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## The suitability of malolactic fermentation for the Cviček wine

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### ABSTRACT

Cviček is a traditional, light red Slovenian wine made by blending the grapes, must or wine of red and white grapevine varieties. The malic acid concentration in Cviček wine is relatively high, so the use of malolactic fermentation (MLF) during its production was studied in two consecutive vintages, particularly with respect to the varieties with which it might be appropriate. The concentrations of organic acids were analysed using HPLC and UV-Vis detection. The simple ranking test and directional difference test were used for sensory assessments. They showed that partial MLF, concerning 15-50% of the wines in the final blend, contributed to a balanced acidity of Cviček. A suitable technique concerned either the MLF of Blaufränkisch or that of Blaufränkisch and white wine together. The MLF of Blauer Kölner or white wine alone was not appropriate. Despite our analysis of only a limited number of bottled wines, it appeared that MLF is already used in Cviček production and the resulting quality is promising.

**Key words:** wine, acidity, biological deacidification, *Oenococcus oeni*, sensory evaluation

### IZVLEČEK

#### PRIMERENOST UPORABE JABOLČNO MLEČNOKISLINSKE FERMENTACIJE V PRIDELAVI CVIČKA PTP

Cviček PTP je tradicionalno rdečkasto vino, ki ga pridelujejo z mešanjem grozdja, mošta ali vina rdečih in belih sort žlahtne vinske trte. Vsebnost jabolčne kisline v njem je precej velika, zato smo v dveh zaporednih letih proučevali uporabo jabolčno mlečnokislinske fermentacije (JMKF) s poudarkom na izboru sort, ki bi bile primerne za izvedbo JMKF. Vsebnost organskih kislin smo merili s HPLC in UV-Vis detektorjem. Za senzorično ocenjevanje smo uporabili test razvrščanja in test parov. Pokazali smo, da je delna uporaba JMKF (na 15-50 % vina v končni zvrsti) pozitivno vplivala na uravnoteženost kislinske zaznave vina cviček PTP. Primerna sta bila bodisi JMKF vina modra frankinja, bodisi modra frankinja in belo vino skupaj. Uporaba JMKF samo pri vinu žametovka ali belem vinu ni bila primerna. Čeprav smo analizirali le omejeno število stekleničenih vin, se zdi, da pridelovalci že uporabljajo JMKF v pridelavi cvička PTP in kakovost procesa je obetavna.

**Ključne besede:** vino, kislina, biološki razkis, *Oenococcus oeni*, senzorično ocenjevanje

## 1 INTRODUCTION

Cviček is a traditional, light red Slovenian wine made by blending the grapes, must or wine of red and white grapevine varieties. Some researches have been already performed on the wine, namely an investigation of the yeast community on grapes and during alcoholic fermentation relative to the three most important grapevine varieties used in its production (Raspor et al., 2002; Raspor et al.,

2009). The total acidity (expressed as tartaric acid) in Cviček wine should be between 6.0 to 9.5 g l<sup>-1</sup>. The addition of acids to must or wine is not allowed (Pravilnik o vinu ..., 2006). The level of malic acid in Cviček is relatively high as it tends to be dependent on grapevine variety and climate. However, malolactic fermentation (MLF, also referred to as biological deacidification) has rarely

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been applied during the production of Cviček wine. Davis et al. (1985) considered that it was difficult to describe MLF as being distinctly desirable or undesirable in terms of final wine quality. A grey area exists where the benefits and drawbacks depend on the region of production, grape variety, wine composition, winemaking techniques and style objectives of the winemaker. Chemical deacidification is more common in Cviček production, during which the use of potassium and calcium carbonates and tartrates is allowed.

