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The Role of On-line Redox Potential Measurement in *Sauvignon blanc* Fermentation

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Summary

The aim of this work was to study the changes of the principal compounds of *Sauvignon blanc* grape must and wine during fermentation at the temperatures of 15, 18 and 24 °C and to compare the obtained results with the changes of redox potential. Among the changes of redox potential value, the levels of oxygen partial pressure, biomass, concentrations of reductive sugars (glucose and fructose), ethanol, glycerol and pH value, a good correlation was found. Redox potential was found as a valuable indicator of yeast metabolic activity in wine fermentation that enables an insight and control of the fermentation process.

Key words: redox potential, *Sacharomyces cerevisiae*, *Sauvignon blanc*, grape juice fermentation

Introduction

In living organisms oxidation – reduction systems play so intimate and so essential part that life itself might be defined as a continuos oxidation – reduction reaction *(1)*.

Redox potential in microbial cultures is a sum of all oxidation – reduction processes in particular fermentation, where metabolism in microbial cells plays one of the most significant roles. It is caused by the existence of reversible oxidation – reduction couples, irreversible reductors, and the action of the free oxygen and free hydrogen *(2)*. It is dependent on pH value, dissolved oxygen concentration, equilibrium constant and oxidation – reduction potentials in a liquid *(3)*.

The measurement of redox potential is relatively fast, accurate and reliable and its values give an insight into oxidation as well as reduction ability of wine, which are useful factors in monitoring wine fermentation. Measured values of redox potential give information on redox reaction in wine, which have an influence on its quality and stability *(4)*.

During ripening, storing and aging of wine, oxidation and reduction processes affect the character and taste of wine to a considerable extent. The intensity of the oxidation and reduction can be measured as the redox potential. The value of the redox potential follows the Nernst equation and E depends on the ratio of the activity of oxidised and reduced components of a certain substance, on the number of electrons involved in the redox reaction and on the constant of the formal potential of the redox system E^0 *(5)*.

$$E = E^0 + \frac{RT}{zF} \ln \frac{\sum a_{ox}^{\nu_{ox}}}{\sum a_{red}^{\nu_{red}}} \qquad /1/$$

where E is the electrode potential in V, E^0 is the standard potential of the redox system in V, R is the gas constant, T is temperature in K, F is Faraday constant,

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a_{ox} and a_{red} are activities and stoichiometric coefficients of oxidised and reduced forms, while ν_{ox} and ν_{red} are corresponding stoichiometric coefficients.

The degree of oxidation and reduction also depends on the temperature and pH value. Therefore, Clark (1923) introduced the term rH, which combines the relationship between *E* and pH. The pH value is the negative logarithm of the concentration of hydrogen ions, while the rH value is the negative logarithm of the partial pressure of hydrogen, when the system is in equilibrium, $rH = -\log(H^+)$.

From this (at 25 °C):

$$rH = \frac{E}{0.029\,V} + 2\ pH \qquad /2/$$

The value of *E* is calculated from the equation:

$$E = E_{meas.} + E_{ref.} \qquad /3/$$

where $E_{ref.}$ is the potential of the calomel electrode; $E_{meas.}$ is the measured potential of the platinum electrode in V *(6)*. Later it was seen that the assumptions made were not correct in every case, therefore Clark himself demanded that the term rH should no longer be used *(5,7)*.

Redox potential of wine reflects the rate and intensity of oxidation and reduction processes that take place. Wine represents a complex redox system due to its chemical composition. Generally speaking wines show reducing character. Since the value of redox potential is in close relation with a number of reactions taking place in wine, being either spontaneous or induced, it enables the control of wine making process *(4)*.

The practical significance of redox potential and oxygen content at various stages of winemaking was examined by Mazzoleni *(8)*.

Many of chemical, enzymatic and biological processes in wine are correlated with the oxidative state of wine. Redox potential measurement assesses the ability of life of microorganism, growth as well as the physiological activity in defined environment.

