UDC 663.2:663.257
ISSN 1330-9862

(FTB-1202)

preliminary communication

Reduction in Acidity by Chemical and Microbiological Methods and Their Effect on Moslavac Wine Quality

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Received: November 13, 2002
Revised version: April 30, 2003
Accepted: June 9, 2003

Summary

Changes in chemical composition and sensory properties caused by chemical and microbiological methods of deacidification in Moslavac (syn. Furmint) wines were investigated. Alcoholic fermentation of Moslavac musts was carried out with two different strains of the yeasts *Saccharomyces paradoxus*. There were no marked differences in chemical composition among the wines. Compared to the control microbiological deacidification of wines by *Oenococcus oeni* resulted in a complete decomposition of malic acid, total acidity decrease of 1.55 g/L and pH value increase of 0.14 units. There were no significant differences in volatile acidity, tartaric and citric acid concentrations between the control and malolactically fermented wine. Chemically deacidified wines contained similar total acidity but higher pH values than malolactically fermented wines. Compared to control wines, the tartaric acid concentration decrease of 2.13 g/L and the pH value increase of 0.5 units were determined. The sensory quality of Moslavac wines of high acidity was improved by the application of deacidification methods. Chemically deacidified wines were assessed as the best whereas the quality of the control wines was judged inferior.

Key words: malolactic fermentation, chemical deacidification, acidic compositions, sensory properties, wine

Introduction

To produce a high-quality dry wine, it is important to obtain a fine balance between the various chemical constituents, especially between the alcohol and acid content. L-tartaric and L-malic acid represent 70–90 % of the total grape acidity (*1*), which is one of the basic physicochemical parameters of wine (*2*,*3*) and an important factor in the taste of dry wines. A wine with low acidity and high pH has a distinctly flat taste, whereas a wine with high acidity and low pH has a sour taste (*4*). Deacidification is a reduction in the titrable acidity. The objective of this oenological practice is production of balanced wines from the sensory point of view. In theory, acidity and pH adjustment could be conducted at almost any stage during vinification. Nevertheless, postfermentative correction is probably optimal. During fermentation, deacidification often occurs spontaneously

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due to acid precipitation or to yeast and bacterial metabolism. Thus, the method and extent of deacidification needed are difficult to perform before the end of fermentation (*3*). There is a number of methods of wine deacidification; biological deacidification carried out through malolactic fermentation (MLF) or maloethanolic fermentation (MEF) *(5)* and chemical deacidification by the addition of neutral potassium tartarate, potassium hydrogen carbonate or calcium carbonate. During MLF, lactic acid bacteria such as *Oenococcus oeni* convert malic acid into lactic acid and CO_2, whereas MEF is carried out mostly by yeast species such as *Schizosaccharomyces pombe* and the strains of *Saccharomyces* that convert malic acid into pyruvate by means of an intracellular malic enzyme *(6)*. The effects of MLF on the sensory properties of wine were once the subject of controversy, but it is now generally admitted that specific wine attributes are altered and that complexity is modified through the appearance of unique flavours and odours (*7,8*). Standard chemical methods for adjusting the acidity include direct addition of $CaCO_3$ or $KHCO_3$ to the juice or wine to remove a predetermined amount of tartaric acid because the carbonates do not react significantly with malate (*9,10*). After that the wine still contains at least 1 g/L of tartaric acid. Among the methods of deacidification, the $KHCO_3$ treatment is interesting because it does not add anything foreign to the wine. All that occurs is an increase in K^+ concentration and a decrease in both acid and tartarate. Furthermore, the treatment can be accomplished without the increase of pH above 3.60, avoiding the complications of high pH reactions. The main disadvantage of this method is the fact that it may not be effective in all cases *(10)*. Moslavac (syn. Furmint) is one of the white grape varieties with high to very high acidity. Over the last ten years the total acid concentration in Moslavac grape must from the northwestern vineyards of continental Croatia has always been high, ranging between 7.1 and 9.7 g/L (unpublished data). Pospisilova *(11)* reported that Moslavac wine is refreshing, not aromatic and always with very high acidity. The main objective of this study was to examine the effect of chemical and microbiological deacidification methods on chemical composition and sensory properties of Moslavac wine.

