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Deacidification of wine using wine yeast and bacteria starter cultures

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Present investigations deal with the malic acid reducing capacity of selected wine yeast strains of the species Saccharomyces cerevisiae *and bacteria starter cultures of the species* Oenococcus oeni*. Deacidification capacity and influence of bacteria present in seven of ten commercial dry yeast strains compared to pure culture of yeast strains were evaluated on the basis of 'Rheinriesling' must fermentations. Analysis of malic acid reduction in must and wine showed basically yeast borne activity which was shown to be amplified in musts with pH-value 3.1 and 3.4 respectively. Some of the investigated dry yeast preparations were found to contain low amounts of bacteria cultivable on appropriate media. Results concerning the malic acid degradation velocity in red wines did not correlate with the amount of bacteria cell number in dry yeast preparations. Mash from grapes of the 'Blauer Portugieser' cultivar was fermented with two commercial dry yeast preparations. Malolactic fermentation variants were induced using three different bacteria starter cultures, simultaneously to a spontaneous fermentation variant. At 16 °C malolactic fermentation finished successfully several days later compared to variants at 21 °C. Both yeast and bacteria reduced monomeric anthocyanins in red wines. After four months of storage on fine lees, the influence of microorganisms on phenols and biological stability was evaluated. Bacteria developed during spontaneous malolactic fermentation showed highest viability using temperatures of 21 °C and 16 °C compared to* Oenococcus oeni *starter culture. During wine storage on fine lees, the amino acid tyrosine increased to the highest amount compared to arginine and lysine. Amounts of caffeic acid, a precursor for volatile phenols, cannot be related to yeast or bacteria strain influence.*
Keywords: Wine, deacidification, yeasts, malolactic fermentation, starter cultures, *Saccharomyces cerevisiae, Oenococcus oeni*

Abbau der Äpfelsäure mit Weinhefen und Bakterien-Starterkulturen. *Die äpfelsäureabbauende Kapazität von selektionierten Stämmen der Art* Saccharomyces cerevisiae *und von Bakterien der Art* Oenococcus oeni *sind Gegenstand vorliegender Untersuchungen in Weinen der Sorte 'Rheinriesling' und 'Blauer Portugieser'. Die Analysen bestätigten, dass der Äpfelsäureabbau teilweise auf die metabolische Aktivität der untersuchten Hefen zurückzuführen ist. Diese Aktivität trat in Mosten mit einem pH-Wert von 3.4 im Vergleich zu Mosten mit einem pH-Wert von 3.1 verstärkt auf. In sieben von zehn Trockenhefepräparaten wurden Bakterien nachgewiesen. Die Kinetik des biologischen Säureabbaus im Rotwein der Sorte 'Blauer Portugieser' korrelierte nicht mit der Höhe der nachgewiesenen Bakterienzellzahlen. Trauben der Sorte 'Blauer Portugieser' wurden mit zwei kommerziellen Hefestämmen vergoren. Der anschließend durchgeführte biologische Säureabbau mit drei Bakterien-Starterkulturen im Vergleich zu spontanem biologischen Säureabbau verlief in allen Varianten erfolgreich, war jedoch bei 16 °C um einige Tage verzögert im Vergleich zu 21 °C. Hefen und Bakterien reduzierten die monomeren Anthocyane in den Rotweinen in unterschiedlichem Ausmaß. Nach einer Lagerung von vier Monaten auf der Feinhefe wurde der Einfluss der Mikroflora auf die biologische Stabilität und auf die Phenole evaluiert. Die Bakterien-Spontanflora wies im Vergleich zu* Oenococcus oeni *Starterkulturen die höchste Lebendzellzahl auf. Der Gehalt der Aminosäure Tyrosin stieg am stärksten an. Auch die Konzentration an Arginin und Lysin nahm zu. Kaffeesäure kann auf Grund mikrobiologischen Einflusses in flüchtige Phenole umgewandelt werden.* Saccharomyces cerevisiae *oder* Oenococcus oeni *hatten keinen Einfluss auf Zu- oder Abnahme des Kaffeesäuregehaltes in den Weinen.*
Schlagwörter: Wein, Entsäuerung, Hefen, biologischer Säureabbau, Starterkulturen, *Saccharomyces cerevisiae, Oenococcus oeni*

Dégradation de l'acide malique par des levures de vin et des cultures starter de bactéries. *La capacité des souches sélectionnées du genre* Saccharomyces cerevisiae *et des bactéries du genre* Oenococcus oeni *de dégrader l'acide malique fait l'objet des présents examens des vins des cépages 'Rheinriesling' et 'Blauer Portugieser'. Les analyses confirment que la dégradation de l'acide malique est partiellement due à l'activité métabolique des levures examinées. Cette activité s'est manifestée plus intensément dans des moûts d'un pH de 3,4 que dans les mo«ts d'un pH de 3,1. Dans quelques préparations de levure sèche, on a détecté des bactéries qui ne jouent très vraisemblablement aucun rźle à la fermentation. La cinétique de la dégradation biologique de l'acide dans le vin rouge du cépage 'Blauer Portugieser' ne présente aucune corrélation avec le nombre des bactereis détectés dans les souches commerciales. Les macérations avec les raisins du cépage 'Blauer Portugieser' ont été effectuées avec deux souches de levure commerciale. La dégradation biologique de l'acide à l'aide de trois cultures starter de bactéries s'est déroulée de manière efficace par comparaison à la dégradation biologique spontanée de l'acide, mais a été retardée de quelques jours à une température de 16 °C par rapport à une température de 21 °C. Les levures et les bactéries réduisaient d'une manière différente les anthocyanes monomères dans les vins rouges. L'influence de la microflore sur la stabilité biologique et sur les phénols a été évaluée après un stockage de quatre mois sur lies fines. Comparée à* Oenococcus oeni *des cultures commerciales, la flore spontanée présentait l'indice de germination le plus élevé. La teneur en acide aminé tyrosine présentait la plus forte augmentation. La concentration d'arginine et de lysine a également augmenté. Graçe à l' influence microbiologique, l'acide caféique peut ńtre transformé en phénols volatils.* Saccharomyces cerevisiae *ou* Oenococcus oeni *n'ont eu aucune influence sur l'augmentation ou la baisse de la teneur des vins en acide caféique.*
Mots clés: vin, désacidification, levures, fermentation malolactique, cultures starter, *Saccharomyces cerevisiae, Oenococcus oeni*

New enological procedures contribute to accurate wine making and a higher quality on the wine market. Processing standards are getting higher, demanding precise winemakers' skills and process monitoring from vineyard to filled bottles.