The most important change that results from the activity of lactic acid bacteria (LAB) in wine is a reduction in wine acidity due to the decarboxylation of L-malic acid to form L-lactic acid. LAB may also influence aroma and flavour through the production of volatile secondary metabolites and modifications to grape and yeast-derived metabolites (Davis et al., 1985). Laaboudi et al. (1995) demonstrated differences between young MLF and non-MLF wines resulting from the lower acidity level of MLF wines, but their flavour was not significantly modified. However, certain LAB also degrade citric acid into various products of metabolism, amongst which acetoin compounds, diacetyl, acetoin and 2,3-butanediol play important roles. A moderate diacetyl content is desirable in wine; however, excessive acetic acid, the synthesis of glucane, biogenic amines and ethyl carbamate precursors are not (Beneduce et al., 2010; Liu, 2002; Lonvaud-Funel, 1999). In terms of the colour of red wines, the concentrations in monomeric anthocyanins were shown to fall during MLF (Garcia-Falcon et al., 2007; Mazza et al., 1999). However, García-Falcón et al. (2007) confirmed the absence of a correlation between changes in colour density and monomeric

anthocyanins content in two young Spanish red wines during a year of storage; suggesting that co-pigmentation and polymerization with other phenolic compounds prevailed over pigment degradation. Among other factors, the colour of anthocyanins is dependent on the pH of the wine (Brouillard et al., 1978; Vivar-Quintana et al., 2002), which rises during MLF. LAB also consume pyruvic acid and thereby limit the production of vitisin A, which forms from pyruvic acid and malvidin-3-glucoside and contributes to colour stability (Asenstorfer et al., 2003). Together with the fact that monomeric anthocyanins may account for more than 85% of total anthocyanins in Cviček wine, considerable caution should therefore be adopted when applying MLF.

Of the chromatographic methods, liquid chromatography (HPLC) is the most widely employed to determine the organic acids present in wine (Lopez and Gomez, 1996; Mato et al., 2005) and it was also used in our study. Attribute difference tests are used for the sensory evaluation of a single attribute, e.g. acidity, by comparing one sample with one or more others. Various tests should be used in this context, depending on the number of samples under analysis (Meilgaard et al., 1999).

During a two-year experiment, the use of MLF was studied in the production of Cviček wine, particularly with respect to the main grapevine varieties used and, consequently, the proportions in the final blend. Furthermore, the use of MLF at an industrial scale was investigated through the analysis of organic acids and sensorial evaluations of bottled Cviček wines.

## 2 MATERIALS AND METHODS

### 2.1 Biological deacidification

Eighteen, 25-litre samples of wine, vintage 2008 were collected after alcoholic fermentation and transferred to our microvinification cellar. Three of the main wines used to produce Cviček: a white wine (a blend of different white grapevine varieties, containing a majority of 'Königstraube' and 'Welschriesling'), 'Blaufränkisch' (red wine) and 'Blauer Kölner' (red wine) were sampled in

duplicate at their sites of production (three producers). Inoculated MLF was applied to one of these duplicate samples: *Oenococcus oeni* commercial strain VP 41 (Lallemand Inc., Rexdale, Ontario, Canada) was used (10 mg l<sup>-1</sup>) and the temperature of the wine was maintained at between 20 °C and 22 °C for 10-14 days. For the other sample of each wine, free SO<sub>2</sub> was maintained at 20-30 mg l<sup>-1</sup>. The experimental procedure described for 2008 was only completed

in six wines of 2009 vintage (one producer). MLF was performed as described above, except for Blauer Kölner wine.

## 2.2 Determining the concentrations of organic acids

MLF was followed by the identification and quantification of citric, lactic, malic and tartaric acids in the wines, using the reference method with an Agilent 1100 HPLC with a DAD detector (Agilent Technologies, Palo Alto, USA). The wine was filtered prior to injection using a Minisart® RC25 syringe filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany) with a pore size of 0.45 µm. The organic acids were separated using an Aminex HPX-87H ion-exchange resin column, with dimensions of 300 x 7.8 mm i.d. (Bio-Rad Laboratories, Hercules, CA) and detected with UV-Vis absorption spectroscopy at 210 nm. An isocratic technique was used with sulphuric acid (0.0125 mmol l<sup>-1</sup>) as the mobile phase at a constant flow rate of 0.5 ml min<sup>-1</sup>. The injection volume was 10 µl, the column temperature 65 °C and the time of analysis 30 min. Quantification was performed by comparing the peak areas of the sample and those of the external standards in a linear relationship (Lopez and Gomez, 1996; OIV, 2013).