With the addition of sulphur dioxide into the grape juice redox potential will be reduced and the fermentation processes will slow down. There are big differences in requirements among some yeast species for redox potential ratio. The addition of H_2O_2 increases the rate of fermentation processes *(9)*.

The sphere of yeast activity is within the limits of –200 mV and 350 mV. Above 350 mV oxygen acts toxically and inhibitory, and below –200 mV the concentration of dissolved oxygen is too low for normal life conditions of yeast *(10)*.

Materials and Methods

Microorganism

Selected dry yeast (*Saccharomyces cerevisiae*, Uvaferm, SLO) was used in all of the experiments. Reactivation of the yeast in a water diluted *Sauvignon blanc* must at 30 °C was used. The inoculum was equivalent to 0.3 g of dry yeast per litre of must.

Fermentation substrate

Sauvignon blanc grape juice from wine-growing region Ljutomersko-Ormoške Gorice was used as the fermentation media in all the experiments. The musts, fermented on the laboratory scale measurement, were not sulphured before the beginning of the fermentation.

Bioreactor

All laboratory scale experiments were performed in three identical standard type configuration 10-litre working volume Stirred Tank Reactors (Infors AG, CH-4103 Bottmingen). The reactors were equipped with a sterilised Ingold pH electrode, Ingold redox electrode (platinum as indicator and calomel electrode as reference electrode were used), Ingold pO_2 electrode and pCO_2 electrode, together with automatic temperature control unit (Mettler Toledo). Bioreactor head space was aerated through each fermentation with N_2 to prevent oxidation. Three series of experiments were performed. The first one at 15, the second at 18 and the third at 24 °C. In all the reactors mixing of 100 rpm was applied. The experiments were performed in four repetitions at each temperature. For the presentation of the results the average value (X) was used *(11)*:

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n} \qquad /4/$$

where: *n* = number of the experiments and X_i = value of the i-th measurement.

Analytical methods

Reductive sugars and alcohol in wine and grape must were analysed by HPLC. Validated methods proposed by Bio-Rad were used *(12)*.

Measurements of the concentrations of reductive sugar, ethanol, glycerol and the increase of biomass concentration were measured off-line every 24 hours. Samples were filtered through a 0.45 μm membrane and analysed using 300 mm × 7.8 mm Aminex HPX-87H organic acid analysis cation exchange column (Bio-Rad laboratories). Elution was performed at 65 °C. The mobile phase was 0.005 M H_2SO_4 in redistilled water. The pump was operating at a flow rate of 0.5 mL/min ($0.008 \cdot 10^{-3}$ L/s). The injection volume was 20 μL. The eluting compounds were monitored by a fixed wavelength ultraviolet (UV-VIS) detector at 210 nm. This detector was connected in series with a refractive index (RI) detector. Glucose, fructose, glycerol and ethanol were detected by RI. The peaks were quantified using external standard calibration. The components were identified by a comparison of their retention times with those of the standards. Quantification was performed using external standards prepared from pure compounds.

For the evaluation of the method standard deviation (SD) and relative standard deviation (RSD) values were calculated *(13)*.

Biomass

Biomass was determined gravimetrically after 5 min centrifugation at 4 000 rpm and 24 h drying at 105 °C. The volume of 20 mL of fermentation broth was used.

Results

Three series of experiments of *Sauvignon blanc* wine fermentation at temperatures 15, 18 and 24 °C were performed. The results of each run were summarised and presented as the most typical courses.

In the first experiment fermentation was performed at 15 °C. In the lag phase the adaptation of microorganisms to environmental conditions in the medium took place. It was the longest part of all three cases that took place from the inoculation up to 30 hours of fermentation. It is supposed that in this phase more acetaldehyde was produced. During that time the redox potential decreased from 210 to 160 mV. Ethanol was not produced yet, because the enzyme alcohol dehydrogenase was still not available.

