te pojedinih organskih kiselina što je pridonijelo poboljšanju senzornih svojstava i kvalitete vina Kraljevina u kojima je provedena malolaktična fermentacija.

Ključne riječi: Kraljevina, malolaktična fermentacija, hlapivi spojevi

DETALJNI SAŽETAK

Glavni cilj ovoga istraživanja bio je utvrditi utjecaj kontrolirane malolaktične fermentacije na sastav hlapivih spojeva u vinima Kraljevine. U istraživanju je korišteno grožđe autohtone sorte Kraljevina branog na dva proizvodna lokaliteta. Alkoholna fermentacija provedena je primjenom autohtonih sojeva kvasca iz kolekcije Zavoda za mikrobiologiju, Agronomskog fakulteta Sveučilišta u Zagrebu. Malolaktična fermentacija provedena je upotrebom selekcionirane mliječno kiselih bakterija Uvaferm Alpha, Lallemand. Nehlapive komponente analizirane su primjenom tekućinske kromatografije dok su hlapivi spojevi analizirani metodom plinske kromatografije. Vina su senzorno ocijenjena metodom 100 pozitivnih bodova. Dobiveni rezultati istraživanja ukazali su na signifikantne razlike u sadržaju acetaldehida, nekih viših alkohola, etil estera slobodnih i vezanih monoterpena te pojedinih organskih kiselina što je prodonijelo poboljšanju senzornih svojstava i kvalitete vina Kraljevina u kojima je provedena malolaktična fermentacija. Rezultati senzornog ocijenjivanje vina pokazali su da su vina koja su prošla malolaktičnu fermentaciju bila superiornija u odnosu na kontrolna vina. Vina u kojim je prošla MLF bila su punija, zaokruženijeg okusa te kompleksne retronazalne arome. Rezultati istraživanja ukazali su i na značajan utjecaj vinogradarskog položaja na kvalitetu dobivenog vina.

INTRODUCTION

Malolactic fermentation (MLF) is a secondary fermentation in wine during which L-malic acid is degraded to L-lactic acid and carbon dioxide. MLF usually occurs after yeasts have completed the primary alcoholic fermentation and is important for the deacidification of high acid wines [14]. During MLF the bacteria can also affect the final aroma balance by modifying fruity aromas and producing aroma active compounds by themselves [7]. MLF may occur spontaneously by lactic acid bacteria (LAB) naturally present in wine or may be induced by the addition of one or more strains of commercial wine LAB. Different studies have focused on the biosynthesis of aroma compounds during MLF and the concomitant organoleptic consequences [12]. Maicas et al. [15] demonstrated that MLF noticeably changes major and minor volatile compounds that are beneficial to wine flavor during MLF. The observation that the impact of bacterial metabolism on the aroma characteristics of wine varies largely according to the type of grape employed for winemaking [9] suggests that varietal aroma compounds may be involved in the flavor modification related to MLF. The ability of lactic acid bacteria to reveal bound aroma compounds by the release of glycosidic enzymes active under winemaking conditions may also contribute to the modification of the flavor character of wine after MLF. Grimaldi et al. [6] observed a significant ß-glucosidase activity in several O. oeni strains, whereas Boido et al. [1] reported a decrease of glycosilated aroma compounds during MLF of Tannat wine. Acetaldehyde is one of the most important sensory carbonyl compounds formed during vinification and mainly originates from yeast metabolism during alcoholic fermentation [13]. The purpose of this work was to investigate the volatile compounds and sensory properties changes in Kraljevina wines from two different locations due to the malolactic fermentation.

MATERIAL AND METHODS

Yeast and lactic acid bacteria

For alcoholic fermentation S. paradoxus strain RO54 from the wine yeast collection of the Department of microbiology, Faculty of Agriculture was used, while Oenococcus oeni strain used for MLF was Uvaferm Alpha, Lallemand.