Balance of tartaric acid and malic acid is important for further product stability.

Acidification and deacidification of must or wine represent useful tools to adjust the quality. Malic acid will be degraded by chemical or microbiological procedures in case of high vintage acidity or for biological stabilization of red wines.

Microbiological tools such as dry yeasts of the species *Saccharomyces cerevisiae* used for alcoholic fermentation and bacteria starter cultures of the species *Oenococcus oeni* both degrade malic acid. Metabolism of malic acid by yeast leads to the formation of carbonic acids like pyruvate, L-lactic acid and relatively high amounts of D-lactic acid, succinic acid and acetic acid. Homofermentative and heterofermentative lactic acid bacteria metabolize malic acid (Ribéreau-Gayon et al., 1998). Malic acid degradation can be generated spontaneously in case of native bacterial flora development, but accurately selected bacteria starter cultures do emphasize dominance of *Oenococcus oeni* species. Product diversity of supplied bacterial cultures increases process reliability. Addition of high cell numbers of 10^6 cfu (colony forming units)/ml are recommended. Present experiments with must of 'Rheinriesling' investigate the capacity of *Saccharomyces cerevisiae* to degrade malic acid amounts during alcoholic fermentation. Dry yeast preparations contain bacteria contaminants at amounts of 10^2 cfu/g to 10^5 cfu/g of dry yeast (Radler et al., 1985). Bacterial contaminants in dry yeast preparations could contribute to malic acid reduction.

High numbers of viable bacteria cells in wine might cause off-flavours like bitterness and buttery taste or deteriorate red colour. Analysis of bacteria viability during storage is necessary because viable starter cultures are a potential risk for wine stability.

Certain substances can cause wine aging damages and therefore it is of interest to analyse their concentrations with respect to dry yeast and bacteria starter culture variants. The influence of the fine lees microflora on wine quality can be evaluated on the basis of amino acids, phenols and anthocyanins.

Some amino acids are released to wines during lees contact. On one hand, amino acids could be used by bacteria for their nutrition. On the other hand, sulphuric molecules like cysteine could damage wine flavour.

The uncoloured ethylcaffeic acid is a cinnamic acid. Cinnamic acid alterates the colour of wine towards yellow or brown. *Saccharomyces cerevisiae*, lactic acid bacteria and *Dekkera bruxellensis* produce cinnamate decarboxylase and are able to transform caffeic acid to undesired volatile phenols.

Monomeric anthocyanin concentrations indicate colour stability. Some yeast strains preserve monomeric antho-

cyanins during alcoholic fermentation (BERGER et al., 2000) and are therefore useful for red wine production (FUSTER et. al., 2002).

Materials and Methods

Malic acid degradation during alcoholic fermentation

1st Experiment: Fresh 'Riesling' must (pH-value 3.1):

Fresh 'Rheinriesling' must (5 l per variant), pH-value 3.1, was inoculated with 10 g/hl of following eleven rehydrated commercial dry yeast strains: *L 1033*, *L 71 B*, *EC 1118, L RC272*, *L 2056, RHST* (all: Lallemand, Canada), *Oenoferm Klosterneuburg, Oenoferm Bouquet, Oenoferm Tipico, Oenoferm Interdry* (all: Erbslöh-Geisenheim, Germany), and *Fermicru VR5* (Gist Brocades, Netherlands).
Additionally one variant was inoculated with a pure native selected *Saccharomyces cerevisiae* culture named „Rheinriesling Eiswein", starting from a single colony on Wallerstein-agar plates (VWR, USA), cultivated over night at 28 °C in 250 ml liquid YEP medium (20 g peptone, DIFCO, USA; 20 g glucose; 10 g yeast extract, VWR, USA; aqua dest. ad 1000 ml; autoclaved at 121 °C for 15 min.). After centrifugation at 2500 rpm, the medium was discarded. The pellet was resuspended in pasteurized must and total cell number was adjusted in fresh must to 10^7 cfu/ml.

2nd Experiment: Pasteurized 'Riesling' must (pH-value 3.4):