Materials and Methods

Vinification

Moslavac white wine grapes obtained from the continental wine region of Croatia, subregion Prigorje were harvested during the 2001 season, destemmed and crushed. The free-run juice was treated with 75 mg/L of SO_2 and allowed to settle overnight. The juice was racked and the must divided into four 100-L steel tanks. Before the beginning of fermentation the sugar level was corrected by the addition of 3.4 kg sugar per 100 L of must. Alcoholic fermentation of musts was carried out with two different strains of the yeasts *Saccharomyces paradoxus*. Two lots of must were inoculated with *S. paradoxus* strain 88 and two other lots with *S. paradoxus* strain 54. Both strains were obtained from the Department of Microbiology, Faculty of Agriculture, University of Zagreb. The experiment was carried out under the cellar conditions (12 °C). Since strong sulphiting was used in order to control the microbial growth and inactivation of spoilage organisms of grapes, we supposed that the must before inoculation was free of undesirable yeasts and bacterial contaminants. During alcoholic fermentation the temperature did not exceed 18 °C. Complete sugar degradation in all wines was finished within 40 days. After that the wines were decanted and, depending on the yeast they were fermented with, were divided into 12 lots of 15 L each for two repetitions of three different treatments. The first treatment represented control wines and the third included chemical deacidification with $KHCO_3$. These wines were sulfited with 50 mg/L of SO_2 and stored at 12 °C in the cellar. The second treatment included malolactic fermentation of wines with starter culture *Oenococcus oeni*, Uvaferm Alpha, Lallemand. 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 wine samples were sulfited with 100 mg/L of SO_2 and stored under the cellar conditions. Chemical deacidification of wines was carried out with $KHCO_3$ (trade name Kalinat) at the same level of total acidity as in the malolactically fermented wines. The deacidified wines were stabilised at 4 °C for three weeks. Before bottling the wines were filtered and stored in the cellar at 12 °C. The samples of all treatments were chemically analysed after the alcoholic fermentation, after malolactic fermentation, before chemical deacidification and before bottling. Bottled samples were tested by sensorial evaluation.

Chemical analyses

The common analyses of basic wine components were carried out on the must and wines using standard methods (*12*). Organic acid (citric, tartaric, malic and lactic) analyses were performed on the HPLC (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 a 300 x 7.8 mm i.d. Aminex HPX 87H organic acid analysis cation exchange column (Bio-Rad Laboratories) heated to 65±1 °C *(13–17)*. The mobile phase was 0.065 % H_3PO_4 in double glass distilled water *(16)*, with a flow rate of 0.6 mL/min. The content of organic acids was determined by measuring the absorbance at 210 nm. Acids were quantified by integration of peak height and compared to an external standard. Standard solution contained the mixture of citric, tartaric, malic and lactic acid in distilled water. The concentration of the organic acids in external standard corresponded to the concentrations in Moslavac must and wines. Organic acids in the standard solution were of analytical grade with minimum purity 99 % (Fluka Chemie AG).

Statistical analyses

One-way analysis of variance and least significant difference (LSD) comparison test were used to statistically interpret mean differences in mean values if any, at 95 % accuracy level. Statistical analysis was done on the chemical parameters of total samples fermented by both *S. paradoxus* strains in three treatments before bottling.

Sensory analysis

The wines from the 2001 harvest season were subjected to sensory evaluation by the paired sample test, the method of 100-point O.I.V. / U.I.O.E. method (*18*) and by descriptive analyses with a panel of 9 judges. The determination of statistical significance was done according to literature (*19*).

Results and Discussion

Chemical composition of Moslavac must and wine after alcoholic fermentation is shown in Table 1. Both *Saccharomyces* strains metabolised the total must sugar content in the same period of time confirming their good fermentation abilities as reported in earlier experiments (*20,21*). There were no marked differences in chemical composition among the wines fermented with *Saccharomyces paradoxus* strains applied in these investigations.

Concentration of acidic compounds

Just after settling

Initial must contained high total acidity and very high concentrations of tartaric and malic acids, whereas lactic acid was not detected (Table 1).

