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STUDY OF CHANGES ORGANIC ACIDS IN RED WINES DURING MALOLACTIC FERMENTATION

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Abstract

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The aim of this contribution is to be able to describe the movement of organic acids in red wine during malolactic fermentation. Wines from Znojmo wine region were represented by varieties of Svatovavřinecké (Saint Laurent), Rulandské modré (Pinot Noir), Zweigeltrebe, Frankovka (Lemberger) and Dornfelder. The grapes went through the same way of wine making and after completion of alcoholic fermentation were inoculated with pure culture of lactic acid bacteria *Oenococcus oeni*. Samples were taken for chemical analysis during biodegradation of acids within the range of 2 to 4 days and they were measured using a device WineScan FT 120. Chemical analysis detected changes in the concentrations of the following parameters: total acidity, lactic, malic, tartaric and citric acids. The total content of acids statistically significantly ($P = 0.05$) differed only between samples of Svatovavřinecké T 66 and Zweigeltrebe T 2.
The differences of average mass concentrations of lactic, malic and citric acids were not statistically relevant. Nevertheless, statistically relevant difference in the concentration of tartaric acid from all other wines was detected in a sample of SV T 66 which also reached the highest average value (5.18 g/l).

wine, fermentation, organic acids, Oenococcus oeni

The wine is a noble beverage which is made by fermentation of cider or crushed grapes *(Vitis vinifera)*. The character of wine is affected by range of factors in which we could include the location of vineyard, type, agricultural engineering, the course of the year and the production technology. In case of lack of sun and also cold summer weather in our geographical conditions we might encounter the production of less quality grapes, which results in the lower content of fermentable saccharides and the higher content of organic fluids, mostly of malic acid which presence is not covetable. However, there is a possibility to decrease the excessive content of acids. First of all, it is related to biodegradation of acids when lactic fermentation bacteria transform malic acid into lactic acid.

Important oenological characteristics of wine lactic acid bacteria, such as the malolactic activity and the production of volatile organic acids, may be differently affected by the presence of phenolic acids, depending on the bacterial species or strain (Campos *et al.*, 2009; Frudíková, Malík, 2009).

The organic acids largely participate in the constitution, stability and sensorial qualities of the wine. Their preserving quality also gives the wine a better microbiological and physico-chemical stability. The red wines resist much lower acidities, because the presence of the phenolic compounds enforces the acid savor and participates in their keeping during the ageing process (Tita *et al.*, 2006).

Among acids which are the oxidation product in breaking down sugars, the cider is mostly comprised of tartaric and malic acid and then in small amount of citric, succinic, malonic, fuamric and glycolic acid. Lactic acid and volatile acids such as acetic and butyric are generated in wine by microbial activity. Tartaric and malic acid are generated mostly in the initial phases of grapes development. However, the content of grapes is decreased during maturing both due to a cutback in production and also decomposition in breathing process. Kumšta, 2007 introduces up to 8 g/l of tartaric acid in unripe grapes and around 14 g/l of malic acid, the content of tartaric acid in overripe grapes is decreased to 4–6 g/l and malic acid to 4–10 g/l. The important factor in terms of quality of future wine is the proportion between malic and tartaric acid. The tartaric acid always outweighs malic acid in good years. In contrast, the content of malic acid is considerably increased in bad weather conditions as well as in the year 2010 (Kumšta, 2011). The modification of malic acid is made in two ways, either chemical – double deacidification or in biological way such as malolactic fermentation. It is convenient to adjust this proportion by virtue of chemical analysis with the help of malolactic fermentation, in particular, when it comes to red wines which spring from the northern regions. The lactic bacteria are the most appropriate – *Oenococcus oeni* (Delfini, Formica, 2001).

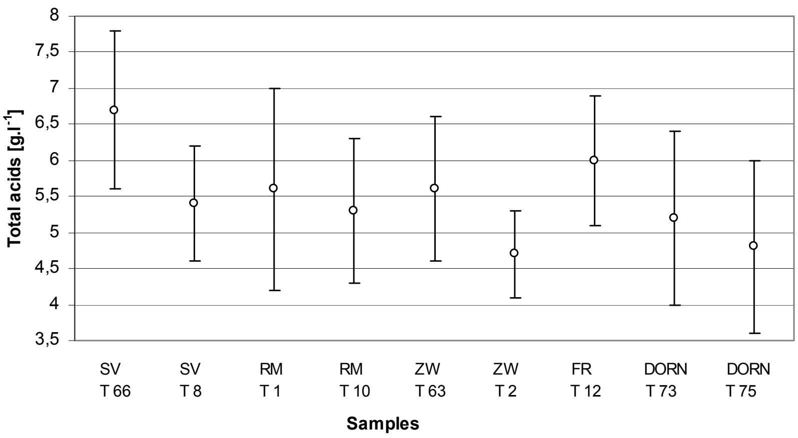
The aim of this contribution is to be able to describe the movement of malolactic fermentation in a number of types of red wines which spring from Znojmo wine region.