Each 10 g/hl of yeast strain *L71 B, EC 1118*, (all: Lallemand, Canada), *Oenoferm Bouquet, Oenoferm Tipico* and *Oenoferm Klosterneuburg* (all: Erbslöh-Geisenheim, Germany) were added to 600 g pasteurized 'Rheinriesling' must (pH-value 3.4). Fermentation lasted ten days in both experiments. Malic acid was analysed by ionchromatography (Dionex, USA) in untreated must and in wines immediately after alcoholic fermentation.
Usually, number of viable bacteria cells accompanying dry yeast preparations come to numbers less than 10^5 cfu/g of dry yeast compared to *Saccharomyces* cell numbers of 10^{10} cfu/g of dry yeast (RADLER et al., 1985). By direct plate counting, starting from rehydrated dry yeast or fermenting musts, aerobic or facultative anaerobic bacteria were not detectable. To promote multiplication of potential contaminants bacterial growth was initiated in appropriate medium. 0.5 g of each dry yeast were cultivated in MRS (Man, Rogosa, Sharpe)-liquid medium (10 g peptone, 10 g meat extract, 5 g yeast extract, 20 g glucose, 1 g Tween 80, 2 g K_2HPO_4, 5 g CH_3COONa x 3 H_2O, 2 g diammonium citrate, 0.2 g $MgS0_4$ x 7 H_2O, 0.05 g $MnSO_4$, VWR, USA; adjusted to pH-value 5.4, aqua dest. ad 1000 ml, 80 µg/ml cycloheximide, VWR, USA, added to sterilized medium) supporting multiplication of the species *Oenococcus, Lactobacillus* and *Pediococcus.* Cells were incubated at 28 °C for 72 hours. Numbers of viable cells were determined on Orange-Serum-agar plates (VWR, USA) supplied with 80 µg/l of cycloheximide (VWR, USA) to suppress yeast multiplication.

Malolactic fermentation

Mash from the grape cultivar 'Blauer Portugieser' was fermented with two different yeast strains, *L 2056* (Lallemand, Canada) and *Oenoferm Klosterneuburg* (Erbslöh Geisenheim, Germany). After three days the spontaneous mash fermentation variant was inoculated with 10 g/hl of each dry yeast strain. Three different bacteria starter cultures, *Viniflora oenos* (*Loen. A* Chr. Hansen, DK,), *Bitec pro Vino* (*Loen. B,* Erbslöh Geisenheim, Germany), and *Uvaferm MBR alpha* (*Loen. C* Lallemand, Canada) were added to dry young wines. Viable cell numbers were determined in wines by plate counting on Orange-Serum-agar plates (VWR, USA) as follows: *Viniflora oenos* viable cell number 3.2 x 10^8 cfu/l in wine; *Bitec pro Vino* 1.3 x 10^6 cfu/ l in wine; *Uvaferm MBR alpha* 7.4 x 10^5 cfu/l in wine. Volumes used for experiments were 5 l for each variant. Malolactic fermentation was initiated at 21 °C and 16 °C and monitored by analysis of malic acid down to residual amounts of less than 0.1 g/l. Four weeks after accomplished alcoholic fermentation and first racking, wines were kept on fine lees for further four months at cellar temperature (13°C to 17 °C). Numbers of viable bacteria were then determined in wines of all variants using the plate counting method on Orange-Serum-agar plates (VWR, USA). Amino acids (BERGER et al., 2000), monomeric anthocyanins (EDER et al., 1990) and phenolic compounds (VRHOVŠEK et al., 1997) were determined by HPLC. Colour intensity was measured using photometrical sample analysis (HP Hewlett Packard 8452A diode array spectrophotometer) at wavelengths of 420, 520 and 620 nm.

Results and discussion

Influence of yeast strain on malic acid degradation during alcoholic fermentation

Influence of pH-value in musts on deacidification

1st Experiment

Variants of fresh must (pH-value 3.1) and pasteurized must (pH-value 3.4) were fermented with 10 g/hl of eleven rehydrated commercial dry yeast strains as previously described and a single colony of yeast strain named *Rheinriesling Eiswein* (RR Eiswein). All strains but *Oenoferm Tipico* displayed malic acid degrading features (Fig. 1). The major part of the initial malic acid amount of 2.3 g/l in fresh 'Rheinriesling' must was reduced and converted to lactic acid by *L 71B* (1 g/l), by L RC212 and *Oenoferm Klosterneuburg* (0.6 g/l). Strains *RHST* and *Oenoferm Tipico* showed the lowest malic acid degrading activity (0.2 g/l and 0 g/l, resp.). *Oenoferm Tipico* had no malic acid metabolizing activity at pH-value 3.1, but 0.6 g/l were reduced at pH-value of 3.4.

With fresh must (pH-value of 3.1), results indicate three groups of yeasts with different degrading levels of malic acid:

1. strains with high malic acid degrading activity, corresponding to 43 % degradation (*L 71B, L RC 212, Oenoferm Klosterneuburg, Oenoferm Bouquet*),
2. strains reducing malic acid from 15 % to 35 % (*EC 1118, L 2056, Oenoferm Interdry, Fermicru VR 5*) including the native selected pure strain *Rheinriesling Eiswein*,
3. strains showing very low malic acid degrading activity of 0 % to 10 % (*RHST, Oenoferm Tipico*).

These characteristics will be useful mostly for white wines.

2nd Experiment

In pasteurized 'Rheinriesling' must containing an initial malic acid amount of 1.8 g/l, all strains increased their deacidifying capacity from 0.6 g/l to 1 g/l of wine. In