The transient period, where yeast metabolism turned from aerobic to anaerobic fermentation phase, lasted from 24th to 48th hour of fermentation. In this part only a slight change (10 mV) of redox potential occurred. In this part metabolic activity of yeast was significantly reduced. From 24 to 48 hours of fermentation concentrations of glucose and fructose stayed nearly constant. Microbial activity in this period was balanced with production of glycerol.

The exponential growth phase, from 48 up to 96 hours of fermentation, followed. In this phase ethanol production started and the decrease of redox potential from 150 to –4 mV followed it.

At 96 hours of fermentation, the decrease of redox potential from –4 to –10 mV indicated that metabolic activity and microbial growth turned slowly to the stationary growth phase, where biomass reached concentration of 6.5 g/L. Ethanol concentration in this phase stayed nearly constant 80 g/L. Ethanol concentration slowly inhibited microbial growth and autolysis of yeast cells started (Fig. 1).

At 18 °C yeast growth was more intensive compared to fermentation at 15 °C. In lag phase, from the inoculation up to 24 hours of fermentation, related to higher fermentation temperature, the adaptation of microorganism to environmental conditions in the medium was shorter than in previous one. This part was followed by a decrease of redox potential from 200 to 142 mV.

The transitional period, where yeast metabolism turned from aerobic to anaerobic fermentation phase, was indicated from 24 up to 48 hours of fermentation. In this part only a slight change (10 mV) of redox potential occurred and metabolic activity of yeast was significantly reduced. From 24 to 48 hours of fermentation, concentrations of glucose and fructose stayed nearly constant. Microbial activity in this period was balanced with production of glycerol. The exponential growth phase took place from 24 up to 72 hours of fermentation. In this phase ethanol production started and the decrease of redox potential from 142 to 14 mV occurred. From 72 hours up to 144 hours of fermentation, the decrease of redox potential from 14 to –133 mV indicated that metabolic activity and microbial growth turned slowly to the stationary growth phase. Biomass concentration reached the value of 7.3 g/L. Ethanol concentration in this phase stayed nearly constant 85 g/L. The concentration slowly inhibited microbial growth and the autolysis of yeast cells from 144 up to 216 hours of fermentation started (Fig. 2).

The highest fermentation temperature of 24 °C, significantly influenced faster yeast metabolism, which was indicated also in faster redox potential response and glucose and fructose uptake. At fermentation temperature of 24 °C, the minimum of redox potential value of –200 mV was reached after 24 hours. In the transient phase, the available amounts of oxygen at this fermentation temperature where much lower than in previous experiments.

At much more intensive metabolic activity of yeast this transient phase was negligible, so it was not detected neither by redox potential nor by glucose or fructose consumption. In this part all of disposable sugars were consumed and the largest amounts up to 7.0 g/L