## 2.3 Sampling of bottled wines

Twenty bottled Cviček wines of the 2009 vintage were sampled at their production sites. After the determination of organic acids, only twelve samples with different concentrations of malic and lactic acids were subjected to sensory evaluation with respect to their acidic taste. The purpose was to examine the use of MLF at an industrial scale and to verify the results obtained during microvinification experiments relative to the preference for the acidic taste of wines with or without partial MLF.

## 2.4 Blending the wine and chemical analysis

During our experiments, the proportions of each grapevine variety in Cviček wines were as follows: 50% 'Blauer Kölner', 35% white wine and 15% 'Blaufränkisch'. After five months of wine maturation of 2008 vintage, four different blends containing different proportions of biologically deacidified wines were prepared for each producer (3x4 wines): C - control, none of the base wines underwent MLF; W - white wine which underwent

MLF (35% of the final blend); BW - 'Blaufränkisch' and white wine which underwent MLF (50% of the final blend); K - 'Blauer Kölner' which underwent MLF (50% of the final blend).

Concerning the total acidity of the wines and the results of sensory evaluations in 2008, it was once again decided to include 15-50% of biologically deacidified wines in the blends of the vintage 2009, but not to use MLF for the 'Blauer Kölner' wine. The proportions of each grapevine variety ('Blauer Kölner', white wine and 'Blaufränkisch') in the final blend were the same as in the previous vintage. After five months of maturation, four different blends containing different proportions of biologically deacidified wines were prepared (1x4 wines): C – control, none of the wine underwent MLF; B - 'Blaufränkisch' which underwent MLF (15% of the final blend; this blend was not prepared in the previous vintage); W – white wine which underwent MLF (35% of the final blend); BW - 'Blaufränkisch' and white wine which underwent MLF (50% of the final blend).

The levels of organic acids were measured in the wines before and after blending using the reference method described above. The total acidity and pH of the wines were determined using the reference methods published by the OIV (2013). The colour of all wines was evaluated by sensory evaluation alone.

## 2.5 Sensory evaluation

Sensory evaluations of 2008 vintage were performed in our sensory unit by two groups of assessors: one representing wine experts experienced in the sensory evaluation of Cviček (12 assessors) and the second representing consumers (12 assessors also). All wines were served at 12 °C. Ranking tests were used for the following attributes: colour, flavour and acidity. Both panels evaluated three series of four wines. The three series represented the different producers (1, 2, and 3) and the four wines represented the different blends of Cviček (C, W, BW, and K) described above. The assessors ranked the wines from the best – liked extremely (grade 1) to the most inferior – disliked extremely (grade 4) for each of the attributes (Koak et al., 2010). The procedure was applied separately for each attribute, using new samples that were coded

differently. The panelists assessed the samples in random order (Meilgaard et al., 1999).

Wines from 2009 vintage were evaluated using a paired comparison test (two-sided) on three pairs of samples: one wine in each pair was a control (C) and the other was one of the blends described above (B, W, and BW). The samples were offered simultaneously to all subjects who were asked to decide which sample they preferred relative to the attribute being tested (colour, flavour, and acidity). The procedure was carried out separately for each attribute, using new samples that were coded differently. The number of respondents was 13 ( $p_{max} = 75\%$ ,  $\alpha = 0.10$  and  $\beta = 0.50$ ). The minimum numbers of correct responses required for significance at the stated  $\alpha$ -level for the corresponding number of respondents were taken from the table (Meilgaard et al., 1999).

The acidity of the 12 samples of bottled Cviček wines from the 2009 vintage was also evaluated. Eleven assessors (experts) in the sensory panel scored the wines according to their acidic taste on a scale of 1 to 3, where 1 = poor, 2 = good, 3 = excellent (Meilgaard et al., 1999).

## 2.6 Statistical analysis

The results of all sensory evaluations were analysed statistically using the Friedman analysis and the tables for the critical number of correct responses for the directional difference test. The acidity scores for bottled Cviček wines were evaluated by ANOVA using a randomized block design and an LSD multiple comparison procedure at the 95% confidence level (Meilgaard et al., 1999).