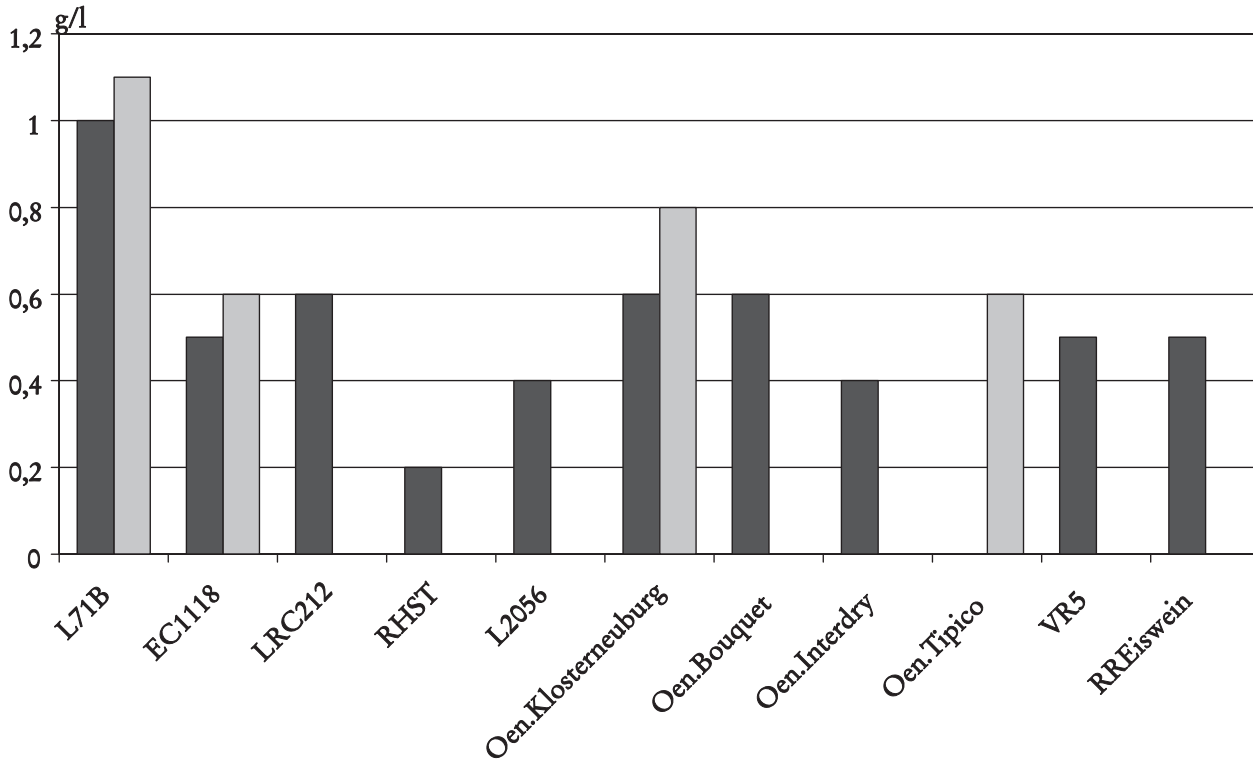

Fig. 1: Malic acid degradation using ten different commercial dry yeast preparations and a pure culture of a new strain named *RR Eiswein* (*Rheinriesling Eiswein*) during alcoholic fermentation of musts with pH-values of 3.1 and 3.4

all variants, fermented with *L 71B, EC 1118, Oenoferm Klosterneuburg,* and *Oenoferm Tipico,* 10 % to 20 % more malic acid were metabolized. In wines fermented with *Oenoferm Tipico,* malic acid reduction increased from 0 % to 26 % in case of pH-value 3.4 compared to pH-value 3.1. These results definitely indicate an influence of the pH-value but also pasteurization on yeast metabolism concerning malic acid degradation.
At ph-value of 3.4, deacidification capacity of *Saccharomyces* was shown to increase. In white wines, deacidification varies according to pH-value of the must. Complete degredation of malic acid has not been achieved. Nevertheless deacidifying characteristics have to be taken into account with respect to treatments for must acidification.

Hygienic quality of wine yeast

In fermentation variants using musts with a pH-value of 3.1, spoilage bacteria are not able to multiply to relevant cell numbers under alcoholic fermentation conditions. Therefore viable cells were not analysed.
After incubation of an aliquot of rehydrated dry yeast and - for selected *Saccharomyces cerevisiae* - an overnight culture from single colony, viable bacteria (cfu/g yeast) were chosen to be determined in MRS after 72 hours incubation (Table 1). MRS was adjusted to pH-value 5.4 to support bacteria multiplication.
In experiments with strains L 71B, *EC 1118, L RC212, RHST, L 2065* and *L 1033*, bacteria developed cell numbers per gram dry yeast to amounts of approximately 2×10^{10} cfu/g to 2×10^{11} cfu/g. Viable cell amounts of 3.9×10^{6} cfu/ml after inoculation with *Oenoferm Klosterneuburg* and 10^{3} cfu/mg dry yeast were analysed in variants inoculated with *Oenoferm Bouquet*, *Oenoferm Interdry, Oenoferm Tipico.*
Bacteria contamination levels did not correlate with scales of malic acid degradation. *Saccharomyces cerevisiae L 71B* caused the highest degradation of 1 g/l, but number of viable bacteria came to 2.5×10^{10} cfu/ml, which is low compared to *EC 1118*, L RC212, *RHST,* and *L 2056.* Strain *L 2056* caused bacteria cell number 10^{11} cfu/ml, but only 0.4 g malic acid per liter were reduced in must with a pH-value of 3.1. Bacteria cell number in variants inoculated with strains *Oenoferm Klosterneuburg, Oenoferm Bouquet, Oenoferm Interdry,* and *Oenoferm Tipico* reached 10^{3} cfu/ml, but malic acid was only reduced from 0.4 mg/l to 0.6 mg/l under given conditions. Strain *Oenoferm Tipico* reduced malic acid only in must with a pH-value of 3.4.

Table 1:
Viable bacteria in ten dry wine yeast preparations and in liquid culture of a single colony from the strain named *RR Eiswein* after three days incubation in MRS (pH-value 5.4)

Yeast strain	bacteria Cfu per g
L1033	5,40E+07
L71B	2,20E+07
EC1118	5,90E+07
LRC212	8,00E+07
RHST	1,20E+08
L2056	1,80E+08
Oen. Klosterneuburg	3,90E+03
Oen. Bouquet	1,00E+00
Oen. Interdry	1,00E+00
Oen. Tipico	1,00E+00
RR Eiswein	0,00E+00

Pure culture *Rheinriesling Eiswein* (RR Eiswein) was free from bacteria. These results - accompanied by the fact, that malic acid degradation occured in pasteurized, bacteria free must independently of bacterial contamination grade - indicate, that malic acid degradation to a large extent is due to *Saccharomyces cerevisiae* capacity.