Fermentation

Kraljevina grapes from two different winegrowing locations (Pušćak, Krč), in Zelina vineyard, were harvested during the 2002 season, destemmed and crushed. The free-run juice was treated with 50 mg/l of SO_2 and allowed to settle overnight. The juice was racked and the must divided into 500-L steel tank where alcoholic fermenation was carried on. After completion of alcoholic fermentation, the wines were racked and divided into 100-litre inox tanks according to the following treatments: A-suppressed MLF with addition of 100 mg SO_2/l, B-inoculated MLF with starter culture Oenococcus oeni. All treatments were carried out in duplicate. For the inoculum of yeast culture S. paradoxus strain was preincubated in sterilized grape must for 48 h at 25 ^{o}C and inoculated at a final level of $5x10^6$ (CFU/ml). The inoculation of Oenococcus oeni strain was at the end of alcoholic fermentation fermentation according to the instructions given by the producer. During alcoholic fermentation the temperature did not exceed 18 °C. Complete sugar degradation was finished within 25 days. Malolactic fermentation was conducted at 22 °C and followed by measuring the concentration of malic acid by the HPLC method. After fourteen days malic acid degradation was completed and the wines samples were sulfited with 100 mg/l of SO_2 and stored under cellar condition.

Chemical analysis

Table 1: Chemical composition of Kraljevina must from two winegrowing locations
Tablica 1: Kemijski sastav mošta Kraljevine s dva vinogradarska položaja

Compounds/Sastojak	**Winegrowing location PUŠĆAK Vinogradarski položaj PUŠĆAK**	**Winegrowing location KRČ Vinogradarski položaj KRČ**
Sugar/Šećer (g/l)	174	175
pH	3.20	3.11
Total acidity/Ukupna kiselost (g/l)*	10.00	10.10
Tartaric acid/Vinska kiselina (g/l)	7.3	7.6
Malic acid/Jabučna kiselina (g/l)	3.2	4.6
Citric acid/Limunska kiselina (mg/l)	210	85

*as tartaric acid/kao vinska kiselina

Table 2: Chemical composition of Kraljevina wines, two winegrowing locations, after alcoholic and malolactic fermentation
Tablica 2: Kemijski sastav vina Kraljevina, dva vinogradarska položaja, nakon alkoholne i malolaktične fermentacije

Compounds/Sastojak	**Winegrowing location PUŠĆAK Vinogradarski položaj Pušćak**			**Winegrowing location KRČ Vinogradarski položaj Krč**		
	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD
	$\overline{x}$			$\overline{x}$		
Total acidity/Ukupna kiselost (g/l)*	6.9	5.8	5%=0.32 1%=0.58	7.0	6.2	5%=0.47 1%=0.87
Volatile acidity/Hlapiva kiselost(g/l)**	0.26	0.30	n.s.	0.35	0.40	n.s.
pH	3.22	3.24	n.s.	3.21	3.26	n.s.
Tartaric acid/Vinska kiselina (g/l)	3.20	3.20	n.s.	2.40	2.30	n.s.
Malic acid/Jabučna kiselina (g/l)	2.20	n.d.		4.10	n.d.	
Lactic acid/Mliječna kiselina (g/l)	0.1	1.7	5%=0.15 1%=0.29	0.1	2.8	5%=0.31 1%=0.58
Citric acid/Limunska kiselina (mg/l)	210	200	n.s.	85	80	n.s.
Succinic acid/Jantarna kiselina (g/l)	0.60	0.65	n.s.	0.70	0.70	n.s.
Glycerol/Glicerol (g/l)	7.9	7.7	n.s.	7.2	7.0	n.s.
Ash/Pepeo (g/l)	1.69	1.60	n.s.	1.86	1.80	n.s.