MATERIALS AND METHODS

We used two samples of types Svatovavřinecké (Saint Laurent) (SV, tank 66 and 8), Rulandské modré (Pinot Noir) (RM, tank 1 and 10), Zweigeltrebe (ZW, tank 63 and 2) and Dornfelder (DORN, tank 73 and 75) and one sample of type Frankovka (Lemberger) (FR, tank 12). The grapes sprang from Znojmo wine region and from other wine growing villages. Wine was processed in Jaroslavice, a branch of winery Znovin Znojmo, a. s. Samples went through process called "vinification" and were treated by the same preparations. We applied pectolytic enzymes for better extraction and stabilization of colour (preparation Depectil extra grade), dosage 2 g/hl. Beet-sugar was added to sweeten only samples of Svatovavřinecké T 66 and T 8 over 22 °NM, the other ciders had the sugar content sufficient. Consequently, we applied pure culture of yeast (preparation Kvasinka Pinot noir MVO 3001). We added potassium bicarbonate ($KHCO_3$) at the end of alcohol fermentation which enabled the reduction of the total acidity and the increase of pH, which also enables to boost the biodegradation of acids (BOK). After completion of alcohol fermentation, the new wine was inoculated with bacteria of lactic fermentation *Oenococcus oeni* (Vitilactic XL). Samples were taken a week after inoculation for measurement within the range of 2 to 4 days till the end of malolactic fermentation. The measurement was taken at the laboratory of company Znovín Znojmo a.s. based in Šatov, using a device WineScan FT 120 on the principle of Fourier Transform

I: *The changes in the concentration of the total acids after completion of biodegradation of acids*

Wine	SV T 66	SV T 8	RM T 1	RM T 10	ZW T 63	ZW T 2	FR T 12	DORN T 73	DORN T 75	Ar. average / Average dev.
Initial value [g/l]	8.3	6.3	7.0	6.5	6.4	5.4	6.8	6.8	6.5	–
Final value [g/l]	6.1	4.9	4.5	4.6	4.8	4.3	5.1	4.8	4.4	–
Abs. variation [g/l]	–2.2	–1.4	–2.5	–1.9	–1.6	–1.1	–1.7	–2.0	–2.1	–1.83 0.34
Rel. value [%]	73.49	77.78	64.29	70.77	75.00	79.63	75.00	70.59	67.60	72.68 3.89



1: *Confidence interval (P = 0.05) for the mass concentration of the total acids*

InfraRed (FTIR), making a use of the whole IR-spectrum. There was observed the content of the total acids and organic acids such as lactic, malic, tartaric and citric.

RESULTS AND DISCUSSION

The total acids

The content of titratable acids during malolactic fermentation was decreased (Tab. I). The reduction of mass concentration added up to on average 1.83 g/l with average deviation of 0.34 g/l. In relative values it represents the reduction to average value 72.68% of its original value. According to Steidl (2002), the concentration of titratable acids by biodegradation will be decreased approximately by a half of the original share of malic acid.

The total content of acids statistically significantly differs only between samples of Svatovavřinecké T 66 and Zweigeltrebe T 2. These two samples also reach the highest (6.72 g/l) and the lowest (4.74 g/l) average values of the total acids.

Tartaric acid

A slight reduction of mass concentration of tartaric acid was detected in all samples during malolactic fermentation (on average by 0.8 g/l, meaning, the reduction to 81.60% in comparison with the beginning of secondary fermentation).

