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Changes in Acidity, some Aroma Compounds and Sensory Properties of Frankovka Wine after Malolactic Fermentation

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Summary

Changes in composition and sensory properties caused by suppressed, natural and induced malolactic fermentation (MLF) using starter cultures of lactic acid bacteria were investigated in Frankovka wine.

Malic acid decomposition was completed in wines of all MLF variants and the result was lowering of total acidity by 2.6 g/L, raising of pH value of wine by 0.12 units and volatile acidity by 0.18 g/L. MLF was not accompanied with tartaric acid degradation, and citric acid concentration was lowered by 0.2 g/L. No changes were noticed in concentration of 1-propanol, isobutanol, isoamyl alcohol, hexanol and 2-phenyl ethanol. During the period of investigations, a significant concentration increase of ethyl lactate and a smaller increase of ethyl acetate were established in wines of MLF variants. Tested wines contained low concentrations of isoamyl acetate, 2-phenylethyl acetate, ethyl caproate, ethyl caprilate, diethyl succinate and acetoin.

Wines of suppressed MLF were of inferior quality compared with malolactic fermented wines, while wines of natural malolactic fermentation were significantly better.

Keywords: malolactic fermentation, acids, volatile compounds, sensory properties, wine

Introduction

Wine is a product made out of grapes and technologically modified, and its quality is mainly the result of a series of biochemical reactions. Among those reactions malolactic fermentation, the conversion of L-malic acid to L-lactic acid and carbon dioxide, plays an important role. The malolactic fermentation of wine results from the metabolic activity of certain strains of lactic acid bacteria. The most notable one is *Leuconostoc oenos*, which has been recently reclassified by Dicks *et al.*(*1*) as *Oenococcus oeni*.

Malolactic fermentation improves the quality of red wines and it is generally important in cool wine producing regions where grapes of high acidity are used (2). The main effect of the malolactic fermentation is a reduction in the titritable acidity. Reduction in wine acidity by malolactic fermentation can vary from 0.1 to 0.3% (*3–5*). Malolactic fermentation increases the pH of wine by 0.1 to 0.3 unit (*3,6*). According to Davis *et al.* (2) malolactic fermentation improves the sensory complexity of wine, producing some compounds that are of considerable importance to the flavor profile of wine. Sauvageot and Vivier (*7*) concluded that MLF in red wines are characterized by animal and vegetable notes. They lose part of their fruity aromas improving the mouthful and length of aftertaste.

Spontaneous MLF generally occurs in wine subsequent to the alcoholic fermentation. It usually takes place within the first year. In some circumstances the fermentation may be considerably delayed, sometimes indefinitely.

In order to stimulate malolactic fermentation in wines rapidly and predictably, oenologists have shown great interest in the use of pure culture inoculation of wines with selected strains of bacteria. Starter cultures of *Leuconostoc oenos* are increasingly used in wine industry to induce malolactic fermentation.

The purpose of this work was to examine the effects of malolactic fermentation on the changes of chemical composition and sensory properties of Frankovka wine.

Material and Methods

Lactic acid bacteria

Two lyophilized cultures of *Leuconostoc oenos*: trade mark Bitec D (Erbslöh Geisenheim, Germany) and trade mark *Viniflora oenos* (Chr. Hansen, Danmark) were reactivated prior to inoculation and added to non-sulfited wines just after alcoholic fermentation.

Vinification

Frankovka grapes used in these experiments were grown in the wine region of continental Croatia, subregion Slavonia. Wine from the 1996 harvest season was made by traditional method of color extraction in the winery of PP Orahovica. Grapes were destemmed and crushed. Must was fermented on the skins in 50000 L stainless steel rototanks at 27 to 30 °C during 80 hours. After completion of alcoholic fermentation, the wines were racked and divided into glass bottles (V = 50 L), according to the following treatments. The first one was suppressed MLF with SO_2 concentration of 100 mg/L, stored at 10 °C. The second treatment included natural MLF, the third MLF was induced with starter culture Bitec D and the last one was induced MLF with *Viniflora oenos*. All treatments were done in triplicate. Malolactic fermentation was conducted at 20 °C and followed by measuring the concentration of malic acid by HPLC method. When MLF was completed, wines were sulfited with 50 mg/L of SO_2. Two rackings were carried out to clarify the wines before bottling. Bottles were stored in a cellar at 10 °C. When analyzed and tasted, the wines had been aged for 2 and 8 months, respectively.

