Influence of Malolactic Fermentation on the Quality of Riesling Wine

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SUMMARY

The composition and sensory properties in Riesling wines of suppressed, natural and induced malolactic fermentation (MLF) were investigated. A Riesling wine from the vintage 1998 was produced by the usual technology for white wines. After completion of alcoholic fermentation, wine was racked and divided into 35 L glass bottles for different treatments. The first treatment was suppressed MLF with SO₂ and storage at 10 °C. The second one included natural MLF and the third MLF was induced with starter culture Oenococcus oeni. After the MLF of the wines was completed, the wines were analysed and sensory tested. The analysis of the organic acids were performed by HPLC and the concentrations of volatile compounds were determined by gas chromatography.

Malic acid decomposition was completed in wines of all MLF treatments and the result was a significant decrease of total acidity up to 1.3 g/L and an increase of the pH value of 0.1 units. No differences in the concentrations of volatile acidity and ethyl acetate were detected between the wines of suppressed, natural and induced MLF.

MLF was not accompanied by tartaric acid degradation. The citric acid concentration was reduced by up to 42 %. The concentrations of 1-propanol, isobutanol, isoamyl alcohol and 2-phenylethanol remained unchanged after malolactic fermentation. The concentrations of isoamyl acetate, isobutyl acetate, ethyl butyrate and ethyl caproate were lower in the MLF wines. These wines contained more ethyl lactate and diethyl succinate. Higher quantities of ethyl caprylate, ethyl caprate, caproic, caprylic and capric acids were determined in the MLF wines.

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

KEY WORDS

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

ACKNOWLEDGEMENTS

Thanks to family Tomac for supporting the experimental programme in their own cellar Vinogradarstvo Tomac, Donja Reka 5, Jastrebarsko, Croatia.
INTRODUCTION

Malolactic fermentation (MLF) is a part of the traditional winemaking techniques for red and white still wines (Henick-Kling et al., 1994). According to Davis et al. (1985) malolactic fermentation may improve the sensory complexity of wine by producing some compounds which are of considerable importance to the flavor profile of wine.

The influence of malolactic fermentation depends on the grape variety and strain of lactic acid bacteria which are responsible for the process of induced or spontaneous MLF. Typical MLF aromas (“buttery”, “nutty”, “sweaty”) can negatively affect the wine quality if they are not in balance with the primary fruit aromas (Henick-Kling et al. 1994). According to the same authors malolactic fermentation contributes strongly to aroma and aftertaste in Chardonnay, while its contribution in a Riesling wine may only be noticed by giving the wine softer and rounder taste. Investigations carried out by Fischer (1998) through inoculation of three different Oenococcus oeni strains in Riesling wines even showed intensified fruity and flowery aroma properties of this grape variety.

Generally spontaneous MLF occurs in the wine subsequent to the alcoholic fermentation. In order to reach a rapid and predictable malolactic fermentation, the use of starter cultures, for example selected strains of Oenococcus oeni, have become of great interest.

The purpose of this work was to examine the influence of malolactic fermentation on the changes of chemical composition and sensory properties of Riesling wines.

MATERIAL AND METHODS

Vinification

Riesling grapes from the vintage 1998 were grown in the continental wine region of Croatia, subregion Plešivica. The wine was produced by traditional vinification for white wine. The composition of Riesling wine after alcoholic fermentation is summarized in Table 1. After completion of alcoholic fermentation, the wine was racked and divided into 35-L glass bottles, according to the following treatments: a) suppressed MLF with addition of 100 mg SO₂/L and stored at 10 °C, b) natural MLF; wines were not sulphurized and the spontaneous process of the MLF was conducted, c) MLF was induced with the starter culture Oenococcus oeni; the lyophilized culture of Oenococcus oeni trade mark Uvaferm ML-D was reactivated prior to inoculation and added to non-sulphurized wines just after the alcoholic fermentation.

All treatments were done in triplicate. Malolactic fermentation was conducted at 20 °C and followed by determination of the concentration of malic acid by the HPLC. After the MLF was completed, the chemical analyses of all variants of wines were performed. MLF wines were sulphurized with 100 mg/L of sulfur dioxide and tested by sensorial evaluation after a storage of 3 months.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Alcoholic - vol.%</th>
<th>Reducing sugar - g/L</th>
<th>Total acidity - g/L (1)</th>
<th>Volatile acidity - g/L (2)</th>
<th>Tartaric acid - g/L</th>
<th>Malic acid - g/L</th>
<th>Lactic acid - g/L</th>
<th>Citric acid - g/L</th>
<th>pH</th>
<th>Free SO₂ - mg/L</th>
<th>Total SO₂ - mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>11.90</td>
<td>&lt;1.00</td>
<td>8.1</td>
<td>0.42</td>
<td>4.2</td>
<td>1.9</td>
<td>0.1</td>
<td>0.26</td>
<td>3.24</td>
<td>n.d.</td>
<td>31.40</td>
</tr>
</tbody>
</table>

(1) as tartaric acid (2) as acetic acid

Chemical analyses

Alcohol, total and volatile acidity, free and bound SO₂ were determined using methods proposed by O.I.V. (1995). Reducing sugar was determined by Rebelein method (Zoecklein et al., 1995) and pH value was measured using the Beckman Expandomatic SS 2 instrument.