The smallest changes occurred in Svatovařinecké T 66 which concentration decreased only by 0.5 g/l, meaning, to 90.74% of the origin quantity. On the contrary, the biggest part of tartaric acid were degraded in a sample of Rulandské modré T 1–1.5 g/l, the reduction to 68.09% of original quantity (Tab. II). Švejcar (1989) recommends that the content of tartaric acid in already made wine does not decrease under 2 g/l. The risk of combining excessive quantity of calcium with other organic acids can be easily prevented and it also causes the origin of insoluble salt which negatively affect the flavour of wine. The content of tartaric acid in all measured samples exceeded the limit of 3 g/l, so the sensory attributes should not be affected.

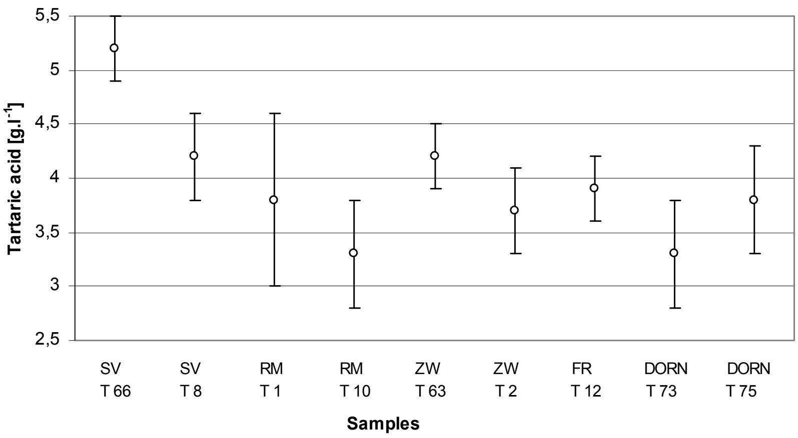
Statistically relevant difference of all wines was detected in a sample of SV T 66 which also reached the highest average value of concentration of tartaric acid (5.18 g/l). ZW T 63 statistically significantly differed from samples of SV T 66, RM T 10 a DORN T 73. The lowest average value of concentration of tartaric acid reached identically RM T 10 and DORN T 73 (3.32 g/l).

Malic acid

Bacteria of lactic fermentation break malic acid down into lactic acid and therefore the reduction

II: *The changes in the concentration of tartaric acid after completion of biodegradation of acids*

Wine	SV T 66	SV T 8	RM T 1	RM T 10	ZW T 63	ZW T 2	FR T 12	DORN T 73	DORN T 75	Ar. average / Average dev.
Initial value [g/l]	5.4	4.5	4.7	3.9	4.4	4.1	4.2	3.9	4.5	–
Final value [g/l]	4.9	3.8	3.2	3.0	3.9	3.4	3.6	3.0	3.6	–
Abs. variation [g/l]	–0.5	–0.7	–1.5	–0.9	–0.5	–0.7	–0.6	–0.9	–0.9	–0.8 / 0.22
Rel. value [%]	90.74	84.44	68.09	76.92	88.64	82.93	85.71	76.92	80.00	81.60 / 5.44



2: *Confidence interval (P = 0.05) for the mass concentration of tartaric acid* of concentration occurred during the process of measurement. The average value was 2.32 g/l (Tab. III). The concentration of malic acid in the relative value decreased on average to 25.06% of its original value. The highest reduction occurred in Svatovavřinecké T 66 (3.2 g/l), the smallest difference was detected in a sample of Zweigeltrebe T 2 (1.6 g/l).

Domine (2005) introduces the duration of biodegradation of acids between 1–4 weeks. This period of time was kept thanks to controlled malolactic fermentation with the help of pure cultures of yeast and the wines were treated after 3 weeks with preparation of SO_2 in order to stop the development of unwanted microflora. Minárik (2008) considers malolactic fermentation to be finished at the moment when bacteria of lactic fermentation stop degrading malic acid and the source of metabolism will become citric acid. The aim of all that is to reduce the mass concentration of malic acid in red wines to 0.5–0.7 g/l. The concentration of malic acid was not reduced to the requested limit in samples Rulandské modré T 1 and Dornfelder T 73 and T 75 which can influence the reduction of sensory attribute.

The differences of average mass concentration of malic acid are not statistically relevant. All samples went through malolactic fermentation at the same time and there are no significant differences between the relative values of malic acid which results in detection of approximately the same dependence in average concentrations. The finding of statistically significant difference between samples would indicate a failure in biodegradation of acids.

