

Effect of Physico-Chemical Factors on Growth of Starter Culture for Wine Malolactic Fermentation

Djelovanje fizikalno-kemijskih parametara na rast starter kulture za jabučno-mliječnu fermentaciju vina

Rajka Božanić, Vjera Runjić-Perić, Ivanka Pavušek and V. Marić

Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 4100 Zagreb, Croatia

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Summary

The induction of the malolactic fermentation (MLF) by inoculating a wine with selected malolactic bacteria has, recently, received immense enologists' attention because of its positive influence on quality and sensory characteristics of wine. The strains of *Leuconostoc oenos* adapted to grow in adverse conditions of fermentation (i.e., low pH values and temperature, high concentrations of SO₂ and ethanol) were the most frequently used.

Since MLF of wines is performed in adverse conditions for bacterial growth in this paper the effects of temperature and pH lower than optimal, and different ethanol and SO₂ concentrations on the growth of strain UR were investigated with optimal value of at least one factor of growth (T and/or pH). The simultaneous effect of these factors combined by Graeco-Latin Square method was also investigated. The optimal conditions determined for the growth of the investigated strain are: 37 °C and pH = 6.5. The growth of *Leuconostoc oenos* UR was good at 25 °C, while lower pH (3.0 and 4.0) and higher ethanol volume fractions ($\varphi = 10$ and 12 %) inhibited bacterial growth significantly. The investigation of SO₂ concentration (30, 50 and 100 mg/L) showed that only the highest SO₂ concentration in medium slightly suppressed bacterial growth after 24 hours of cultivation. The growth of *L. oenos* UR in the conditions combined by Graeco-Latin Square method was significantly suppressed. Namely, after 24 hours of growth under such conditions, the maximal cell concentration ratio (CCR) was only 15 % of CCR value obtained at the same unfavourable pH, but in otherwise optimal conditions. The ratio between sums of squares of separate variables and total sums of squares (expressed as a percent), estimates the inhibitory influence of each variable in Graeco-Latin Square Design on the growth of *L. oenos* UR. It was estimated that the share of medium pH value is the most significant: 73.0 % and 88.4 % after 24 hours and 120 hours of growth, respectively.

Introduction

Lactic acid bacteria make a significant contribution to the quality and stability of wines by conducting malolactic fermentation (MLF). Wine composition and, conse-

Sažetak

Poticanje jabučno-mliječne fermentacije (JMF) u vinima inokulacijom odabranim bakterijama mliječne kiseline zaokuplja sve veću pažnju enologa radi povoljnih utjecaja na kakvoću i senzorske karakteristike vina. U tu svrhu najčešće se koriste sojevi vrste *Leuconostoc oenos* prilagođeni rastu u nepovoljnim uvjetima pod kojima se provodi fermentacija (tj. niske vrijednosti pH i temperature, visoke koncentracije SO₂ i etanola).

U radu su istraženi optimalni uvjeti rasta (pH podloge i temperatura) bakterije *L. oenos* soj UR u MRS podlozi u svrhu proizvodnje starter kulture za JMF. Budući da se JMF u vinima provodi u uvjetima nepovoljnim za bakterijski rast, u radu je istražen i utjecaj temperatura i vrijednosti pH nižih od optimalnih, različitih koncentracija etanola i SO₂ na rast soja UR, uz uvjet da je bar jedan od parametara rasta (T i/ili pH) bio optimalan. Isto je tako istražen i utjecaj tih parametara kombiniranih metodom grčko-latinskog kvadrata. Optimalni uvjeti za rast istraživanoj soji su 37 °C i pH podloge 6,5. Pri 25 °C *L. oenos* UR raste dobro, dok niže vrijednosti pH podloge (3,0 i 4,0) i veći volumni udjeli etanola ($\varphi = 10$ i 12 %) značajno inhibiraju bakterijski rast. Od istraživanih koncentracija SO₂ (30, 50 i 100 mg/L) samo je u podlozi s najvećom koncentracijom SO₂ uočen nešto slabiji rast nakon 24 sata uzgoja. Rast *L. oenos* UR značajno je potisnut u uvjetima kombiniranim prema grčko-latinskom kvadratu. Naime, nakon 24 sata rasta u ovim uvjetima, maksimalni postignuti faktor preživljavanja (CCR) iznosio je samo 15 % od vrijednosti CCR dobivene pri istom nepovoljnom pH, ali uz inače ostale optimalne uvjete rasta. Odnosom suma kvadrata pojedinih varijabli i ukupne sume kvadrata (izražene kao postotak) određen je inhibitorški utjecaj pojedine varijable u grčko-latinskom kvadratu na rast *L. oenos* UR. Udjel vrijednosti pH podloge je najznačajniji: 73,0 % nakon 24 sata, odnosno 88,4 % nakon 120 sati rasta istraživane bakterije.