Deacidification using bacteria starter culture

Viable bacteria

Malolactic fermentation (MLF) was induced by using three different starter cultures described above and further designated as *Loen. A*, *Loen. B*, and *Loen. C*. Precise application needs attention to level of total or free SO_2, and the pH-value in fermented must samples as well as bacteria temperature tolerance and kinetics of MLF (Fuster et al., 2000 und 2002). Number of viable cells developed to 10^{6} cfu/ml in *Loen. A* variants, to 8×10^{5} cfu/ml in *Loen. B*, and 3×10^{5} cfu/ml in *Loen. C* variants.
The initial malic acid amount of 2.9 g/l was totally reduced (Table 2) in all variants incubated at 21 °C or 16 °C within 19 days and 28 days, respectively. A cooler temperature of 16 °C caused a 5 to 10 days retardation. MLF kinetics developed differently depending on bacteria culture. *Loen. C* displayed best MLF velocity,

Table 2:
Residual amount of malic acid at various days ofter malolactic fermentation at 16 °C compared to 21 °C

	g/l malic acid					
Microflora	day 0	day 2	day 4	day 6	day 17	day 19
Lalvin 2056/L. oen. C 21°C	2,9	2,2	1,4	0,2	0,1	
Oen. Rouge/L.oen. C 21°C	2,9	2,4	1,4	1,1	0,3	0,1
Lalvin 2056/L.oen. B 21°C	2,9	2,5	2	1	0,2	0,1
Lalvin 2056/L.oen. A 21°C	2,9	2,6	1,9	0,7	0,1	0,1
Lalvin 2056/spont. MLF 21°C	2,9	2,9	2,9	2,8	2,5	2,6
	0	2	4	6	17	19
Lalvin 2056L. oen. C 16°C	2,9	2,3	2,1	1,6	0,8	0,4
Oen. Rouge/L. oen. C 16°C	2,9	2,6	2,4	2,1	1,5	1,3
Oen. Rouge/L. oen. B 16°C	2,9	2,7	2,2	1,7	1,1	0,6
Oen. Rouge/L. oen. A 16°C	2,9	2,3	2	1,3	0,4	0,2
Oen. Rouge/spont. MLF16°C	2,9	2,8	2,6	2,6	2,3	2,3

taking six days until accomplishing MLF at 21 °C, and more than three weeks to accomplish at 16 °C. Spontaneous MLF did not start within the investigated period. Variants fermented with *L 2056* dry yeast displayed higher MLF velocity compared to *Oenoferm Klosterneuburg* variants.
After first racking, basically a cell number reducing procedure, wines were stored at cellar temperature of 13 °C to 15 °C without sulphiting. To be able to evaluate storage resistance of bacteria starter cultures, numbers of viable cells were determined four months later (Fig. 2). Regarding initial bacteria inoculation, the viable cell number decreased in all variants during storage. Malolactic fermentations realized at 21 °C, residual cell numbers were higher compared to variants fermented at 16 °C. Viable cell number also differed depending on starter culture. This result was evident in variants inoculated with *Loen. A* and particularly evident for *Loen. C*. Resistance to vinification conditions is related to bacteria strain characteristics (Fuster et al., 2001).
Spontaneous flora displayed higher resistance after storage. Numbers of viable cells were higher than 5000 cfu/g dry yeast after MLF at 21 °C and MLF at 16 °C. Starter cultures showed higher sensitivity compared to spontaneous flora and could present better preconditions for final biological stabilization.

Influence on wine quality

The influence of bacteria starter cultures and MLF on monomeric anthocyanins and colour was evaluated. Also amino acids and phenols were analysed after four months of storage in contact with fine lees and residual microflora. Amino acids were released into the wine during storage in contact with fine lees (Berger et al., 1999). Amino acids amounts in wines (Fig. 3) were analysed after MLF. Amounts of released amino acids correlated with yeast strain (Berger et al., 2000). Changes of selected aminoacids lysine, asparagine, tyrosine and ornithine were detected after four months of storage and compared to amounts in the original wine. Ornithine and tyrosine were ten times higher after storage. In variants fermented with the wine yeast *Lalvin 2056*, distinctively higher amounts of tyrosine were found compared to wines fermented with *Oenoferm Rouge*.
In case of *Saccharomyces cerevisiae* late inoculation (after three days of spontaneous fermentation), the release of tyrosine after a storage period of four months was about 20 % lower compared to fermentation initiated with dry yeast immediately in untreated mashes and musts (data not shown).
Choice of bacteria starter culture had no systematic influence on the amino acid amounts in wines.
One of the major phenolics in grapes and wines is caftaric acid (caftaric), a combined molecule of caffeic acid