## 3 RESULTS AND DISCUSSION

During this two-year experiment, the use of MLF in Cviček production was studied, particularly with respect to the varieties used and consequently their proportions in the final blend enabling its application.

### 3.1 2008 vintage

The total acidity, pH, lactic and malic acid levels of the white wine, Blaufränkisch and Blauer Kölner before and after MLF and after blending (C, W, BW and K blends) are shown in Table 1, 2, and 3 (Producers 1, 2, and 3). The total acidity of the control blends ranged from 6.93 g l<sup>-1</sup> (Producer 1) to 7.91 g l<sup>-1</sup> (Producer 3). The choice of wine samples from the 2008 vintage was indeed

excellent because the total acidity of control blends had a range of almost 1.0 g l<sup>-1</sup>. The total acidity of Cviček wine with MLF of Blauer Kölner (K - Producer 1) was lower than the lower limit fixed by the regulations (6 g l<sup>-1</sup>). Together with the increased proportion of biologically deacidified wine in the final blends, the total acidity of the wines decreased; in particular the difference between the controls (C) and wines with MLF of Blauer Kölner (K) ranged from 1.34 (Producer 2) to 1.59 g l<sup>-1</sup> (Producer 3). Consequently, the pH values of the blends with MLF of Blauer Kölner rose from 0.07 (Producer 2) to 0.12 (Producers 1 and 3). At the same time, the malic acid levels fell and those of lactic acid rose.

**Table 1:** Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine, Blaufränkisch, Blauer Kölner, and in the different blends of 2008 Cviček wines – Producer 1. The results of sensory evaluations of the different blends are also shown

Producer 1	Wines for blending					
	White wine (without MLF)	White wine (with MLF)	Blaufränkisch (without MLF)	Blaufränkisch (with MLF)	Blauer Kölner (without MLF)	Blauer Kölner (with MLF)
Before MLF						
<i>Total acidity (g l<sup>-1</sup> of tartaric acid)</i>	7.21	7.18	7.17	7.08	8.93	9.01
<i>pH</i>	3.37	3.37	3.42	3.42	3.23	3.22
<i>Malic acid (g l<sup>-1</sup>)</i>	3.75	3.71	2.91	2.73	3.53	3.04
<i>Lactic acid (g l<sup>-1</sup>)</i>	0.26	0.26	0.30	0.42	0.23	0.15
After MLF						
<i>Malic acid (g l<sup>-1</sup>)</i>	2.96	0.05	2.28	0.19	3.69	0.24
<i>Lactic acid (g l<sup>-1</sup>)</i>	0.20	2.37	0.40	2.05	0.33	2.00
<i>Cviček – blends with and without partial MLF<sup>1)</sup></i>						
	C	W	BW	K		
<i>Chemical parameter</i>						
<i>Total acidity (g l<sup>-1</sup> of tartaric acid)</i>	6.93	6.34	6.32	5.51		
<i>pH</i>	3.30	3.33	3.32	3.42		
<i>Malic acid (g l<sup>-1</sup>)</i>	2.81	1.86	1.70	1.04		
<i>Lactic acid (g l<sup>-1</sup>)</i>	0.63	1.16	1.27	1.80		
<i>Sensory attributes</i>	<i>Panel 1 (experts; n = 12) - Rank sum</i>					
<i>Colour<sup>2</sup></i>	20 (the best)	22	32	46 (the most inferior)		
<i>Flavour<sup>2</sup></i>	24	21 (the best)	28	47 (the most inferior)		
<i>Acidity<sup>2</sup></i>	25	24 (the best)	27	44 (the most inferior)		
	<i>Panel 2 (consumers; n = 12) - Rank sum</i>					
<i>Colour<sup>2</sup></i>	19 (the best)	30	30	41 (the most inferior)		
<i>Flavour<sup>2</sup></i>	19 (the best)	36 (the most inferior)	29	36 (the most inferior)		
<i>Acidity<sup>2</sup></i>	22 (the best)	32	27	39 (the most inferior)		