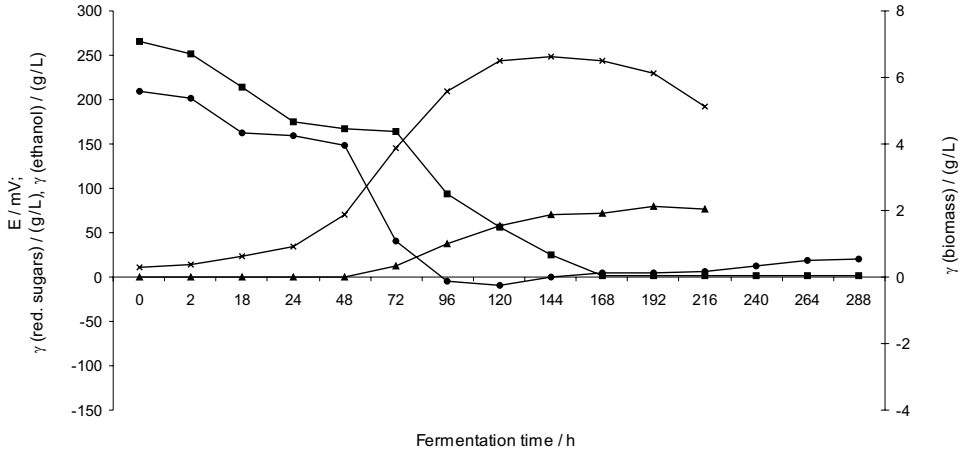

Fig. 1. Time dependence of redox potential (●), reductive sugar concentration γ / (g/L) (■), ethanol concentration γ / (g/L) (▲) (at the left ordinate) and biomass growth concentration γ / (g/L) (×) (at the right ordinate) at fermentation temperature 15 °C

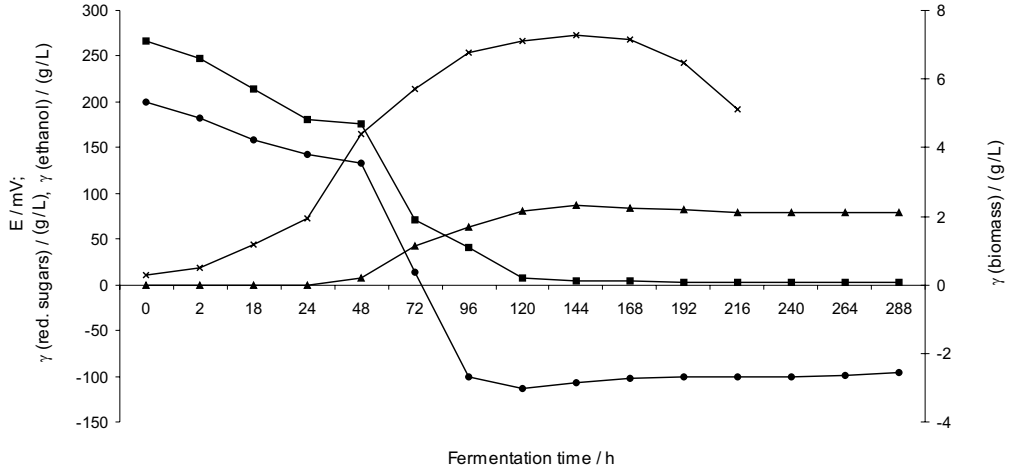

Fig. 2. Time dependence of redox potential (●), reductive sugar concentration γ / (g/L) (■), ethanol concentration γ / (g/L) (▲) (at the left ordinate) and biomass growth concentration γ / (g/L) (×) (at the right ordinate) at fermentation temperature 18 °C

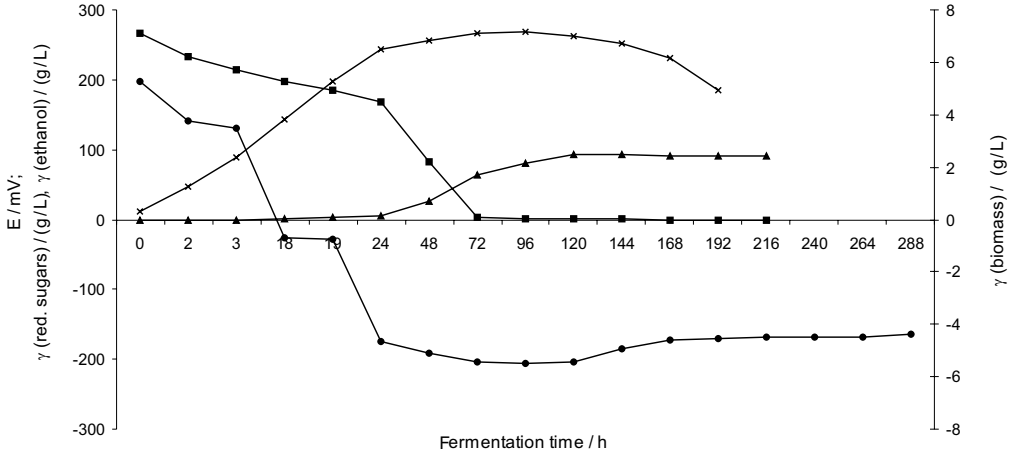

Fig. 3. Time dependence of redox potential (●), reductive sugar concentration γ / (g/L) (■), ethanol concentration γ / (g/L) (▲)(at the left ordinate) and biomass growth γ / (g/L) (×) (at the right ordinate) at fermentation temperature 24 °C