Table 1. Chemical composition of Moslavac must and wine

Compounds	Must after settling	Wine after fermentation	
		Treatments	
		S. paradoxus strain 54	*S. paradoxus* strain 88
Reducing sugars/(g/L)***	176	< 1.0	< 1.0
φ(alcohol)/%	–	10.6	10.2
γ(total acidity)/(g/L)*	11.1	9.4	9.5
γ(volatile acidity)/(g/L)**	–	0.30	0.26
pH	3.00	2.99	2.99
γ(tartaric acid)/(g/L)	7.3	5.8	6.0
γ(malic acid)/(g/L)	4.6	2.9	2.9
γ(lactic acid)/(g/L)	–	0.3	0.2
γ(citric acid)/(g/L)	0.2	0.2	0.2

* as tartaric acid; ** as acetic acid; *** after the addition of 3.4 kg sugar/100 L of must

After alcoholic fermentation

The analysis of the wines (Table 1) showed a uniform decrease in total acidity and the tartaric acid concentration in regard to initial values. We suppose that the decrease in tartaric acid was a result of salt precipitation, which is in line with Ribereau-Gayon *et al.* (*22*) and numerous other authors. An equal decrease in malic acid concentration of 1.70 g/L was achieved in Moslavac wines fermented with two different *S. paradoxus* strains. An experiment by Orlic (*20*) carried out with another cultivar in different microbiological conditions showed that some of *S. paradoxus* strains can metabolise malic acid to ethanol while others, *e.g. S. paradoxus* strain 54, do not have this ability. The results of our investigation presented in Table 1 show that the lactic acid contents were similar in both wines fermented by *S. paradoxus* strain 54 and strain 88. We consider that the ability of *S. paradoxus* strain 54 and strain 88 to degrade malic acid in complex biological substratum such as wine needs further investigations.

After malolactic fermentation

In all malolactically fermented wines malic acid was completely metabolised into lactic acid, whereas its concentration in wines of other treatments remained unchanged (Table 2). Compared with the control, MLF samples contained lower total acidity with the decrease of 1.6 g/L, whereas the pH values increased by only 0.08 units. There were not any essential changes in the tartaric acid concentration and the increase in volatile acidity was low, which is in line with our earlier investigations *(8)*. According to some authors (*23,24*), MLF can cause higher or lower decrease in the citric acid concentration, but our results for Moslavac wine did not confirm that.

Table 2. Concentration of organic acids (g/L) in Moslavac wines after malolactic fermentation

Compounds	Treatments					
	S. paradoxus strain 54			*S. paradoxus* strain 88		
	I	II	III	I	II	III
γ(total acidity)/(g/L)*	9.2	7.6	9.1	9.3	7.7	9.4
γ(volatile acidity)/(g/L)**	0.30	0.38	0.47	0.33	0.37	0.26
pH	3.00	3.08	3.00	2.99	3.08	3.08
γ(tartaric acid)/(g/L)	5.7	5.6	5.8	5.6	5.8	5.7
γ(malic acid)/(g/L)	2.6	n.d.	2.6	2.6	n.d.	2.6
γ(lactic acid)/(g/L)	0.3	1.8	0.3	0.2	1.9	0.2
γ(citric acid)/(g/L)	0.2	0.2	0.2	0.2	0.2	0.2

* as tartaric acid; ** as acetic acid; I control wines; II MLF wines; III wines for chemical deacidification

Before chemical deacidification

When malolactic fermentation was completed, wines of all treatments were kept for two months at cellar temperature (12 °C) before chemical deacidification. In wines of all treatments a decrease in the concentration of tartaric acid of 1.8 g/L was observed, which was the result of salt precipitation at low temperature. There were no changes in concentrations of other organic acids (Table 3). The deacidification of Moslavac wines with $KHCO_3$ was carried out to the same value of total acidity as it was in MLF fermented samples.

After deacidification and before bottling

An analysis made in the period just before bottling showed that the tartaric acid concentration of chemically deacidified wines decreased to 1.95 g/L, while total acidity decreased to 6.55 g/L (Table 4). Furthermore, these changes were linked with an increase in the pH value up to 3.47. Regarding the reduction of total acidity and tartaric acid and the increase of pH, our results are

Table 3. Concentration of organic acids (g/L) in Moslavac wines before chemical deacidification