Organic acid (citric, tartaric, malic and lactic) analyses were performed on the HPLC Modules Hewlett Packard 1050 Series comprised VW detector and HP 3395 Integrator. The chromatographic separations were 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) H₃PO₄ in double glass distilled water with a flow rate of 0.6 ml/min. Detection was by measurement absorption at 210 nm. Acids were quantified by integration of peak height and calibrated with an external standard.

Volatile compounds analysis were performed on Hewlett Packard model 5890 Gas chromatograph fitted with flame ionization detector. For data treatment, a Hewlett Packard model 3396 Series II integrator was used. Higher alcohols (1-propanol, isobutanol, isoamyl alcohol) and ethyl acetate were analysed on distillate from wine using HP101 (Hewlett Packard) column of 50m x 0.32mm and 0.3 µm film thickness. Temperature programming was as follows: 6 min isothermal at 40 °C, then linear temperature rise of 15 °C/min to 200 °C. The carrier gas was nitrogen. 1-Butanol was used as the internal standard.

To determine volatile fatty acids, ethyl esters of fatty acids, higher alcohols acetates and other volatile compounds, the wine (500 mL), to which 1-heptanol was added as internal standard, was...
continuously extracted (10 h) by dichloromethan. The extract was dried over anhydrous sodium sulfate, concentrated to 10 mL and stored prior to GC analysis. The extract (1 µL) was injected (split 1:30) into a FFAP – HP (Hewlett Packard) column of 50 m x 0.32mm and 0.5 µm phase thickness. Temperature programming was: 5 min isothermal at 60°C, then a linear temperature rise of 2.5 °C/min to 190 °C and 20 min isothermal at 190 °C. The carrier gas used was nitrogen.

The determination of statistical significance of analysed compounds was based on ANOVA.

Sensory evaluation of wines
The overall quality of the wines was tested by an experienced panel of 7 judges. The ranking method for sensory differences was used. The determination of statistical significance was based on Appendix I-1 in Amerine and Roessler (1976).

RESULTS AND DISCUSSION
Concentration of organic acids
Table 2. shows organic acids concentrations in the wines of all treatments right upon completion of the malolactic fermentation.

Organic acids transformations are shown in Fig. 1 and 2. Almost in all MLF samples the degradation of malic acid was noted after 34 days. In the inoculated wines it was completed after eight days. In the samples of natural MLF the degradation finished after fourteen days.

In wines of suppressed MLF malic acid concentration remained unchanged.

Comparing to the control, malolactic fermented wines contained significantly lower total acidity with max. decrease of the 1.3 g/L. At the same time the pH values significantly increased for max. 0.11 units (Table 2). Similar results were obtained by Bousbourous et al. (1971) and Pilone et al. (1966). Increase of the volatile acidity was minor. There were no differences among wines of different treatments. According to Rodriguez et al. (1989) and Davis et al. (1985) the levels of volatile acidity were

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Suppressed MLF</th>
<th>Natural MLF</th>
<th>Induced MLF</th>
<th>LSD (P &lt;5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total acidity -g/L (1)</td>
<td>7.6 A</td>
<td>6.9 B</td>
<td>6.8 C</td>
<td>0.1</td>
</tr>
<tr>
<td>Volatile acidity - mg/L (2)</td>
<td>0.48</td>
<td>0.50</td>
<td>0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>Tartaric acid - g/L</td>
<td>3.3</td>
<td>3.3</td>
<td>3.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Malic acid -mg/L</td>
<td>1.8</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>Lactic acid - mg/L</td>
<td>0.1 A</td>
<td>1.3 B</td>
<td>1.4 B</td>
<td>0.1</td>
</tr>
<tr>
<td>Citric acid - mg/L</td>
<td>0.25 A</td>
<td>0.17 B</td>
<td>0.15 C</td>
<td>0.01</td>
</tr>
<tr>
<td>pH</td>
<td>3.26 A</td>
<td>3.34 B</td>
<td>3.35 B</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Note: Different letters (A, B, C) beside the means of compound denote significant difference (P<5%) among treatments. The same letters beside the means of compound denote not significant difference (P<5%) among treatments.

TABLE 2. Concentration of organic acids in Riesling wines

(1) as tartaric acid
(2) as acetic acid

FIGURE 1. Transformation of malic acid

FIGURE 2. Formation of lactic acid
higher in the wines which had undergone a complete malolactic fermentation.

Results showed uniform decrease of tartaric acid concentration regarding initial values in wines of all treatments. We suppose that the decrease of tartaric acid in Riesling wine of all treatments was due to the salts precipitation. According to Wibowo et al. (1985), Fornachon did not establish changes in tartaric acid concentration during MLF. Minarik and Jungova (1995) conclude that controlled MLF with application of *Oenococcus oeni* has no effect on tartaric acid content of wine. On the other hand Pilone et. al. (1966), Bousbouras et al. (1971), Kunkee (1998), suggest that MLF is often accompanied by a small decrease (3 - 30%) of tartaric acid.