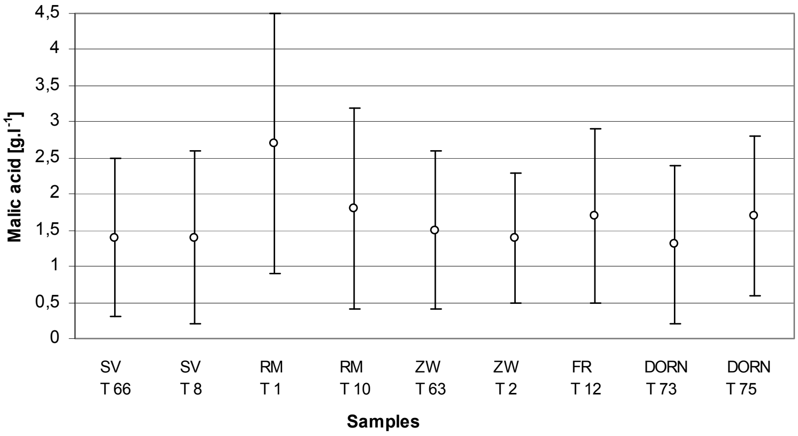
Lactic acid

The lowest concentration of lactic acid (Tab. IV) is detected in Zweigeltrebe T 63 (2.2 g/l), the highest concentration was measured in a sample of Dornfelder T 75 (3.5 g/l). The measured values correspond with the affirmation of Frudíková and Malík (2009) who claim that the content of lactic acid in wines, in which biodegradation occurred, should be within the range of 1.5 to 3.5 g/l. The average increase of concentration of lactic acid was 2.31 g/l and detected the average deviation 0.48 g/l. In the relative values, meaning, the average increase to 729.89% in comparison with the original value and average deviation 364.49%. The increase of lactic acid detected large average deviation which can be caused by various concentrations of malic acid, which is the initial raw material during malolactic fermentation. Havran, Stratil (2008) introduce that 67 g of lactic acid arises from 100 g of malic acid which was not confirmed in our samples. The final concentrations of lactic acid reached lower values.

The differences of the average mass concentrations of lactic acid are not statistically

III: *The changes in the concentration of malic acid after completion of biodegradation of acids*

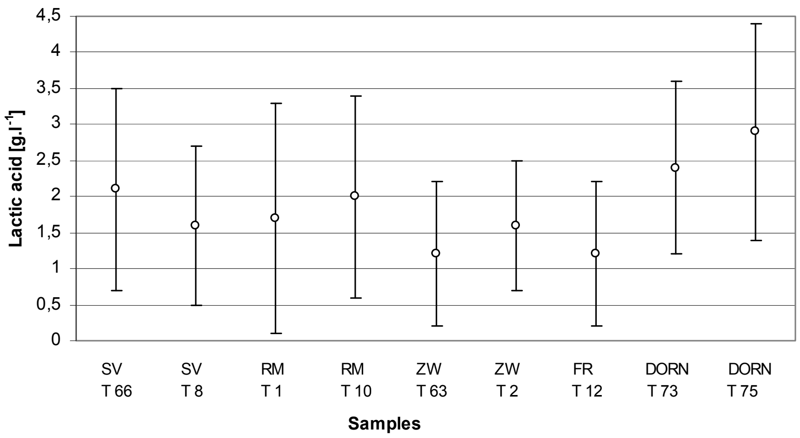
Wine	SV T 66	SV T 8	RM T 1	RM T 10	ZW T 63	ZW T 2	FR T 12	DORN T 73	DORN T 75	Ar. average / Averagedev.
Initial value [g/l]	3.8	2.8	4.3	3.3	2.5	2.3	2.7	2.9	3.3	–
Final value [g/l]	0.6	0.7	1.2	0.7	0.5	0.7	0.4	0.9	1.3	–
Abs. variation [g/l]	−3.2	−2.1	−3.1	−2.6	−2.0	−1.6	−2.3	−2.0	−2.0	−2.32 / 0.43
Rel. value [%]	15.79	25.00	27.91	21.21	20.00	30.43	14.81	31.03	39.39	25.06 / 6.33



3: *Confidence interval (P = 0.05) for the mass concentration of malic acid*

IV: *The changes in the concentration of lactic acid after completion of biodegradation of acids*