*as tartaric acid/kao vinska kiselina; **as acetic acid/kao octena kiselina; n.d. not detected/; n.s. not significant/nesignifikantno

Basic chemical analyses of must and wine were done using methods proposed by O.I.V. [17]. Higher alcohols and acetaldehyde analysis were performed by gas chromatography method by Garbi and Salvagiotto [5]. Volatile fatty acids, ethyl esters of fatty acids, higher alcohol acetates and organic acids were analyzed by methods described by Herjavec et al. [10]. Free and bound monoterpenes were analyzed according to method by Radeka [19]. Organic acid analyses were performed on the HPLC Modules Hewlett Packard 1050 Series comprising a quaternary pump, an online degaser, manual injector, a VW detector linked to a HP (Hewlett Packard) 3395 Integrator. The chromatographic separations were done on Bio Rad Aminex HPX 87H (300 x 7.8 mm i.d) organic acid cation exchange column heated to 65 °C. The mobile phase was 0,065 % (v/v) H_3PO_4 in double glass distilled water with a flow rate of 0.6 ml/min. Detection of organic acids was measured by absorption at 210 nm. Acids were quantified by integration of peak height and calibrated with an external standard. Higher alcohols, ethyl acetate and acetaldehyde analyses were performed on FISION gas chromatograph from distillate of wine and 3-methil-

Table 3: Higher alcohol and acetaldehyde concentrations in Kraljevina wines after alcoholic and malolactic fermentation
Tablica 3: Koncentracija viših alkohola i acetaldehida u vinima Kraljevina nakon alkoholne i malolaktične fermentacije

Compound (mg/l)/ Sastojak	Winegrowing location PUŠĆAK Vinogradarski položaj Pušćak			Winegrowing location KRČ Vinogradarski položaj Krč		
	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD
	$\bar{x}$			$\bar{x}$		
1- Propanol	18	18.5	n.s. n.s.	18.5	18	n.s. n.s.
1-Hexanol	0.42	0.46	n.s. n.s.	0.35	0.35	n.s. n.s.
Isobutanol	44.5	64.5	5%=3.2 1%=5.8	43.5	43.5	n.s. n.s.
Amyl alcohol	59	60	n.s. n.s	46.5	47.5	n.s. n.s.
Isoamyl alcohol	226	227.5	n.s. n.s.	227	228	n.s. n.s.
2-Phenyl ethanol	49	50	n.s. n.s.	45	40	5%=3.2 n.s.
Σ Higher alcohols/Viši alkoholi	396.92	420.96	5%=1.6 1%=2.9	380.90	377.35	n.s. n.s.
Acetaldehyde	19	15.5	5%=0.2 1%=0.4	18.0	16	5%=0.6 1%=1.2

2 butanol as internal standard. Volatile wine components (esters, fatty acids, monoterpens) are determined on CARLO ERBA gas chromatograph, on two capillary columns (CarboWax and DBWax). Sample preparation was made by solid-phase extraction (SPE) using XAD-2 cartridges and elution with pentane: dichlorometane (2:1) solvent. Temperature programming was: 5 min isothermal at 55 °C than linear temperature rise of 2.5 °C/min to 175 °C, (10 min) than linear temperature rise of 2.5 °C/min to 188 °C (10 min), than linear temperature rise of 10 °C/min to 192 °C (55 min). Injector and detector temperature was 250 °C, split mode 1:30, carrier gas used was hydrogen (2 ml/min). Glycerol was analyzed by enzymatic method on UV-VIS spectrophotometer (Boehringer Mannheim No. 148 270).

Sensory analysis

The wines were subjected to sensory evaluation by the 100-point O.I.V./U.I.O.E method [2], with a panel of 12 judges.

Statistical analysis

One-way analysis of variance (ANOVA) and Least Significant Difference (LSD) comparison test of SAS [21] were used to interpret differences in means, if any, at the 95% and 99% confidence level.

RESULTS AND DISCUSSION

Chemical composition of the must and wine

The chemical composition of Kraljevina musts are presented in the table 1. There were no marked differences in sugar content and total acidity content between investigated locations except in the malic acid concentration. Must from winegrowing location Krč had 1.4 g/l more malic acid compared with must from location Pušćak. The table 2 shows the chemical composition of Kraljevina wines before and after MLF. Results were within the normal range of values expected. Significant difference was noted in the total acidity and malic and lactic acid concentrations. There was no marked increase in volatile acidity and pH values. According to [10], [16], and [3] citric acid can be metabolized up to 50% what was not the case in our investigation. The reason for that can be a relatively low concentration of citric acid especially in wines from winegrowing location Krč (table 2). There were no difference in the concentration of succinic acid and glycerol what is in accordance with literature data [8].