Compounds	Treatments					
	S. paradoxus strain 54			*S. paradoxus* strain 88		
	I	II	II	I	II	III
γ(total acidity)/(g/L)*	8.5	6.8	8.4	8.6	6.9	8.6
γ(volatile acidity)/(g/L)**	0.45	0.49	0.48	0.47	0.42	0.44
pH	2.99	3.10	3.00	2.99	3.10	3.08
γ(tartaric acid)/(g/L)	4.1	4.0	4.0	4.2	4.2	4.1
γ(malic acid)/(g/L)	2.5	n.d.	2.5	2.6	n.d.	2.5
γ(lactic acid)/(g/L)	0.3	2.0	0.3	0.3	1.9	0.3
γ(citric acid)/(g/L)	0.2	0.2	0.2	0.2	0.2	0.2

* as tartaric acid; ** as acetic acid; I control wines; II MLF wines; III wines for chemical deacidification

in line with the investigations by Nemanic *et al.* (*25*) and Weger (*26*).

Wines vary considerably in pH, with the values below 3.1 considered sour, and those above 3.7 considered flat (*3*). As shown in Table 1, the pH values of Moslavac wines were very low and a correction in pH was desired. The choice of acidity-correction procedure is influenced considerably by the way it affects the pH. Since tartaric acid is more ionized than malic acid, whithin the usual range of the values of wine pH, an adjustment in the concentration of tartaric acid has a greater effect on pH than an equivalent change in the concentration of malic acid. According to our results, the decrease of total acidity of 1.55 g/L in MLF fermented wines affected the low change of pH value (0.14 units) compared with the control wines (Table 4).

Table 4. Concentration of organic acids (g/L) in Moslavac wines before bottling (mean values of 4 treatments)

Compounds	*Saccharomyces paradoxus*			
	Number of treatments (N = 4)			
	I	II	III	LSD p = 5 %
γ(total acidity)/(g/L)*	8.30^{a}	6.75^{b}	6.55^{b}	0.2262
γ(volatile acidity)/(g/L)**	0.45	0.46	0.45	n.s.
pH	2.97^{c}	3.11^{b}	3.47^{a}	0.0478
γ(tartaric acid)/(g/L)	4.08^{a}	4.08^{a}	1.95^{b}	0.1359
γ(malic acid)/(g/L)	2.58	n.d.	2.65	–
γ(lactic acid)/(g/L)	0.28^{b}	1.98^{a}	0.28^{b}	0.1099
γ(citric acid)/(g/L)	0.2	0.2	0.2	n.s.

* as tartaric acid; ** as acetic acid; I control wines; II MLF wines; III chemically deacidified wines;
Note: Different letters in the superscript of the mean of a compound denote a significant difference among treatments (a, b, c for p = 0.05).
The same letter in the superscript of the mean of a compound denotes an insignificant difference among treatments (a, b, c for p = 0.05); n.d. not detectable; n.s. not significant

Sensory properties of wines

Generally, Moslavac wines from the northwestern vineyards of continental Croatia are characterised by a neutral to less intensive aroma and, regardless of the vintage, by high to very high acidity. The results of wine tasting by the paired sample test and the 100-point method 3 months after bottling are given in Tables 5 and 6. On the basis of the presented data deacidification influenced the quality of Moslavac wine. In comparison with the control, all deacidified wines were of better quality, which was primarily the result of their lower acidity. The results of the paired sample test showed that the differences were significant ($p<0.01$) among the wines produced by the *S. paradoxus* 88 strain. On the other hand, on the basis of the presented data it seems difficult to detect sensory differences in wines obtained by the fermentation with the *S. paradoxus* 54 strain. Regarding the total score obtained by wine tasting with O.I.V. method of 100 points, the control Moslavac wines were the most inferior ones, while the best evaluated were the chemically deacidified wines (Table 6).

Table 5. Results of wine tasting by the paired sample test

Treatments	1	2	3	4	5	6	7	8	9	Total
S. paradoxus strain 54										
I	+									1
III		+	+	+	+	+	+	+	+	8*
I	+		+			+				3
II		+		+	+		+	+	+	6
II					+					1
III	+	+	+	+		+	+	+	+	8*
S. paradoxus strain 88										
I										0
III	+	+	+	+	+	+	+	+	+	9**
I										0
II	+	+	+	+	+	+	+	+	+	9**
II										0
III	+	+	+	+	+	+	+	+	+	9**

Note: * significant level $p< 0.05$; ** significant level $p< 0.01$;
I control wines; II MLF wines; III chemically deacidified wines