quently, wine quality are altered by these bacteria, because some wine components are utilized as substrates for growth, and metabolic end products are excreted into

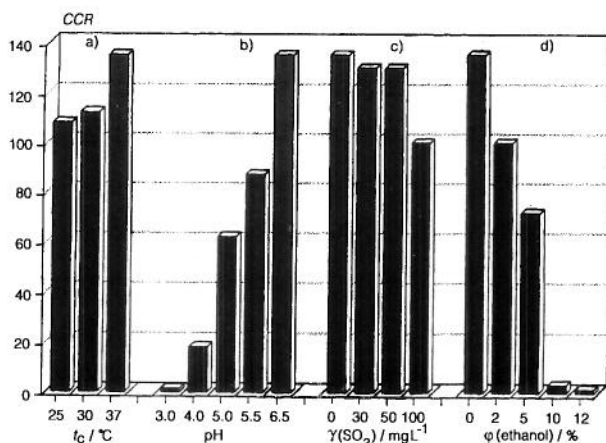


Fig. 1. The effect of separate factors: a) temperature, b) pH, c) ethanol, d) SO_2 on the cell concentration ratio ($\text{CCR} = N_{24}/N_0$) of *L. oenos* UR after 24 hours of growth in MRS broth. $N_0 = 3.2 \times 10^{-7} - 4.5 \times 10^{-7} \text{ mL}^{-1}$ is starting cell concentration; N_{24} is cell concentration at the end of cultivation

Slika 1. Djelovanje pojedinih parametara: a) temperatura; b) pH; c) etanol; d) SO_2 na faktor preživljavanja ($\text{CCR} = N_{24}/N_0$) *L. oenos* UR nakon 24 sata uzgoja u MRS podlozi. $N_0 = 3.2 \times 10^{-7} - 4.5 \times 10^{-7} \text{ mL}^{-1}$ je početna koncentracija stanica; N_{24} je koncentracija stanica na kraju uzgoja

creased by addition of SO_2 in the medium (Fig. 1.d). The cell concentration ratio after 24 and 120 hours of bacterial growth in media combined by the Graeco-Latin Square method (Table 1) are shown in Fig. 2.

Discussion

The extent of physico-chemical factors known to affect the growth of malolactic bacteria (i.e., pH, T , SO_2 and ethanol) greatly depends on the species (3) or even the bacterial strain (8,9). Among leuconostocs the *Leuconostoc oenos* is the species reported to be almost exclusively present in wines, wineries and vineyards (1) and is considered as the malolactic bacterium »par excellence«. Although it was found that the optimal temperature for growth of *Leuconostoc oenos* is between 20 and 25 °C (10,11) and pH between 4.4 and 4.6, the best growth of *L. oenos* UR was obtained at $t_c = 37$ °C, and pH = 6.5. Examining the influence of separate factors on *L. oenos* UR growth at least one factor (pH and/or T) of growth was optimal. The highest negative effect on bacterial growth produced the low pH value of growth medium (below 5.0, Fig. 1.b), and the high alcohol content (above $\phi = 5$ %, Fig. 1.c). However, although the bacterial growth was significantly suppressed, even at the lowest pH and highest alcohol concentration some growth occurred (Fig. 1). The cumulative negative effect of investigated factors is evident. In media with combined factors (Fig. 2) much lower CCR, either after 24 or 120 hours of growth were obtained, compared to the bacterial growth resulted from investigations of separate factors (Fig. 1). For example, the maximal CCR after 24 hours of growth, achieved in medium 8 (Fig. 2), was about 85 % smaller than that obtained in medium with the same pH (pH = 4.0) but without the influence of the other growth inhibiting factors (Fig. 1). This is in accordance with the published data (3,8,12).