of glycerol were produced. The microbial growth reached its stationary growth phase at 72 hours of fermentation. Biomass concentration at this point was 7.1 g/L while redox potential reached its minimum of –206 mV and stayed constant until the end of fermentation. Stationary phase after 96 hours turned to growth declining phase. In this part the largest amounts of ethanol of 90 g/L inhibited microbial growth and yeast autolysis started (Fig. 3).

Related to our experiments, temperature was found to be one of the most influential physical parameters in the fermentation process that rapidly increases the rate of yeast metabolism. It influences significantly the yeast growth and its metabolism and therefore it represents one of the most relevant features for further process control. The effect of temperature on yeast metabolism in wine fermentation is also reflected in redox potential measurements during the process (Fig. 4)

In all of the experiments related to the fermentation temperature, the measured redox potential difference was in the range between 370 and 220 mV, which means that the process changed into the strong reductive environment. At 15 °C the difference between the redox potential at the beginning of the process and the redox potential value at the end of the process, ΔE of 220 mV was obtained after 168 hours, at 18 °C after 120 hours ΔE was 313 mV and at 24 °C already at 72 hours of fermentation ΔE was 370 mV (Fig. 4).

Redox potential values in the initial phase were dependent on the levels of disposable dissolved oxygen in fermentation medium. Biomass concentration at this point reached its maximum of up to 7 g/L dry mass. The process turned at this time from aerobic to anaerobic fermentation phase. All available sugar was utilised at the same time period when redox potential decreased to minimum values. At 24 °C the reductive sugars were consumed after 72 hours, at 18 °C after 144 hours and at 15 °C insignificant concentrations of residual sugar were detected after 168 hours of fermentation.

Redox potential could give relevant information on yeast metabolism during fermentation. At fermentations at 15 and 18 °C the redox potential clearly indicates the

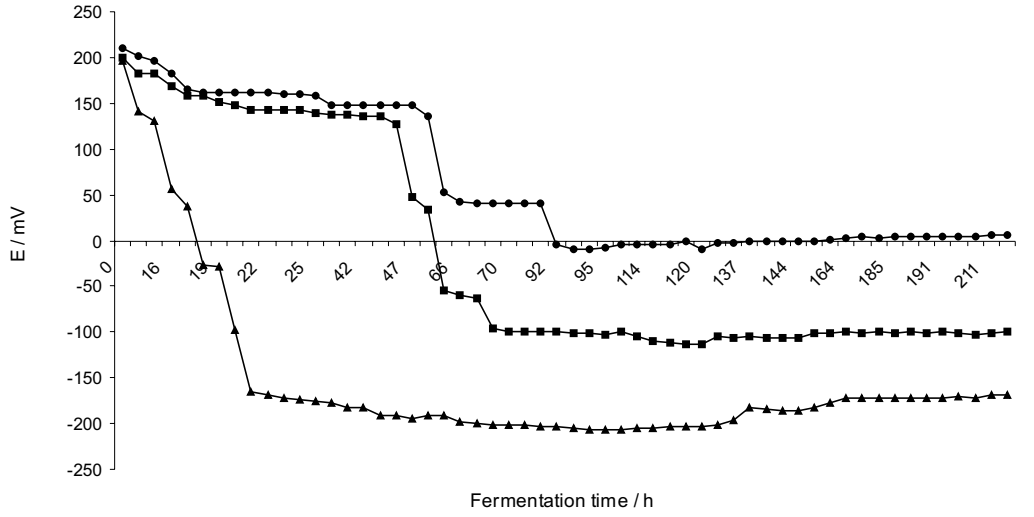

Fig. 4. Effect of time on redox potential values measured on-line during wine fermentation at different temperatures; 15 (●), 18 (■), 24 °C (▲).