Wine	SV T 66	SV T 8	RM T 1	RM T 10	ZW T 63	ZW T 2	FR T 12	DORN T 73	DORN T 75	Ar. average / Averagedev.
Initial value [g/l]	0.2	0.4	0.2	0.5	0.5	0.7	0.5	0.6	0.7	–
Final value [g/l]	2.8	2.4	3.4	3.2	2.2	2.3	2.3	3.0	3.5	–
Abs. variation [g/l]	+2.6	+2.0	+3.2	+2.7	+1.7	+1.6	+1.8	+2.4	2.8	2.31 / 0.48
Rel. value [%]	1 400	600	1700	640	440	329	460	500	500	729.89 / 364.49



4: *Confidence interval (P = 0.05) for the mass concentration of lactic acid*

relevant. The highest average value of concentration reaches a sample of Dornfelder T 75 (2.88 g/l), the lowest average value a sample of Zweigeltrebe T 63 and Frankovka T 12 (1.20 g/l).

Citric acid

The mass concentration of citric acid (Tab. V) which was detected during malolactic fermentation the reduction (on average by 0.17 g/l to 21.7 % of the original value). The complete degradation occurred in samples of Rulandské modré T 1 and T 10. Minárik (2008) claims that lactic bacteria begin to metabolize citric acid at the end of biodegradation of acids. Degradation is linked with generating of acetic acid, diacetyl and other substances unfavourably affecting the bouquet and flavour of wine (Henick-Kling, Park, 1994; Delfini and Formica, 2001).

The differences of the mass concentrations of citric acid were not statistically relevant. The highest average concentration was detected in a sample of Zweigeltrebe T 63 (0.18 g/l), the lowest average value in Dornfelder T 75 (0.07).

V: *The changes in the concentration of citric acid after completion of biodegradation of acids*

Wine	SV T 66	SV T 8	RM T 1	RM T 10	ZW T 63	ZW T 2	FR T 12	DORN T 73	DORN T 75	Ar. average / Averagedev.
Initial value [g/l]	0.27	0.21	0.22	0.20	0.24	0.21	0.24	0.24	0.2	–
Final value [g/l]	0.08	0.07	0.00	0.00	0.12	0.05	0.06	0.06	0.03	–
Abs. variation [g/l]	–0.19	–0.14	–0.22	–0.20	–0.12	–0.16	–0.18	–0.18	–0.17	–0.17 / 0.02
Rel. value [%]	29.63	33.33	0.00	0.00	50.00	23.81	25.00	25.00	3.75	21.17 / 13.28

CONCLUSIONS

By virtue of introduced results we can state that the controlled malolactic fermentation with the help of the pure culture of lactic bacteria *Oenococcus oeni* has a positive influence on the red wines. The malolactic fermentation causes the degradation of malic acid from wine and also its replacement with lactic acid which is better evaluated in sensory way. The other advantages consist in favourable influence on aroma, creating a finer feeling in your mouth, higher microbiological stability and the reduction of quantity of used sulphure dioxide. Nowadays, this method is more and more expanded in practice.

SUMMARY

In red wines which spring from Znojmo wine region such as Svatovavřinecké, Rulandské modré, Zweigeltrebe, Frankovka and Dornfelder were detected the changes of the content organic during malolactic fermentation. The grapes went through vinification and after completion of alcoholic fermentation were inoculated with pure culture of lactic acid bacteria – preparation called Vitilactic XL (bacteria strain – *Oenococcus oeni*) which is used for the controlled process of malolactic fermentation. The chemical analysis during the process of biodegradation determined within the range of 2 to 4 days changes in the concentrations in the content of total acids such as: malic, lactic, tartaric and citric. The final content of acids statistically significantly differed only between samples of Svatovavřinecké T 66 and Zweigeltrebe T 2, samples reached the highest (6.72 g/l) and the lowest (4.74 g/l) average values of the total acids. The differences of average mass concentrations of lactic acid and malic acid were not statistically relevant. A sample of Dornfelder T 75 reached the highest average value of lactic acid (2.88 g/l) and a sample of Zweigeltrebe T 63 and Frankovka T 12 the lowest average value (1.20 g/l). The statistically relevant difference of tartaric acid from all the other wines was detected in a sample of SV T 66 which also reached the highest average value (5.18 g/l). The differences of mass concentrations of citric acid were not statistically relevant. The controlled malolactic fermentation with the help of pure cultures of lactic acids *Oenococcus oeni* has a positive influence on red wines. The malolactic fermentation causes the degradation of malic acid from wine and also its replacement with lactic acid which is better evaluated in sensory way and has a positive influence on sensory attributes of wine.

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