Analytical methods

Ethanol, total and volatile acidity, SO_2 were determined using methods proposed by O.I.V. (*8*). Reducing sugar was determined volumetrically by Lane Eynon method (*9*) and pH was measured on the Beckman Expandomatic SS 2 pH-meter.

Organic acid analyses were performed on the HPLC (Hewlett Packard 1050 series) using UV variable detector at λ = 210 nm. The chromatographic separations were performed on a 300 x 7.8 mm i.d Aminex HPX 87H organic acid cation exchange column (Bio-Rad Laboratories) heated to 65 °C. The mobile phase was H_3PO_4 0.065% (v/v) with a flow rate 0.6 mL/min.

Volatile compounds analysis was done on gas chromatograph (Hewlet Packard 5890) using FID. Analyses of higher alcohols (1-propanol, isobutanol, isoamyl alcohol and ethyl acetate) were performed from wine distillate. Temperature programming was as follows: HP 101 Column, 6 min isothermal at 40 °C, then linear temperature rise of 15 °C min^{-1} to 200 °C.

Analyses of volatile esters, hexanol, 2-phenyl ethanol and acetoin were performed from volatile extracts. Wine (500 mL) was taken in a liquid-liquid upward displacement apparatus and extracted for 10 hours with dichloromethan. The extract was dried over anhydrous sodium sulfate, concentrated to 10 mL and stored prior to GC analysis. Temperature programming was: HP FFAP Column, 5 min isothermal at 60 °C, then linear temperature rise of 2.5 °C min^{-1} to 190 °C and 20 min isothermal at 190 °C. Determination of volatile compounds was done by method of internal standards.

Sensory evaluation of wines

Wines were tested for sensory differences by an experienced panel of 10 judges. Those analyses were made by ranking method in two tasting periods: two months after MLF and eight months after MLF. The determination of statistical significance was done according to literature (*10*).

Results and Discussion

Chemical composition of wine

Chemical composition of wine before and after malolactic fermentation is shown in Table 1.

Concentration of acidic compounds

Decrease of total acidity by up to 2.6 g/L and increase of pH value by up to 0.12 units were established in wines of all MLF variants. Volatile acids concentration in wines of MLF variants were increased by max. 0.18 g/L (Table 1). Organic acids transformation rate is shown in Figs. 1. and 2. Malic acid transformation was completed in all MLF variants, whereas in wines of suppressed MLF variants malic acid concentration remained unchanged. Equal increase in lactic acid concentration was established in wines of all MLF variants. Malolactic fermentation was not accompanied by tartaric acid degradation. Decrease of tartaric acid in suppressed MLF was the result of salts precipitation at low temperature. Citric acid concentration in wines of all MLF variants decreased by 0.2 g/L.

Our results regarding reduction of total acidity and increase of pH values are in agreement with investigations of authors cited by Davis *et al.* (*2*). According to Minarik and Jungova (*11*) total degradation of malic acid greatly depends on *Leuconostoc oenos* strain and on

Table 1. Chemical composition of wine Frankovka before and after MLF

Compounds	Variants				
	Start	suppressed MLF	natural MLF	Bitec D	*Viniflora oenos*
φ (Alcohol) / %	10.7	10.70	10.78	10.70	10.87
γ (Reduc. sugar) / g L^{-1}	2.75	2.50	2.50	2.50	2.40
γ (Total acidity) / g L^{-1}*	8.60	7.7	6.2	6.2	6.0
γ (Volatile acidity) / g L^{-1}**	0.23	0.30	0.41	0.36	0.35
γ (Tartaric acid) / g L^{-1}	3.4	2.6	3.3	3.3	3.3
γ (Malic acid) / g L^{-1}	3.0	2.8	0.0	0.0	0.0
γ (Lactic acid) / g L^{-1}	0.5	0.4	3.9	4.0	4.1
γ (Citric acid) / g L^{-1}	0.8	0.6	0.6	0.6	0.6
pH	3.51	3.5	3.62	3.62	3.62