<sup>1)</sup> C – control blend (all wines without MLF); W – white wine with MLF (35% of final blend); BW – white wine and Blaufränkisch with MLF (50% of final blend); K – Blauer Kölner with MLF (50% of final blend)

<sup>2)</sup> Samples differing significantly regarding that parameter. The critical value (LSD<sub>rank</sub>) of the multiple comparisons was 12.4. Any two samples whose sums differed by more than 12 were rated significantly different at  $\alpha = 0.05$

**Table 2:** Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine, Blaufränkisch, Blauer Kölner, and in the different blends of 2008 Cviček wines – Producer 2. The results of sensory evaluations of the different blends are also shown

<u>Producer 2</u>	<i>Wines for blending</i>					
	White wine (without MLF)	White wine (with MLF)	Blafränkisch (without MLF)	Blafränkisch (with MLF)	Blauer Kölner (without MLF)	Blauer Kölner (with MLF)
Before MLF						
<i>Total acidity</i> (g l <sup>-1</sup> of tartaric acid)	9.38	9.32	7.79	7.90	8.54	8.76
<i>pH</i>	3.17	3.18	3.40	3.43	3.23	3.24
<i>Malic acid</i> (g l <sup>-1</sup> )	4.72	4.81	2.93	3.17	2.92	3.25
<i>Lactic acid</i> (g l <sup>-1</sup> )	0.15	0.16	0.19	0.27	0.14	0.14
After MLF						
<i>Malic acid</i> (g l <sup>-1</sup> )	4.44	0.10	2.40	0.21	3.31	0.25
<i>Lactic acid</i> (g l <sup>-1</sup> )	0.13	3.45	0.24	1.85	0.36	2.48
<i>Cviček – blends with and without partial MLF<sup>1)</sup></i>						
	C	W	BW	K		
<i>Chemical parameter</i>						
<i>Total acidity</i> (g l <sup>-1</sup> of tartaric acid)	7.61	6.46	6.37	6.27		
<i>pH</i>	3.24	3.33	3.33	3.31		
<i>Malic acid</i> (g l <sup>-1</sup> )	3.29	1.83	1.70	1.54		
<i>Lactic acid</i> (g l <sup>-1</sup> )	0.53	1.57	1.71	1.80		
<i>Sensory attributes</i>	<i>Panel 1 (experts; n = 12) - Rank sum</i>					
<i>Colour</i> <sup>2)</sup>	14 (the best)	30	30	46 (the most inferior)		
<i>Flavour</i>	26 (the best)	30	26 (the best)	38 (the most inferior)		
<i>Acidity</i> <sup>2)</sup>	35	23	22 (the best)	40 (the most inferior)		
	<i>Panel 2 (consumers; n = 12) - Rank sum</i>					
<i>Colour</i> <sup>2)</sup>	22 (the best)	30	26	42 (the most inferior)		
<i>Flavour</i>	24 (the best)	31	29	36 (the most inferior)		
<i>Acidity</i>	27 (the best)	31	29	33 (the most inferior)		

<sup>1, 2)</sup> See notes to Table 1

**Table 3:** Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine, Blaufränkisch, Blauer Kölner, and in the different blends of 2008 Cviček wines – Producer 3. The results of sensory evaluations of the different blends are also shown

<u>Producer 3</u>		<i>Wines for blending</i>				
	White wine (without MLF)	White wine (with MLF)	Blaufränkisch (without MLF)	Blaufränkisch (with MLF)	Blauer Kölner (without MLF)	Blauer Kölner (with MLF)
Before MLF						
<i>Total acidity</i> (g l <sup>-1</sup> of tartaric acid)	9.43	9.40	8.34	8.31	9.00	9.02
<i>pH</i>	3.14	3.16	3.29	3.30	3.25	3.24
<i>Malic acid</i> (g l <sup>-1</sup> )	4.91	5.12	2.97	3.12	3.64	3.90
<i>Lactic acid</i> (g l <sup>-1</sup> )	0.19	0.28	0.20	0.26	0.27	0.17
After MLF						
<i>Malic acid</i> (g l <sup>-1</sup> )	4.70	0.06	2.14	0.30	3.80	0.12
<i>Lactic acid</i> (g l <sup>-1</sup> )	0.10	3.48	0.30	1.31	0.37	2.60