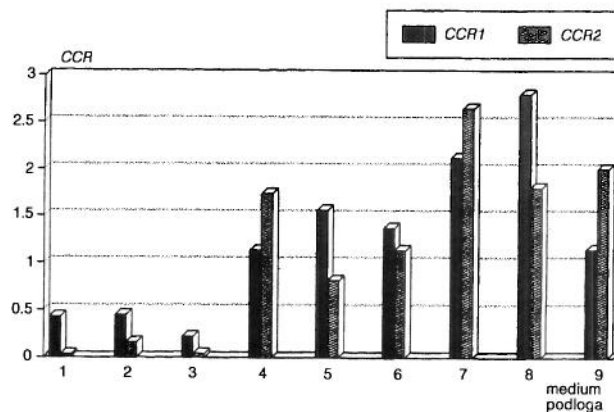


Fig. 2. The influences of SO_2 , ethanol, starting pH and temperature interactions (media 1-9) on the cell concentration ratios (CCR) of *L. oenos* UR in MRS broth after 24 ($\text{CCR}_1 = N_{24}/N_0$) and 120 ($\text{CCR}_2 = N_{120}/N_0$) hours of cultivation

Slika 2. Utjecaj djelovanja različitih koncentracija SO_2 , etanola, početnih vrijednosti pH podloge i temperatura (podloge 1-9) na faktore preživljavanja (CCR) bakterije *L. oenos* soj UR u MRS podlozi nakon 24 ($\text{CCR}_1 = N_{24}/N_0$) i 120 ($\text{CCR}_2 = N_{120}/N_0$) sati uzgoja

Namely, the negative effect of pH lowering is increasing when the medium contains SO_2 , because at lower pH values (below 5.0) the free SO_2 concentration (known antimicrobial agent in wine), increases (13). According to the literature (8,14) alcohol tolerance of malolactic bacteria is greatly affected by temperature and pH, and it decreases as the temperature rises and pH decreases. Ethanol is known as the main inhibitor of bacterial growth, but *L. oenos* UR can grow in medium with $\phi = 10$ % of ethanol (Fig. 1.d), even at pH below optimal value and in the presence of the other inhibiting factors (Fig. 2, medium 7). In combined factors conditions the best cell concentration ratios were achieved in medium 8 and 7 after 24 and 120 hours of growth, respectively. Results presented in Fig. 2 indicate that *L. oenos* UR is able to adapt and even grow in unfavorable conditions. Namely, the CCR values obtained after 120 hours of growth in media 4, 7 and 9 (Fig. 2) were by 35, 20 and 43 % higher than after 24 hours of growth in the same media, respectively. The ratio between sums of squares of individual variables (SS_{Rows} , $\text{SS}_{\text{Columns}}$, $\text{SS}_{\text{Latin letter}}$ and $\text{SS}_{\text{Greek letter}}$) and total sums of squares ($\text{SS}_{\text{Total}} = 5.67$ for N_{24}/N_0 and $\text{SS}_{\text{Total}} = 7.32$ for N_{120}/N_0), expressed as a percent, was used to estimate the negative influence of each variable in Graeco-Latin Square Design on the growth of *L. oenos* UR. The obtained values for pH, SO_2 , ethanol and temperature are 73.0, 12.7, 10.6 and 3.4 % (for N_{24}/N_0) and 88.4, 6.7, 1.9 and 2.9 % (for N_{120}/N_0), respectively.

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