* as tartaric acid, ** as acetic acid

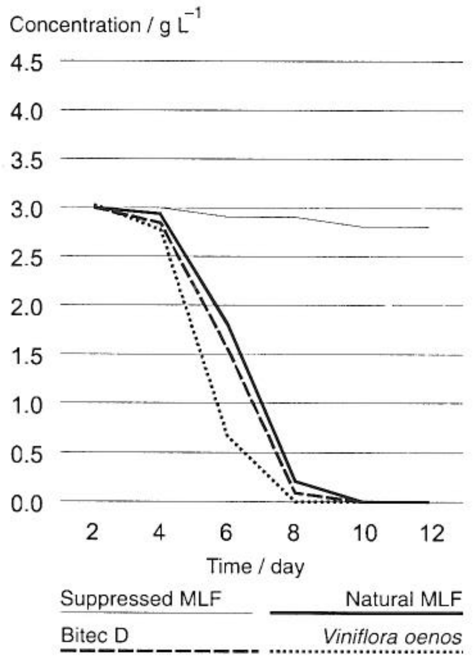

Fig. 1. Transformation of malic acid

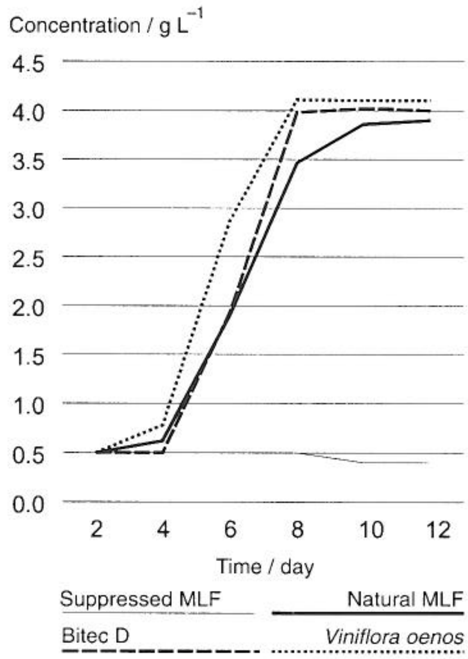

Fig. 2. Formation of lactic acid

the application time of starter culture. According to our results, by the application of *Viniflora oenos* starter culture total transformation of malic acid and strong increase of lactic acid was established. According to the same author, the most efficient degradation of malic acid was reached by addition of starter culture into young wine after the end of the alcoholic fermentation. According to Wibowo *et al.* (*12*), Fornachon did not establish changes in tartaric acid concentration during MLF. Minarik and Jungova (*11*) conclude that controlled MLF with application of *Viniflora oenos* has no effect on tartaric acid content and volatile acidity of wine which is in accordance with our investigations. Opposite to this, Delfini and di Stefano (*13*), Nielsen and Prahl (*14*) and Pilone and Kunkee (*15*) observed the increase in concentration of volatile acids with *Leuconostoc oenos*. Malolactic fermentation can cause decrease of citric acid according to Sato *et al.* (*16*), Nielsen and Prahl (*14*), and Webb (*17*) noted complete metabolization of citric acid. According to Dittrich *et al.* (*18*), decrease of this acid can be up to 50%, which is in accordance with our investigations.

Concentration of volatile compounds

Higher alcohols. Concentrations of higher alcohols in initial wine are shown in Table 2. After malolactic fermentation, changes in concentration of certain investigated higher alcohols were not observed. No changes were observed even after storage of bottled wines. Delfini and di Stefano (*13*) concluded that higher alcohols have minimal variations regarding the absolute values, which did not allow to draw out any conclusions. Our results are in accordance with the above mentioned. In contrast, Davis *et al.* (*2*) noted an increase in n-propanol and n-hexanol, and Radler and Gelwarth (*19*) concluded that *Leuconostoc oenos* in synthetic medium with ethanol produces small quantities of propanol and n-hexanol.

Volatile esters. After malolactic fermentation, there was an increase in the concentration of ethyl lactate up to 37 mg/L in relation to wines of suppressed MLF. After 8 months there was further increase of ethyl lactate up to 57 mg/L. There were no differences in concentration of ethyl lactate among wines of natural or initiated MLF in any given period of investigations, respectively. Also, there were no observed differences regarding applied starter cultures. Small increase in concentration of ethyl lactate was noted in wines of suppressed MLF after the period of 8 months.