Concentration of higher alcohols and acetaldehyde

Higher alcohols are considered to be produced from amino acids or from hexoses through pyruvate and appear to contribute to the flavor of wines. In wines which had undergone the MLF no significant increase was recorded

Table 4: Concentrations of volatile ester compounds and fatty acids in Kraljevina wines after alcoholic and malolactic fermentation
Tablica 4: Koncentracija hlapivih estera i masnih kiselina u vinima Kraljevine nakon alkoholne i malolaktične fermentacije

Compound (mg/l)/ Sastojak	Winegrowing location PUŠĆAK Vinogradarski položaj Pušćak			Winegrowing location KRČ Vinogradarski položaj Krč		
	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD
	$\overline{x}$			$\overline{x}$		
Ethyl acetate	17.0	20.5	5%=0.4 1%=0.8	22.5	25.5	5%=0.7 1%=1.5
Isobutyl acetate	0.02	0.03	n.s. n.s.	0.09	0.07	n.s. n.s.
Isoamyl acetate	0.32	0.35	n.s. n.s.	2.77	1.49	5%=0.1 1%=0.2
Hexyl acetate	0.01	0.03	n.s. n.s.	0.03	0.01	n.s. n.s.
2-Phenylethyl acetate	0.08	0.08	n.s. n.s.	0.39	0.20	5%=0.8 n.s.
Σ Acetate esters/ Acetatni esteri	0.43	0.49	n.s. n.s.	3.28	1.77	5%=0.4 1%=0.8
Ethyl butyrate	0.10	0.10	n.s. n.s.	0.24	0.31	n.s. n.s.
Ethyl caproate	0.19	0.19	n.s. n.s.	0.63	0.53	n.s. n.s.
Ethyl caprylate	0.32	0.34	n.s. n.s.	1.14	0.95	5%=0.1 n.s.
Ethyl caprate	0.08	0.07	n.s. n.s.	0.25	0.22	n.s. n.s.
Σ Ethyl esters of fatty acids/ Etil esteri masnih kiselina	0.69	0.70	n.s. n.s.	2.26	2.01	5%=0.1 n.s.
Ethyl lactate	0.90	5.50	5%=0.66 1%=1.22	1.12	5.73	5%=0.4 1%=0.8
Diethyl succinate	0.49	0.47	n.s. n.s.	0.48	0.82	5%=0.2 n.s.
Caproic acid	1.15	1.16	n.s. n.s.	3.65	3.24	5%=0.1 1%=0.2
Caprylic acid	2.25	2.55	5%=0.3 n.s.	7.29	6.55	5%=0.5 n.s.
Capric acid	0.29	0.51	5%=0.11 1%=0.2	1.76	1.60	5%=0.1 n.s.
Σ Fatty acids/ Masne kiseline	4.23	4.67	n.s. n.s.	12.70	11.39	5%=0.8 n.s.

what is in agreement with previous results by [10], [12]. Significant difference was noted in the acetaldehyde concentrations in wines from both winegrowing locations. Lactic acid bacteria are able to metabolize acetaldehyde what is confirmed in our investigation and is in agreement with literature data [11], [18].

Concentration of volatile aroma compounds

The results are shown in tables 4, 5 and 6. Significant differences were noted in the concentrations of ethyl acetate and ethyl lactate. After MLF wines from both winegrowing locations had 2.5 to 3.0 mg/l more ethyl acetate and about 5 mg/l more ethyl lactate than control

Table 5: Free monoterpene concentration in Kraljevina wines after alcoholic and malolactic fermentation
Tablica 5: Koncentracija slobodnih monoterpena u vinima Kraljevine
nakon alkoholne i malolaktične fermentacije