Table 6. Results of wine tasting by the 100-point O.I.V. / U.I.O.E. method

	Treatments					
	S. paradoxus strain 54			*S. paradoxus* strain 88		
	I	III	II	I	III	II
Total score	73.57	82.43	78.86	74.00	81.29	76.88

According to the descriptive sensory analyses, the control Moslavac wines had a very simple, markedly sour and inharmonious taste and a pronounced vegetal aroma. Malolactic fermentation resulted in a better wine quality compared with the control Moslavac wines. The wines of MLF treatments expressed roundness in taste with a complex retronasal aroma, as most of the tasters evaluated, these wines had somewhat unbalanced aroma with less pronounced varietal flavours. The way in which MLF contributes to the wine aroma and its structure depends a lot on the grape variety from which it is produced. Henick-Kling *et al*. (*27*) reported that fruity aromas of the Chardonnay wine are not destroyed by MLF, which Herjavec *et al*. (*8*) also found in Riesling wine. Apparently, in a more aromatic wine some flavour contributed by MLF is more difficult to detect (*27*). We presume that the strong characteristic aromas of MLF are not considered complementary to the delicate aromas of Moslavac grape variety.

Chemical deacidification resulted in the best evaluated wines, which were round in taste, with a harmonious balance between alcohol and acidity and with a nice, medium long finish. Certainly, lower content of tartaric acid also contributed to the softer taste of these wines. Besides more harmonious and nicer mouth quality than the control wines, chemically deacidified wines were characterised by delicate and recognised floral and fruity aroma. According to von Nida and Fischer (*28*), the use of $KHCO_3$ does not have an influence on wine flavour modifications, whereas $CaCO_3$ and double salt deacidified wines can have a cardboard and flat taste. The differences in the nature of aroma and intensity between the control and chemically deacidified wines are difficult to explain because all wines were stored under the same conditions. We suppose that there was some loss of the aroma compounds in the control wines. According to R. S. Jackson (*3*) the hydrolysis of volatile esters occurs more rapidly at low pH values.

Conclusions

The reduction in acidity was successful using the applied methods of wine malolactic fermentation and chemical deacidification. There were no marked differences in chemical composition among the wines fermented with *S. paradoxus* strain 54 and strain 88. In comparison with the control, the deacidified wines were of better quality, which was primarily the result of their lower acidity. The results of this study indicate that the applied methods for deacidification influence the composition and sensory properties of Moslavac wines. The best general quality of Moslavac wine was obtained by chemical deacidification. The $KHCO_3$ method was effective in reducing the tartaric acid concentration and in marked increase of the pH value, which resulted in the soft taste of these wines. These wines were characterised by delicate and recognised varietal floral and fruity aroma. Microbiological deacidification by *Oenococcus oeni* also resulted in modified taste of the wines, but characteristic aromas of MLF are not considered complementary to the delicate aromas of Moslavac wine. It can be concluded that the quality of Moslavac wines of high acidity can be significantly improved by the application of the appropriate deacidification method.

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Smanjenje kiselosti mikrobiološkim i kemijskim metodama i njihov utjecaj na kvalitetu vina Moslavac

Sažetak

Istraživane su promjene kiselinskog sastava i senzorskih svojstava vina Moslavac, otkiseljavanog pomoću kemijskih i mikrobioloških metoda. Alkoholno vrenje mošta provedeno je sa dva soja 54 i 88 *Saccharomyces paradoxus*. Dobivena vina nisu se bitno razlikovala po kemijskom sastavu. U usporedbi s kontrolnim vinom, nakon malolaktičnog vrenja došlo je do potpune razgradnje jabučne kiseline, smanjena je ukupna kiselost za 1,55 g/L i povećana pH-vrijednost za 0,14 jedinica. Nisu ustanovljene značajne razlike u hlapljivoj kiselosti te koncentraciji vinske i limunske kiseline. Uz podjednaku ukupnu kiselost, kemijski otkiseljena vina sadržavala su za 2,13 g/L manje vinske kiseline, a pH je bio viši za 0,5 jedinica u usporedbi s kontrolnim vinom. Primijenjenim metodama otkiseljavanja poboljšana su senzorska svojstva vina Moslavac, koja karakterizira velika kiselost. Najbolje kakvoće bila su kemijski otkiseljena, a najslabije su ocijenjena kontrolna vina.