  

<i>Cviček – blends with and without partial MLF<sup>1)</sup></i>				
	C	W	BW	K
<i>Chemical parameter</i>				
<i>Total acidity</i> (g l <sup>-1</sup> of tartaric acid)	7.91	6.63	6.57	6.32
<i>pH</i>	3.20	3.30	3.29	3.32
<i>Malic acid</i> (g l <sup>-1</sup> )	3.44	2.17	1.97	1.58
<i>Lactic acid</i> (g l <sup>-1</sup> )	0.47	1.49	1.64	1.92
<i>Sensory attributes</i>				
	<i>Panel 1 (experts; n = 12) - Rank sum</i>			
<i>Colour</i> <sup>2)</sup>	25	21 (the best)	31	43 (the most inferior)
<i>Flavour</i>	30	27 (the best)	27 (the best)	36 (the most inferior)
<i>Acidity</i> <sup>2)</sup>	35	22 (the best)	23	40 (the most inferior)
	<i>Panel 2 (consumers; n = 12) - Rank sum</i>			
<i>Colour</i> <sup>2)</sup>	30	25	20 (the best)	45 (the most inferior)
<i>Flavour</i>	27 (the best)	27 (the best)	30	36 (the most inferior)
<i>Acidity</i> <sup>2)</sup>	33	27	21 (the best)	39 (the most inferior)

<sup>1, 2)</sup> See notes to Table 1

The results of sensory evaluations with respect to wine colour, flavour and acidity are shown in Tables 1, 2, and 3. A lower score means a higher quality for the attribute evaluated.

**Colour.** Panel 1 (experts) determined the colour of blends containing biologically deacidified Blauer Kölner (K – all three producers) as being inferior to all the other blends (Tables 1, 2, and 3). At the same time, the control blends (C) had a better colour than blends with MLF of Blaufränkisch and white wine (BW) (Producer 1) or blends containing either white wine with MLF (W) or

Blaufränkisch and white wine together (BW) (Producer 2). Panel 2 (consumers) also found the colour of K blends to be inferior to the other blends (Producers 2 and 3), while for Producer 1, only the colour of the control blend was significantly better.

**Flavour.** Panel 1 found the flavour of the K blend to be inferior to that of the other blends (Producer 1), while the blend from Producer 2 scored significantly lower than the control and BW blend. The same trend towards lower scores (by both panels) for the K blend could be also seen for Producer 3. Panel 2 found the flavour of K blend to

be significantly inferior for Producers 1 and 2 when compared to the control. For Producer 1, the W blend also received a significantly lower score than the control.

**Acidity.** Panel 1 ranked the acidity of the W and BW blends as being better than the control and K blends (Producers 2 and 3). For producer 1, the K blend was evaluated as being inferior to the other three blends in terms of acidity. Panel 2 scored the acidity of the control and BW blend higher than the K blend (Producer 1). This panel did not recognize any differences in acidity between the wines from Producer 2. For Producer 3, the W and BW blends displayed a more appropriate acidity than the K blend. At the same time, the consumers also ranked the acidity of the BW blend as being better than the control.

### 3.2 2009 vintage

The total acidity, pH, lactic and malic acid levels of the two replicates of white wine and Blaufränkisch (before and after MLF) and one replicate of Blauer Kölner, are shown in Table 4. These parameters were also determined for Cviček wines after blending (C, B, W, and BW). The total acidity in the blends fell from 6.40 g l<sup>-1</sup> (C) to 5.60 g l<sup>-1</sup> (BW). The total acidity of the W and BW blends were lower than the limit fixed by the regulations (6 g l<sup>-1</sup>), but a sensory comparison with the control blend was nonetheless performed. By increasing the proportion of biologically deacidified wine in the final blends, the pH of the wines rose from 3.15 (C) to 3.22 (BW); malic acid levels fell, while lactic acid levels rose.