Compound (mg/l)/ Sastojak	Wine growing location PUŠĆAK Vinogradarski položaj Pušćak			Wine growing location KRČ Vinogradarski položaj Krč		
	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD
	$\overline{x}$			$\overline{x}$		
trans-Furan linalool oxide	n.d.	n.d.		n.d.	n.d.	
cis-Furan linalool oxide	0.4	1.1	5%=0.11 1%=0.2	0.7	2.7	5%=0.14 1%=0.26
trans-Pyran linalool oxide	0.5	1.8	5%=0.25 1%=0.51	0.7	2.1	5%=0.33 1%=0.61
cis-Pyran linalool oxide	1.6	2.0	5%=0.05 1%=0.11	1.1	1.1	n.s.
Σ Linalool oxides/ Linalol oksidi	**2.5**	**4.9**	5%=0.6 1%=1.2	**2.5**	**5.9**	5%=0.25 1%=0.51
Linalool	3.3	2.4	5%=0.25 1%=0.51	3.4	2.2	5%=0.15 1%=0.30
α-Terpineol	1.3	1.2	n.s.	0.9	1.0	n.s.
Citronellol	n.d.	n.d.		n.d.	n.d.	
Nerol	n.d.	n.d.		n.d.	n.d.	
Geraniol	1.3	0.9	5%=0.06 1%=0.12	1.3	0.9	5%=0.05 1%=0.11
HO-diol (I)	4.3	3.5	5%=0.11 1%=0.20	4.3	2.6	5%=0.33 1%=0.61
HO-diol (II)	1.9	1.8	n.s.	2.0	2.0	n.s.
Σ Free monoterpenes/ Slobodni monoterpeni	**14.6**	**14.7**	n.s.	**14.4**	**14.6**	n.s.

wines what is in accordance with data published by [15], [11]. Kraljevina wines from investigated winegrowing locations differ in concentrations of acetate esters. Only in the wines from location Krč significant decrease in isoamyl acetate and 2-phenylethyl acetate was noted. One can find different literature data of MLF influence on ester concentration changes. According to [25] a decrease of isoamyl acetate was noted in MLF wines. Contrary to that Laurent et al. [12] noticed large increase of isoamyl acetate and no variation in the amount of ethyl esters of fatty acids such as ethyl butyrate, caprylate and caprate. According to [11] diethyl succinate can be synthesized by lactic acid bacteria. Our results partially confirm that statement because significant increase was noted only in wines from winegrowing location Krč.

The complex array of aroma and flavor compounds found in wine largely originates from grape, yeast metabolism during alcoholic fermentation and oak when used. Bacterial metabolism during MLF contributes to wine flavor by the formation of additional compounds and the modification of grape, yeast and oak derived compounds [15], [12]. Many volatile compounds can be released from their flavorless glycoconjugate precursors by acid or enzymatic hydrolysis [22]. Even thou the monoterpene concentration in wine are stabile during the storage time the changes can occur. According to [20] oxidation or some other transformation pathway can influence the changes in monoterpene alcohol

Table 6: Bound monoterpene concentration in Kraljevina wines after alcoholic and malolactic fermentation
Tablica 6: Koncentracija vezanih monoterpena u vinima Kraljevine nakon alkoholne i malolaktične fermentacije

Compound (mg/l) Sastojak	Winegrowing location PUŠĆAK Vinogradarski položaj Pušćak			Winegrowing location KRČ Vinogradarski položaj Krč		
	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	LSD
	$\overline{x}$			$\overline{x}$		
trans-Furan linalool oxide	1.7	1.8	n.s.	1.5	1.3	5%=0.06 1%=0.12
cis-Furan linalool oxide	1.5	0.7	5%=0.11 1%=0.20	0.8	0.9	n.s.
trans-Pyran linalool oxide	<0.5	<0.5	n.s.	<0.5	<0.5	n.s.
cis-Pyran linalool oxide	<0.5	<0.5	n.s.	<0.5	<0.5	n.s.
Σ Linalool oxides/ Linalol oksidi	**4.2**	**3.5**	5%=0.14 1%=0.26	**3.3**	**3.2**	n.s.
Linalool	0.9	0.7	n.s.	1.3	0.7	5%=0.14 1%=0.26
α-Terpineol	0.6	0.9	n.s.	0.7	<0.5	n.s.
Citronellol	9.5	8.2	5%=0.11 1%=0.20	6.7	6.6	n.s.
Nerol	1.7	0.9	5%=0.33 1%=0.61	0.7	0.7	n.s.
Geraniol	3.4	2.6	5%=0.11 1%=0.20	2.7	1.3	5%=0.33 1%=0.61
HO-diol (I)	2.9	2.8	n.s.	2.6	2.8	n.s.
HO-diol (II)	<0.5	<0.5	n.s.	<0.5	<0.5	n.s.
Σ Bound monoterpenes Vezani monoterpeni	**23.7**	**20.2**	5%=0.33 1%=0.61	**18.5**	**16.3**	5%=0.16 1%=0.29