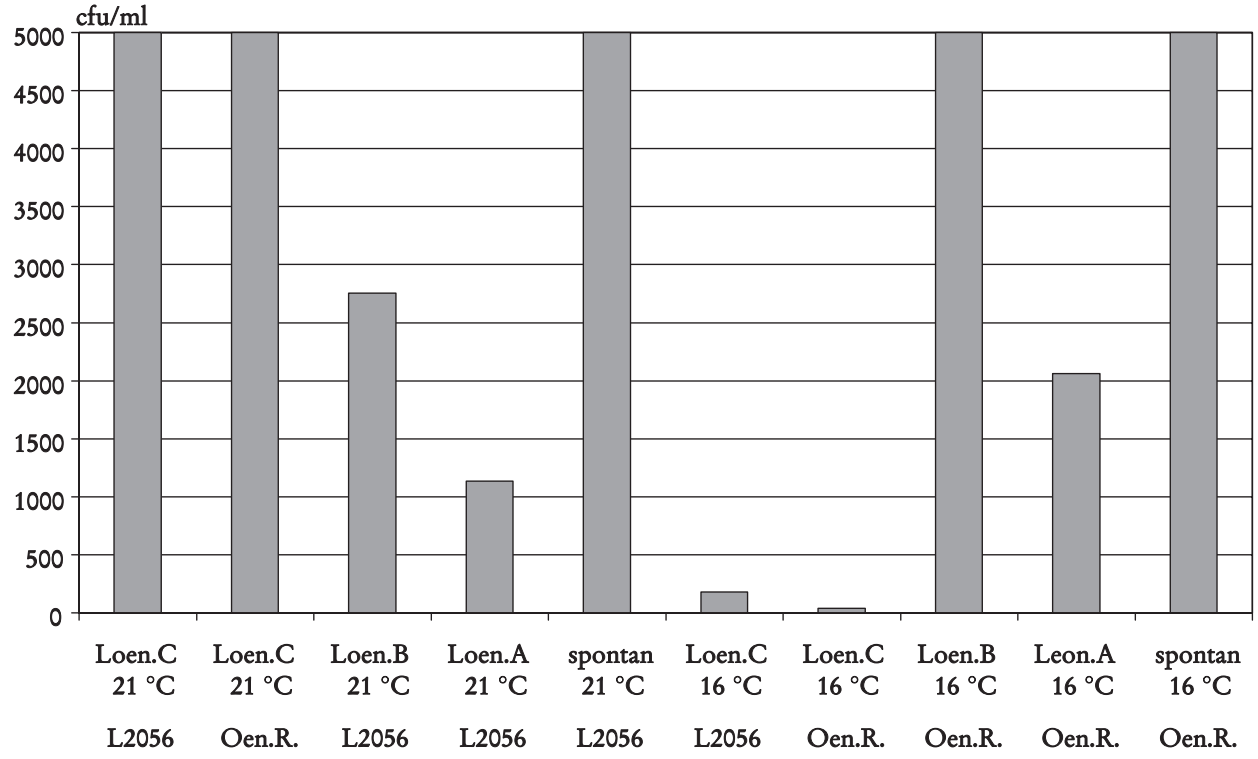

Fig. 2: Viable bacteria (cfu/ml) in 'Blauer Portugieser' after four months' storage

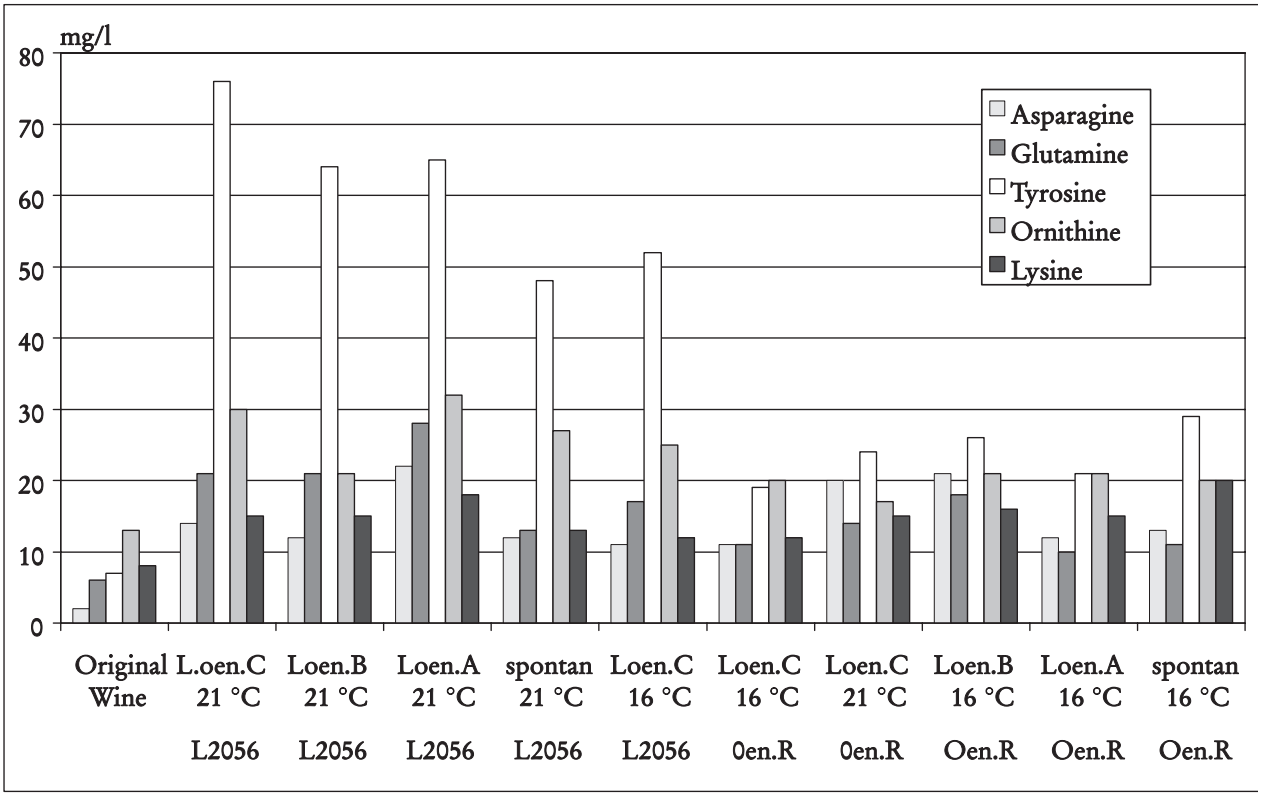

Fig. 3: Content of selected amino acids in wines of 'Blauer Portugieser' after four months' storage