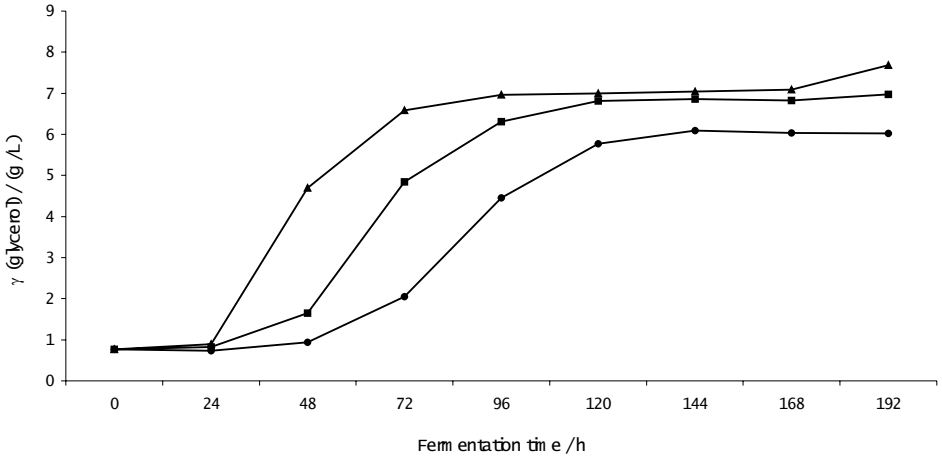

Fig. 5. Effect of temperature on the increase of glycerol concentrations during wine fermentation performed at different temperatures; 15 (●), 18 (■), 24 °C (▲).

transient phase where microbial growth turns from aerobic to anaerobic growth as well as the rapid growth rate of yeast at 24 °C, where this phase is very short and larger quantities of glycerol were detected. Costenoble *et al*. reported that *Saccharomyces cerevisiae* produced large amounts of glycerol as an osmoregulator, as a redox sink, at low oxygen availability *(14)*.

The glycerol production kinetics is biphasic, with a high production rate during the growth phase and less intensive in the stationary phase. In the time period, when the metabolism was interrupted, as in the case of fermentations at 15 and 18 °C, the glycerol production started with the first detectable amounts between the first and the third day, dependent on the fermentation temperature. At 15 °C first detectable concentrations of glycerol were measured approximately 72 hours after the beginning of the fermentation; at medium temperature of 18 °C glycerol appeared after 48 hours from the inoculation. At fermentation at 24 °C, the increase of glycerol concentration started after only 24 hours from the inoculation. The investigations of glycerol kinetics have shown similar results which are in agreement with the results of Liebert *(15)* (Fig. 5).

The time delay in glycerol production was noted dependending on different fermentation temperatures. The increase in glycerol concentration occurred faster at the highest fermentation temperature (Fig. 5).

Glycerol is one of the most significant parameters that significantly influence the wine harmony and its quality. It was found that the quantities of glycerol are induced by fermentation process temperature and therefore related to higher yeast activity. Fermentation temperature of 24 °C was found to be the most favourable for higher glycerol production. At higher fermentation temperature microbial activities are significantly faster, as well as the consumption rates of glucose and fructose. This factor also appears significantly in the fast changes of redox potential. The formation of ethanol and glycerol increased exponentially with time and were directly growth related. The increase in ethanol

and glycerol concentrations started at the same time at given fermentation temperature.