concentrations mainly due to their transformation to terpene oxides (pyran and furan cis- and trans-form). Results shown in table 5 pointed out significant increase in linalool oxides probably connected with decrease in linalool concentrations. Decrease in geraniol and HO-diol (I) concentrations was also probably connected with their transformation to hotrienol and 8-hydroxy geraniol, geranyl acetate and citronellyl acetate [23]. Vivas et al. [24] have demonstrated that wine (lactic) bacteria, in particular O. oeni are able in red wines to cleave glucose moiety from the malvidin-3-glucoside and use it as a carbon source. Resent work by D´Incecco et al. [4] have shown that lactic acid bacteria can have β-glycosidase activity. According to literature data and results shown in table 6 we can presume that in Kraljevina wines that have undergone MLF enzymatic hydrolysis and liberation of one part of bound monoterpenes occurred. Significant decrease in geraniol, citronellol and nerol concentrations in wines from location Pušćak was noted while in the wines from location Krč decrease was in geraniol and linalool concentrations.

Sensory properties of wines

Results of wine tasting by 100-point O.I.V/U.I.O.E. method are given in Table 7 indicating a substantial effect on the sensory properties of Kraljevina wines. On the basis of presented data wines that had undergone MLF were of better overall quality compared to control wines. Most of the tasters noted that MLF wines were very full and round in taste, with a complex retronasal aroma. The difference between investigated winegrowing locations was also noted. According to the results wines from location Krč were better evaluated (higher total score)

Table 7: Results of Kraljevina wine testing by 100-point O.I.V./U.I.O.E. method (mean value results of 10 judges)
Tablica 7: Rezultati senzornog ocijenjivanja vina Kraljevina metodom 100 bodova (srednja vrijednost od 10 ocijenjivača)

	Wine growing location PUŠĆAK Vinogradarski položaj Pušćak		**Wine growing location KRČ Vinogradarski položaj Krč**	
	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*	Treatment A *Alcoholic fermentation*	Treatment B *Malolactic fermentation*
Total score/ Ukupni zbroj	80.3	82.7	82.7	84.2

compared to wines from location Pušćak. One of the reasons is probably higher concentration of some volatile components analyzed in wines from location Krč (acetate esters, ethyl esters of fatty acids, fatty acids)

CONCLUSIONS

Kraljevina wines from both winegrowing locations that underwent malolactic fermentation were of better quality than wines where MLF was suppressed. It can be stated that lactic acid bacteria implication in winemaking is not only due to malic acid degradation but also to notable modification of the final wine volatile composition. Changes in acetaldehyde, some higher alcohols, ethyl esters, free and bound monoterpenes and acids were noted. Some of this changes, it can be assumed contributed to enhance the sensory properties and quality of Kraljevina wines that have undergone MLF.

REFERENCES

[1] Boido E., Lloret A., Medina K., Carrau F., Dellacasa E., Effect of ß-glycosidase activity of Oenococcus oeni on the glycosylated flavor precursors of Tannat wine during malolactic fermentation, J. Agric. Food Chem. (2002) 50: 2344-2349.

[2] Crettenand J., Tasting cards in international wine competitions, Journal International des sciences de la vigne du vin, special issue (1999) 99-106.

[3] Dittrich H.H., Sponholz W.R., Wunisch B., Wipfler M., Zur Veränderung des Weines durch Bakteriellen säureabbau, Wein-Wiss. (1980) 35: 421-429.

[4] D´Incecco N., Bartowsky E., Kassara S., Lante A., Spettoli P., Henschke P., Release of glycosidically bound flavour compounds of Chardonnay by Oenococcus oeni during malolactic fermentation, Food Microbiology (2004) 21: 257-265.