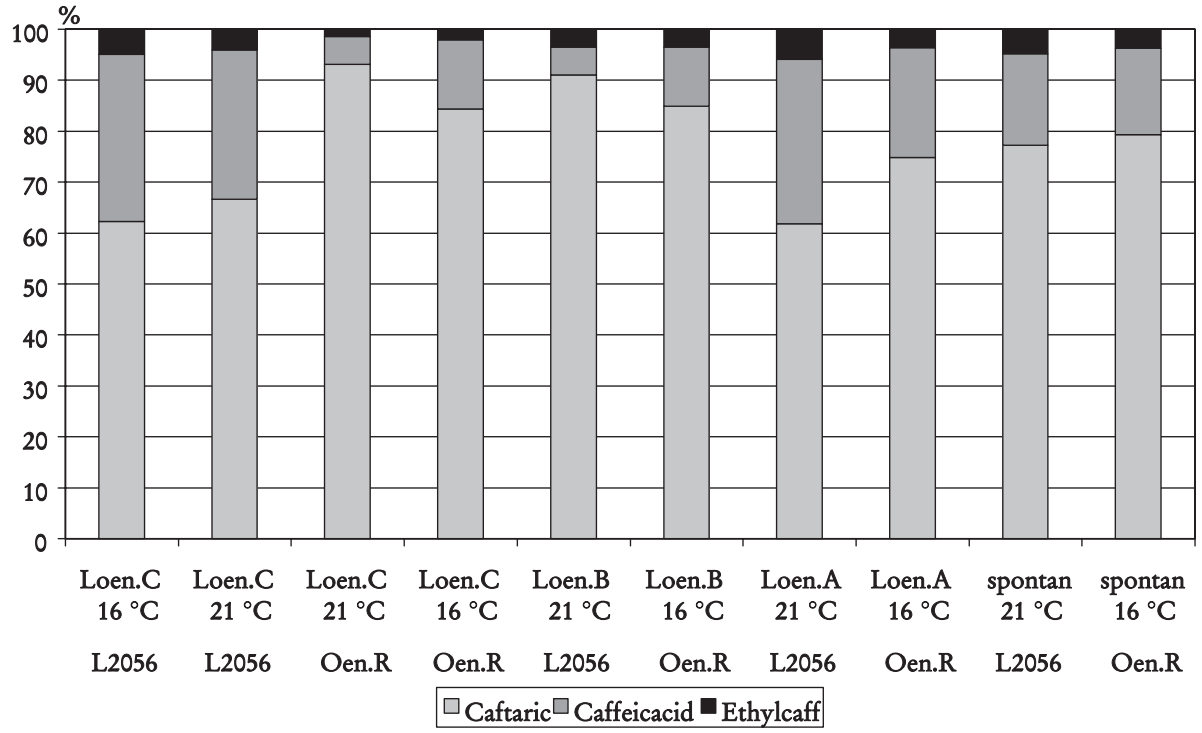

Fig. 4: Percentage of caftaric acid and its derivatives in wines of 'Blauer Portugieser' after four months' storage

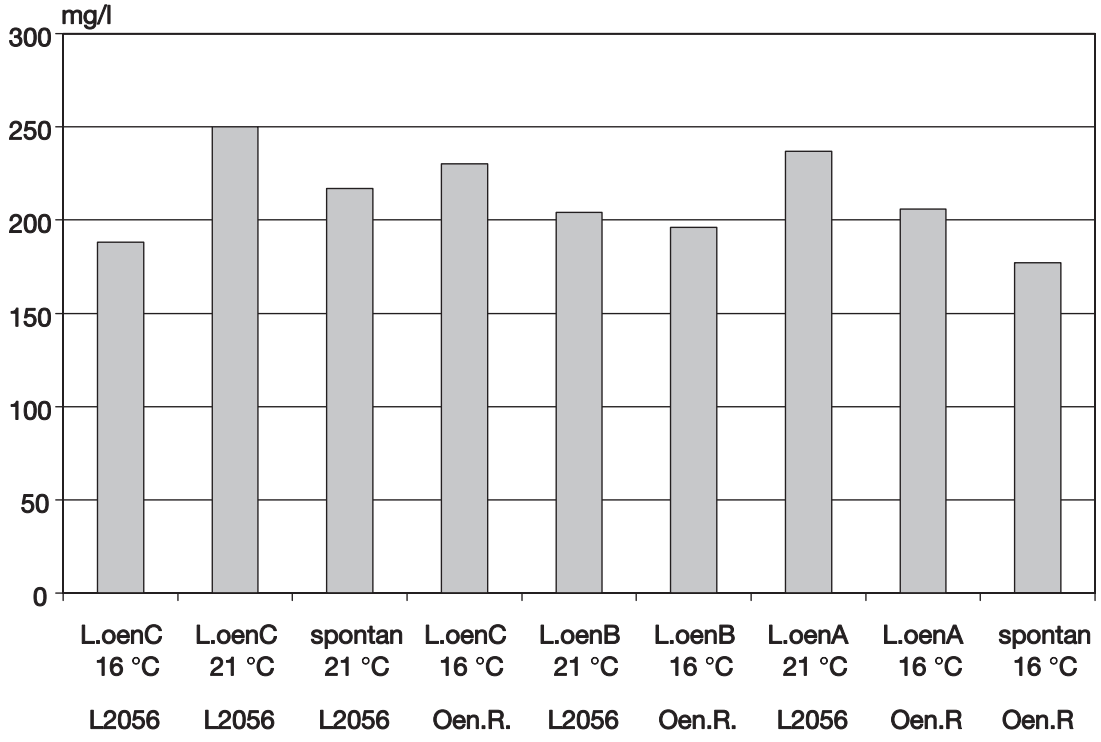

Fig. 5: Monomeric anthocyanins in wines of 'Blauer Portugieser' after four months' storage