**Table 4:** Total acidity, pH and malic and lactic acid levels before and after MLF in the two replicates of white wine and Blaufränkisch and one of Blauer Kölner, respectively, and in the different blends of 2009 Cviček wines. The results of sensory evaluations comparing the control and other blends are also shown

Wines for blending					
	White wine (without MLF)	White wine (with MLF)	Blaufränkisch (without MLF)	Blaufränkisch (with MLF)	Blauer Kölner (without MLF)
Before MLF					
<i>Total acidity</i> <i>(g l<sup>-1</sup> of tartaric acid)</i>	8.01	8.04	8.21	8.28	7.52
<i>pH</i>	3.18	3.18	3.25	3.24	3.28
<i>Malic acid (g l<sup>-1</sup>)</i>	3.59	3.57	2.23	2.36	1.83
<i>Lactic acid (g l<sup>-1</sup>)</i>	0.23	0.29	0.25	0.22	0.22
After MLF					
<i>Malic acid (g l<sup>-1</sup>)</i>	3.50	0.60	2.05	0.10	1.73
<i>Lactic acid (g l<sup>-1</sup>)</i>	0.20	1.95	0.30	1.76	0.27

Cviček – blends with and without partial MLF <sup>1)</sup>						
	C	B	W	BW		
Chemical parameter						
<i>Total acidity</i> <i>(g l<sup>-1</sup> of tartaric acid)</i>	6.40	6.10	5.70	5.60		
<i>pH</i>	3.15	3.16	3.21	3.22		
<i>Malic acid (g l<sup>-1</sup>)</i>	2.30	2.06	1.01	0.80		
<i>Lactic acid (g l<sup>-1</sup>)</i>	0.24	0.47	0.85	1.16		
Sensory attributes	Panel 1 (experts; n = 13) – Number of preferred choices in directional difference test					
	C	B	C	W	C	BW
<i>Colour</i>	6	7	3	10b <sup>2)</sup> (better)	8	5
<i>Flavour</i>	6	7	5	8	6	7
<i>Acidity</i>	3	10b (better)	3	10b (better)	3	10b (better)
	Panel 2 (consumers; n = 13) - Number of preferred choices in directional difference test					
	C	B	C	W	C	BW
<i>Colour</i>	7	6	10 (better)	3b	5	8
<i>Flavour</i>	6	7	10 (better)	3b	6	7
<i>Acidity</i>	2	11a (better)	11 (better)	2a	5	8

<sup>1)</sup> C – control (without MLF); B – Blaufränkisch with MLF (15% of final blend); W – white wine with MLF (35% of final blend); BW – white wine and Blaufränkisch with MLF (50% of final blend)

<sup>2)</sup> The samples differed significantly from the control for the parameter at  $\alpha = 0.05$  (a) or  $\alpha = 0.10$  (b), respectively. Higher number of preferred choices denotes better wine for that sensory attribute

The results of sensory evaluations by both panels are shown in Table 4. A higher number of preferred choices in the directional difference test indicated a higher quality of the parameter being assessed in the chosen wine. Parameter quality differed significantly when 10 (10% level) or 11 (5% level) assessors preferred the same blend.

**Colour.** Panel 1 assessed the colour of the W blend as being significantly better than the colour of the control, in complete contrast with the findings of panel 2.

**Flavour.** Panel 1 did not distinguish between the flavour of blends with partial MLF and the control. By contrast, panel 2 evaluated the flavour of the W blend as being significantly worse.

**Acidity.** Panel 1 assessed the acidity of blends with partial MLF (B, W and BW) as being significantly better than the control. Panel 2 assessed the acidity of the B blend as being significantly better, but the W blend as being significantly worse, than the control.