Two months after the end of the malolactic fermentation in wines of all variants, small and even increase in concentration of ethyl acetate was detected. The same tendency was kept during the period of bottled wines storage. Concentration of isoamyl acetate, ethyl caproate, ethyl caprilate and diethyl succinate was significantly lower in initial wine after the alcoholic fermentation. Concentration of these components varied during the period of investigations. Nevertheless, definitive conclusions about the rate of these changes are not easy to draw due to their low concentrations. Isobutyl acetate was not detected in wines, and ethyl butyrate was detected in wines two months after the end of malolactic fermentation.

Davis *et al.* (*2*) and Pilone and Kunkee (*15*) reported an increase in the concentration of ethyl lactate in wines after the malolactic fermentation, which is in agreement with our investigation. Pilone and Kunkee (*15*) established that the quantity of diethyl succinate and diethyl caprilate increases depending on type of lactic acid bacteria and on procedure of vinification. Wibowo *et al.* (*12*) report great increase in the concentrations of ethyl acetate and diethyl succinate and small decrease of 2-phenylethyl acetate, isoamyl acetate and ethyl caproate.

Table 2. Mass concentration of volatile compounds in wines

Compounds	Start	Variants							
		suppressed MLF		natural MLF		Bitec D		*Viniflora oenos*	
		a	b	a	b	a	b	a	b
		Mass concentration / mg/L^{-1}							
1-Propanol	22	25	25	25	25	26	25	25	25
Isobutanol	66	66	67	66	68	67	68	67	67
Isoamyl alcohol	298	306	303	298	308	302	306	305	305
Hexanol	0.92	0.93	0.82	0.84	0.94	0.86	0.89	0.95	0.87
2-Phenyl ethanol	37	43	39	34	37	36	36	38	34
Ethyl acetate	13	21	29	21	36	21	33	23	37
Isoamyl acetate	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2
Isobutyl acetate	*	*	*	*	*	*	*	*	*
Phenyl ethyl acetate	0.05	*	*	*	*	*	*	*	*
Ethyl lactate	4.1	6.4	9.0	31	57	29	55	37	55
Ethyl butyrate	*	0.06	0.06	0.05	0.05	0.05	0.06	0.06	0.05
Ethyl caproate	0.1	0.1	0.08	0.06	0.08	0.07	0.08	0.08	0.08
Ethyl caprilate	0.08	0.09	0.08	0.04	0.08	0.06	0.07	0.05	0.07
Diethyl succinate	*	0.14	0.12	0.05	0.12	0.05	0.13	0.06	0.12
Acetoin	1.0	1.0	1.0	2.9	2.5	3.4	2.8	3.7	2.9

a = 2 months after MLF, b = 8 months after MLF

Delfini and di Stefano (*13*) established no variations in the concentration of fatty acid esters, except ethyl caprilate, which had a significant increase.

Acetoin. In relation to the initial wine, an increase in the concentration of acetoin was found in all variants of malolactic fermentation. Concentration of acetoin was lowered during the period of bottled wines storage. The quantities of acetoin found in investigated wines are somewhat lower than those established by Radler (*20*). The same author found approximately 4.3 ppm of acetoin in German non MLF wines, and double of that concentration in MLF wines. Similar results regarding the increase of acetoin during the MLF were reported by Pilone and Kunkee (*15*) and Wibowo *et al.* (*12*). Kunkee *et al.* (*21*) concluded that wines with more acetoin have undergone MLF, but did not establish significant correlation between acetoin concentration and MLF.

Sensory characteristics of wines

Results of two tasting periods-two months after MLF and eight months after MLF- are given in Table 3. The data suggest that wines of suppressed MLF have inferior quality compared with malolactic fermented wines ($p<1\%$). During both periods the wines of natural malolactic fermentation were significantly better evaluated ($p<5\%$). There was no significant difference in quality between wines of induced malolactic fermentation during both periods of evaluation.