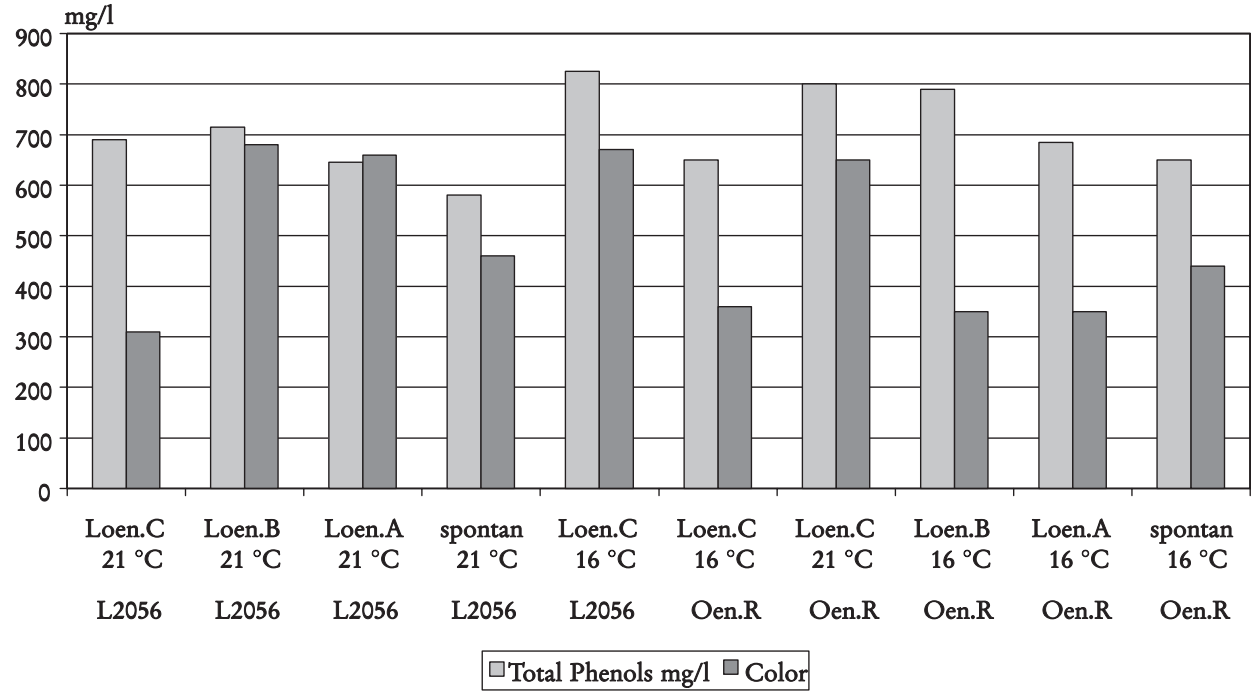

Fig. 6: Total phenols and colour intensity in wines of 'Blauer Portugieser' after four months' storage

and tartaric acid. During the winemaking process, caftaric is splitted into caffeic acid and tartaric acid. Ethylcaffeic acid develops during storage. Present experiments demonstrate a high variability in caffeic acid (Fig. 4) and phenols (Fig. 6) released in all variants, but no tendency for an influence of yeast strain, bacteria culture or temperature. Development of these molecules might rather be attributed to processing (VRHOVŠEK et al., 1997).

Monomeric anthocyanin amounts analysed after MLF correlate with microflora (Fig. 5). In MLF variants, the bacterial strain *Loen. C* and *Loen. A* had lowest anthocyanin reducing properties. Regarding period of contact, the influence of the used dry yeast strain seems to be higher than the influence of selected or spontaneous bacterial microflora. In variants fermented with *L 2056,* amounts of anthocyanins were slightly higher than in variants fermented with *Oenoferm Rouge.*

After MLF at a temperature of 21 °C, colour stability was higher compared to conditions of 16 °C (Fig. 6).

Conclusions

Investigated strains of the species *Saccharomyces cerevisiae* display different deacidifying capacities depending on must acidity. A pH-value of 3.4 supports *Saccharomyces* malic acid reduction compared to pH-value 3.1. Concerning bacterial contamination found in dry yeast, present investigations disclosed three quality groups of dry yeast:

1. high contamination level,
2. low contamination level, and
3. dry yeast preparation free or nearly free of bacterial contaminants.

Nevertheless, malic acid degradation intensity was not found to correlate with high numbers of viable bacteria. This and the fact, that pure culture of native *Saccharomyces* isolates also reduces malic acid, demonstrates *Saccharomyces cerevisiae* to be responsible for partial malic acid metabolism. Danger of possible uncontrolled effects due to bacterial contaminants in dry yeast preparations seems attenuated during alcoholic fermentation, but might be more important in case of sparkling wine production after sugar addition and second fermentation at temperatures higher than 20 °C. Such physiological conditions promote contaminants multiplication.

Bacteria starter cultures showed differences in their biotechnological quality. Malolactic fermentation was accomplished at low temperatures (16°C) within usual time delay. *Oenococcus* starter cultures showed slight

differences of anthocyanin reducing characteristics, whereas *Saccharomyces* strains displayed distinctive colour-preserving characteristics.

Furthermore, the use of bacteria starter cultures offers advantages regarding biological stabilization. Inoculated commercial bacteria strains showed reduced vitality compared to spontaneous flora. Relative resistance of spontaneous flora could be responsible for sulphurous acid consuming molecule formation. Inhibition of its development can be achieved with early sulphurization or by using lysozyme (CAI GAO et al., 2002) in must to reduce undesired bacteria flora at an early stage or in young wines.

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