Based on the results obtained with the vintage 2008, it could be seen that if the total acidity of the

control blend was low (Producer 1), then the use of MLF was not necessary. As the acidity rose in control wines, the acidity of blends involving either the MLF of white wine, or of white wine and Blaufränkisch together, was preferred by the experts, a finding that was confirmed for the 2009 vintage. However, this preference was not clearly confirmed by the consumers in either 2008 or 2009. It was certainly possible to confirm that the MLF of Blauer Kölner was not appropriate to Cviček production as it caused significant changes to the colour, flavour and acidity of the wine. As for the MLF of white wine alone, a cautious attitude should also be adopted, because the consumer panel did not prefer that blend.

### 3.3 Analysis of bottled Cviček wines

The malic and lactic acid contents and the results of sensory evaluations of wine acidity by the panel of 11 experts are shown in Table 5. Some correlation between the concentrations of malic or lactic acid, respectively, and the sensory scores for wine acidity, could be seen. The preference for an acidic taste increased as the malic acid content in the wine fell to a concentration of  $0.76 \text{ g l}^{-1}$  in sample 10, which obtained significantly the highest sensory score. For the two samples with lower malic acid content, the sensory scores were lower again.

**Table 5:** Malic and lactic acid levels and the results of acidity scores for 12 bottled Cviček wines (2009 vintage)

Wine sample	1 <sup>1)</sup>	2	3	4	5	6	7	8	9	10	11	12
<i>Organic acids</i>												
Malic ( $\text{g l}^{-1}$ )	2.08	2.06	1.98	1.97	1.87	1.85	1.72	1.49	1.10	0.76	0.53	0.42
Lactic ( $\text{g l}^{-1}$ )	0.17	0.62	0.98	0.33	0.49	0.64	0.29	0.83	0.77	0.90	1.73	1.67
<i>Sensory attribute</i>												
Acidity (1-3)	2.00a <sup>2)</sup>	2.00a	2.18a	2.00a	2.00a	2.09a	2.36ab	2.27ab	2.27ab	2.73b	2.09a	2.18a

<sup>1)</sup> Samples are presented in order from the highest to the lowest concentration of malic acid in the wine. Eleven experts in the sensory panel scored the wines according to the suitability of their acidic taste on a scale of 1 to 3, where 1 = poor, 2 = good, 3 = excellent

<sup>2)</sup> Mean scores followed by the different letters are significantly different at the 95% confidence level –  $\text{LSD}_{95\%} = 0.48$

The analysis of organic acids in bottled wines showed that some producers applied MLF in the production of Cviček in 2009. Its positive influence on wine acidity, as shown in microvinification experiments, was confirmed,

while sample 10 of bottled Cviček received the best score; this contained malic and lactic acid levels that were very similar to those in our blend involving the MLF of Blaufränkisch and white wine together in 2009.

## 4 CONCLUSIONS

It was possible to conclude that a limited use of MLF in Cviček production, namely concerning 15-50% of the wine in the final blend, exerted a positive impact on its acidity. The higher the expected total acidity in the final blend, the greater the proportion of base wines that could be biologically deacidified. MLF should be used on either Blaufränkisch or on Blaufränkisch and white wine together. The upper limit of total acidity at which MLF is considered to be necessary is dependent on each vintage. In this case, measuring

the concentration of malic acid could also be used, since we determined its concentration to be inappropriate if it exceeded  $3.0 \text{ g l}^{-1}$  (2008) or  $2.0 \text{ g l}^{-1}$  (2009) in the final blend. However, it is clear that MLF does not only modify the acidic taste; colour and flavour attributes are also affected (Liu, 2002; Lonvaud-Funel, 1999), as was also shown by our study. Consequently, producers should be cautious when considering the use of MLF, because an excessive rise in the pH negatively influences the colour of light red wines

(Brouillard et al., 1978; Vivar-Quintana et al., 2002). MLF is not always beneficial and may cause undesirable changes to the sensory properties of wine. Greater attention therefore needs to be paid to managing the MLF with respect to the production of diacetyl and other metabolites that

might influence the aroma, flavour and healthiness of the wine (Bartowsky, 2009; Bauer and Dicks, 2004). However, despite our analysis of only a limited number of bottled wines, it appeared that MLF is already used in Cviček production and the resulting quality is promising.

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