Webb (*22*) was the first to report that MLF has an effect on the change of sensory properties of wine. Effects of the malolactic fermentation greatly depend on the cultivar, species and strain of lactic acid bacteria, and degree of wine maturity (*13*), but it is not easy to define them by sensory analysis. Pilone and Kunkee (*15*) concluded that the initially small sensory and chemical differences become more distinct in aged wines. Better

Table 3. Results of the sensory evaluation of wine

Variant	2 months after MLF completion		8 months after MLF completion	
	order	sum	order	sum
Natural MLF	1	11*	1	15*
Bitec D	2	24	2	23
Viniflora oenos	3	32	3	25
Suppressed MLF	4	33	4	37**

quality of MLF wines according to our results corresponds to the data given by Giannakopoulos *et al.* (*5*) and Laboudi, cited in Sauvageot and Vivier (*7*). On the contrary, Kunkee *et al.* (*21*) and Van Wyk (*23*) did not find any differences in quality. Using two *Leuconostoc oenos* strains Minarik and Jungova (*11*) could not determine differences in sensory properties of wines, similarly to Beelman *et al.* (*24*) after a six-month of ageing period, which is in accordance with our results. On the other hand, Giannakopoulos *et al.* (*5*), experimenting with two *Leuconostoc oenos* strains, found the differences in the quality of wines. The sensory evaluation (ranking method) took place six weeks after MLF completion, as well as eight months after bottling.

High titratable acidity, with relatively considerable fraction of malic acid in Frankovka wines, vintage 1996, resulted in a markedly sour and inharmonious taste.

According to our findings, the significantly better quality of MLF Frankovka wines was primarily the result of their changed (lower) acidity. Laboudi, according to Sauvageot and Vivier (*7*) reports similar data for Pinot noir wines of Burgundy.

Frankovka wines that underwent natural malolactic fermentation were characterized, besides lower acidity, with more complex, rounded and matured aroma than wines of suppressed MLF.

Conclusions

Malolactic fermentation is desirable in Frankovka wine. It significantly improves sensory properties of wine. Decrease in total acidity of MLF wines by 2.6 g/L resulted in the pH increase of only 0.12 units. The negligible change of the pH value in relation to the total decrease of malic acid, could be important for the further microbiological stability of wine. In wines of natural and induced MLF significant increase of ethyl lactate was established. Ethyl acetate and volatile acidity increased slightly. Changes in concentrations of other volatile compounds were minor. Wines of natural MLF showed better sensory properties than wines of malolactic fermentation induced by addition of starter cultures. It would be, therefore, useful to determine present indigenous species and strains of lactic acid bacteria.

On the basis of the presented results it can be concluded that controlled malolactic fermentation in Frankovka wine is possible even in practical winemaking by application of recommended optimal conditions.

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Promjene kiselosti, nekih komponenti arome i senzornih svojstava vina Frankovka nakon malolaktičnog vrenja

Sažetak

Istraživane su promjene sastava i senzornih svojstava vina Frankovka, uzrokovane u pokusima spriječenog, prirodnog i induciranog malolaktičnog vrenja dodatkom mikrobnih kultura mliječno-kiselih bakterija. Potpuna razgradnja jabučne kiseline u vinima svih varijanti malolaktičnog vrenja rezultirala je smanjenjem ukupne kiselosti za 2,6 g/L, povisivanjem pH-vrijednosti vina za 0,12 jedinica i hlapljive kiselosti za 0,18 g/L. Malolaktično vrenje nije bilo praćeno degradacijom vinske kiseline, a koncentracija limunske kiseline smanjena je za 0,2 g/L. Nije ustanovljena promjena koncentracije 1-propanola, izobutanola, izoamilnog alkohola, heksanola i 2-fenil etanola. Tijekom istraživanog razdoblja u vinima varijanti malolaktičnog vrenja ustanovljeno je bitno povećanje koncentracije etil-laktata i manje povećanje etil-acetata. Pokusna su vina sadržavala male koncentracije izoamil-acetata, 2-feniletil-acetata, etil-kaproata, etil-kaprilata, dietil-sukcinata i acetoina. Vina spriječenog malolaktičnog vrenja bila su slabije kakvoće u usporedbi s vinima malolaktičnog vrenja. Signifikantno boljim su ocijenjena vina prirodnog malolaktičnog vrenja.