[5] Gabri G., Salvagiotto R., Dosamento gas-cromatografico simultaneo della acetaldeide, del metanolo, dell´acetato e del lattato di etile, e degli alcoli superiori nei distillati alcolici, Vini d´Italia (1980) 124: 37-43.

[6] Grimaldi A., McLean H., Jiranek V., Identification and partial characterization of glycosidic activities of commercial strains of the lactic acid bacterium, Oenococcus oeni, American Journal of Enology and Viticulture (2000) 41: 362-369.

[7] Henick-Kling T., Control of malo-lactic fermentation in wine: energetics, flavour modification and methods of starter culture preparation, Journal of Applied Bacteriology Supplement (1995) 79: 29-37.

[8] Henick-Kling T., Acree T.E., Modification of wine flavor by malolactic fermentation. In: The management of malolactic fermentation and quality of wine, Lallemand, (1980) Verona, 17-22.

[9] Henick-Kling T., Acree T.E., Krieger S.A., Laurent M.H., Edinger W.D., Modificazioni del gusto indotte dalla fermentazione malolattica, Vignevini (1994) 21: 41-47.

[10] Herjavec S., Tupajić P., Majdak A., Influence of malolactic fermentation on the quality of Riesling wine, Agriculture Conspectus Scientificus (2001) 66: 59-64.

[11] Jackson, R.S., Wine science: Principles and Applications. Academic Press, S.L Taylor, University of Nebraska, 1994.

[12] Laurent M.H., Henick-Kling T., Acree T.E., Changes in the aroma and odor of Chardonnay wine due to malolactic fermentation, Wein-Wissenschaft (1994) 49: 3-10.

[13] Liu S.Q., Pilone G.J., An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications, Int. J. Food Sci. Technol. (2000) 35: 49-61.

[14] Lonvaud-Funel A., Lactic acid bacteria in the quality improvement and depreciation of wine, Int. J. Gen. Mol. Microbiol. (1999) 76: 317-331.

[15] Maicas S., Gil J.V., Pardo I., Ferrer S., Improvement of volatile composition of wines by controlled addition of malolactic bacteria, Food Res. Int. (1999) 32: 491-496.

[16] Nielsen C.J., Prahl C., Lonvaud-Funel A., Malolactic fermentation in wine by direct inoculation with freeze-dried Leuconostoc oenos cultures, American Journal of Enology and Viticulture (1996) 47: 42-48.

[17] O.I.V. Compendium of International Methods of Wine and Must Analysis, Vol. 1, Paris, 2007.

[18] Osborne J.P., Orduna de Mira R., Pilone G.J., Liu S.Q., Acetaldehyde metabolism by wine lactic acid bacteria, FEMS Microbiology Letters (2000) 191:51-55.

[19] Radeka, S., Maceracija masulja i primarne arome vina Malvazije istarske. Dissertation, (2005) Faculty of Agronomy, University of Zagreb, Croatia.

[20] Rapp A., Guntert M., Changes in aroma substances during the storage of white wines in bottles. In: The Shelf Life of Foods and Beverages, Elsevier, 1986, pp. 141-167.

[21] SAS/STAT User,s Guide, version 8 Vol. 2.,SAS. Institute, (1997), Cary, NC., USA.

[22] Sefton M.A., Francis I.L., Williams P.J., The volatile composition of Chardonnay juices: a study by flavor precursor analysis, American Journal of Enology and Viticulture (1993) 44: 359-370.

[23] Strauss C.R., Wilson B., Gooley P.R., Williams P.J., Biogeneration of aromas, American Chemical Society, Washington DC, 1986, pp. 222-225.

[24] Vivas N., Lonvaud-Funel A. Glories, Y., Effect of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium, Food Microbiology (1997)14: 291-300.

[25] Wibowo D., Eschenbruch R., Davis R. C., Lee T.H., Fleet G.H., Occurence and growth of lactic acid bacteria in wine: a review, American Journal of Enology and Viticulture (1985) 36: 302-313.