Discussion

Growth and metabolic activity of yeast cells can be represented with the series of oxidation-reduction reactions. The intensity and relation between the oxidation and reduction processes which took place during the alcohol fermentation of grape juice, can be measured by *on-line* redox potential measurements. For wine fermentation oxidation and reduction processes are of great importance. They affect the character and the taste and therefore the quality of wine. Redox potential is also an indicator of the oxidative status in a growth medium in which several reactions take place. It is a parameter that can give valuable information about the metabolism taking place in microbial cultures. The knowledge of the required levels of redox potential for optimal fermentation course enables the control of technological processes leading them in desirable direction.

Wine yeast fermentation is characterised by four growth phases, lag, exponential, stationary and growth declining phase. Lag phase is the answer of microorganism to the environmental conditions and its duration is strongly related to the temperature of fermentation. In this phase a microorganism has to adapt to new environment and temperature. Three extended lag phases were detected: it took 22 hours at 15 °C, 26 hours at 18 °C, and 1 hour at 24 °C. In fermentation at 15 and 18 °C lag phase was followed by intermediate phase for 24 hours and at 24 °C for two hours. In this phase, which is strongly related to the fermentation temperature, growth metabolism has to switch from glucose to fructose and acetaldehyde is produced. High levels of acetaldehyde may cause growth inhibition of yeast and the consumption rate of glucose. Soon after the enzyme alcohol dehydrogenase was synthesized, the conversion of acetaldehyde to ethanol started. All of the available oxygen in the liquid phase was consumed and soon after the anaerobic phase started. In this phase first the production of ethanol was detected and the phase of relatively constant redox potential ended.

The growth in the exponential growth phase was followed by fast drop of redox potential. This fast change was balanced by production of glycerol. The maximum content of glycerol was indicated at the lowest state of the redox potential; 15 °C (6.0 g/L after 148 hours), 18 °C (6.8 g/L after 120 hours) and 24 °C (7.5 g/L after 96 hours). The amount of glycerol was related to the difference in the decrease of redox potential. The amounts of produced ethanol were also related to this finding. At 15 °C its maximum was 75 g/L, at 18 °C, 85 g/L while at 24 °C it rised up to 95 g/L.

The production of ethanol and glycerol is related to the state of the available biomass. The amount of biomass is nearly the same in all experiments (7.2–7.5 g/L) but the activity of its metabolism is different. When ethanol production reached its steady state, yeast metabolism stopped and minimum of redox potential was detected. Soon after the stationary growth phase in the period of the highest production of the ethanol, microbial growth turned to autolysis and the growth declined phase.

Conclusions

From our experiments temperature was found to be one of the most influential physical factors in the fermentation process. Temperature rapidly increases the rate of yeast metabolism. It has strong influences on the yeast growth and its metabolism and therefore it represents one of the most important features that enable further process control. Redox potential measurements can be a useful indicator for monitoring the temperature effect on the yeast metabolism in wine fermentation.

In wine fermentation redox potential could give relevant information on yeast metabolism that takes place. At fermentations at 15 and 18 °C redox potential clearly indicates the transitional phase where microbial growth turns from aerobic to anaerobic as well as the rapid growth rate of yeast at 24 °C, where this phase was very short and larger quantities of glycerol were detected. *Saccharomyces cerevisiae* produces large amounts of glycerol as an osmoregulator, as a redox sink, at low oxygen availability *(14)*.

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Uloga mjerenja redoks-potencijala tijekom fermentacije mošta *Sauvignon blanc*

Sažetak

U radu je proučavana promjena glavnih sastojaka mošta *Sauvignon blanc* tijekom fermentacije pri temperaturama od 15, 18 i 24 °C a dobiveni su rezultati uspoređeni s promjenama redoks-potencijala. Utvrđena je dobra korelacija između promjena vrijednosti redoks-potencijala i parcijalnog tlaka kisika, količine biomase, koncentracije reduktivnih šećera (glukoza i fruktoza), etanola, glicerola i pH-vrijednosti. Redoks-potencijal je vrlo koristan pokazatelj metaboličke aktivnosti kvasca tijekom fermentacije vina koji omogućuje uvid i praćenje procesa vrenja.