In comparison with wines of suppressed MLF, wines of natural and induced MLF had a significant decrease in citric acid concentration of up to 42%. Regarding Cogan et al. (1981), in some MLF wines citric acid can be completely metabolized. Nielsen et al. (1996) also noted complete metabolism of citric acid. According to Dittrich et al. (1980), decrease of this acid can reach 50%, which is in accordance with our investigations.

Composition of volatile aroma compounds in wines

The results in Table 3 show that there were no significant differences among wines of different treatments in concentrations of 1-propanol, isobutanol, isoamyl alcohol and 2-phenyl ethanol. Davis et al. (1985) noted an increase in 1-propanol in MLF wines. Radler and Gelwarth (1971) also conclude that *Oenococcus oeni* in a synthetic medium with ethanol produces small quantities of 1-propanol. Laurent et al. (1994) did not find any changes in isoamyl alcohol and 2-phenyl ethanol concentrations after malolactic fermentation.

Wines of all treatments had similar concentrations of ethyl acetate, and that could lead to conclusion that MLF had no influence on concentration of this ester. These results could be related to unchanged volatile acidity in the wines after the malolactic fermentation. Opposite to our results, Wibowo et al. (1985) reported great increase in concentration of ethyl acetate.

Although the differences were not significant, MLF wines had lower concentrations of isoamyl acetate in respect to wines of suppressed MLF. At the same time, wines of induced MLF had the lowest concentration of this ester. Isobutyl acetate decrease in MLF wines was similar to that of isoamyl acetate. Wines of all treatments contained similar concentrations of fatty acids ethyl esters. Although the differences among certain esters were small and not significant in compared wines, results suggest that concentrations of ethyl butyrate and ethyl caproate were lower while ethyl caprilate and ethyl caprate concentrations were higher in MLF wines. One can find different literature data of MLF influence on ester concentration changes. According to Wibowo et al. (1985) a decrease of isoamyl acetate was noted in MLF wines. Contrary to this, De Wet, Van der Merwe and Etievant (Laurent et al., 1994) noticed large increases of isoamyl acetate in MLF wines. Laurent et al (1994) found no variation in the amount of ethyl esters of fatty acids such as ethyl butyrate, ethyl octanoate and ethyl decanoate caused by the MLF.
In the wines of MLF treatments an increase of ethyl lactate was determined. Diethyl succinate concentrations were also higher in MLF wines with the highest levels in the induced samples. Davis et al. (1985) and Pilone and Kunkee (1966) reported an increase in concentration of ethyl lactate in wines after the malolactic fermentation. Pilone and Kunkee (1966) found the small percentage of diethyl succinate in wines. Its variations in MLF wines depended on the type of lactic acid bacteria. Wibowo et al. (1985) found a strong increase in diethyl succinate concentrations in wines after malolactic fermentation.

Results presented in Table 3 indicate that Riesling wines after malolactic fermentation contained higher concentrations of fatty acids. The highest and significant difference was found in concentrations of caprylic acid.

Sensory properties of wines

Results of wine tasting by ranking method after MLF are given in Table 4. On the basis of presented data significantly the best overall quality had the wines that underwent the natural malolactic fermentation. Besides the pronounced varietal and fruity aroma the wines were very full and round in taste, with a complex retronasal aroma and a fresh, agreeable, medium long finish. Most of the tasters ranked the wines of suppressed MLF as the lower in the quality due to the somewhat unbalanced taste and with less complexity in aroma.

Investigations carried out by Henick-Kling et al. (1994) showed that, among experienced tasters, the preference for the Riesling MLF or the non-MLF wines was split 50:50. According to the same authors, the results of the Riesling tasting also seemed to indicate that the fruity flavors of the wine are not destroyed by MLF as is sometimes assumed.

CONCLUSIONS

Riesling wines that underwent malolactic fermentation were of a better quality than wines of suppressed MLF. Wines of natural MLF were significantly superior to other wines. Those wines had a more complex aroma and a rounder and softer taste. A decrease in the total acidity of MLF wines was 1.3 g/L and an increase in pH of only 0.11 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. There were no changes in volatile acidity and in the concentration of ethyl acetate between the MLF and non MLF wines. The wines that underwent malolactic fermentation had less quantities of some acetate esters. Those wines contained a slightly more fatty acids and ethyl caprylate and caprate. In wines of natural and induced MLF significant increase of ethyl lactate and diethyl succinate was established. It can be concluded that malolactic fermentation had desirable effect on the quality of Riesling wines.

REFERENCES


TABLE 4 . Results of sensory evaluation of Riesling wines after MLF

<table>
<thead>
<tr>
<th>Order</th>
<th>Treatments</th>
<th>Rank totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>natural MLF</td>
<td>9*</td>
</tr>
<tr>
<td>2.</td>
<td>induced MLF</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>suppressed MLF</td>
<td>18</td>
</tr>
</tbody>
</table>

Note: Any rank total outside 10–18 range is significant at the P<5%


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