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The Effect of Fermentation Temperature on the Growth Kinetics of Wine Yeast Species

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Abstract: The effect of fermentation temperature (18 and 25 °C) on kinetic and yield parameters of ethanol fermentation by *Saccharomyces cerevisiae* (Zymaflore VL1) and *Saccharomyces cerevisiae* (Uvaferm CM) was examined using the white Emir grape that is grown in the Nevşehir-Ürgüp region of Turkey. Growth of both yeast species varied according to temperature. Kinetic and yield parameters were both temperature dependent. Sensory evaluation showed that the taste panel was able to discern the wines fermented by Uvaferm CM and Zymaflore VL1 at different temperatures. The panel preferred the wine fermented by Uvaferm CM at 18 °C.

Key Words: Emir grape, kinetics, *Saccharomyces*, temperature, wine

Fermantasyon Sıcaklığının Şarap Mayalarının Büyüme Kinetiğine Etkisi

Özet: Fermantasyon sıcaklığının (18 ve 25 °C), *Saccharomyces cerevisiae* 'Zymaflore VL1' ve *Saccharomyces cerevisiae* 'Uvaferm CM' suşlarının etanol fermentasyonunun kinetik ve verim parametreleri üzerine etkisi araştırılmıştır. Denemelerde, Nevşehir-Ürgüp yöresinde yetiştirilen beyaz Emir üzümü kullanılmıştır. Maya suşlarının gelişimi sıcaklığa bağlı olarak farklılık göstermiştir. Kinetik ve verim parametrelerinin sıcaklığa bağlı oldukları belirlenmiştir. Duyusal değerlendirme sonuçları, farklı sıcaklıklarda Zymaflore ve Uvaferm tarafından fermente olmuş şarapların ayırt edilebildiklerini ve 18 °C'de Uvaferm tarafından fermente edilen şarabın tercih edildiğini göstermiştir.

Anahtar Sözcükler: Emir üzümü, kinetik, *Saccharomyces*, sıcaklık, şarap

Introduction

The fermentation of grape juice into wine is a complex biochemical process, during which yeasts utilise sugars and other constituents of grape juice as substrates for their growth, converting them to ethanol, carbon dioxide, and other metabolic end products that contribute to the chemical composition and sensory quality of the wine. Several factors affect yeast growth during alcoholic fermentation, including clarification of grape juice, addition of sulphur dioxide, temperature of fermentation, composition of grape juice, inoculation with selected yeasts, and interactions with other organisms. In

traditional winemaking, fermentation is carried out by indigenous yeasts; however, this is changing, with a shift toward induced fermentation with selected yeast strains (Jackson, 2000). The concept of inoculating grape juice with selected starter cultures of *S. cerevisiae* to encourage rapid, consistent fermentation has become widely accepted within the wine industry (Fleet and Heard, 1993; Garcia et al., 2004).

White wines are often fermented in the range of 10-20 °C. Nevertheless, some European wineries still prefer fermentation temperatures between 20 and 25 °C. In recent years, there has been a preference by some

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Abbreviations: G: Generation time (h), t_d : Doubling time (h), Y_{XS} : Growth yield coefficient (g biomass/g substrate), Y_{PS} : Product yield coefficient (g ethanol/g substrate), μ : Specific growth rate (h^{-1}) calculated from ln biomass vs. time graph, μ^* : Specific growth rate (h^{-1}) calculated from ln viable cell vs. time graph, β : Growth-associated ethanol production constant (g ethanol/g biomass)

winemakers to ferment white wines at lower temperatures to enhance the production and retention of flavour volatiles. Such trends have required the selection and use of *S. cerevisiae* strains, which exhibit good growth rates at low temperatures (Fleet and Heard, 1993; Jackson, 2000). The temperature of fermentation can affect the development of different *Saccharomyces* strains. The yield of ethanol and other fermentation by-products are also related to temperature (Torija et al., 2003). Temperature can affect the sensitivity of yeasts to alcohol concentration, growth rate, rate of fermentation, viability, length of lag phase, enzyme and membrane function, etc. Because yeast strains differ in response to temperature, the optimum temperature for vinification can vary widely (Jackson, 2000).

Since fermentation temperature affects the quality of the wine produced from a given cultivar, there may be an optimum temperature to produce the most pleasing result. In this study, the growth kinetics and fermentation behaviour of wine yeast species of *Saccharomyces cerevisiae* (Zymaflore VL1) (Bordeaux, France) and *Saccharomyces cerevisiae* (Uvaferm CM) in response to temperature were investigated, using a white grape variety of Emir, Turkey.

Materials and Methods

Yeast species

The wines were produced using commercial yeast species of *Saccharomyces cerevisiae* (Zymaflore VL1) (Bordeaux, France) and *Saccharomyces cerevisiae* (Uvaferm CM) (Lallemand, France.)

Fermentation Trials

The grapes used in this study were white Emir grapes grown in the Cappadocia region of Turkey. The incoming grapes were passed through a roller-crusher without destemming and were pressed at 1 atm in a horizontal press. Then, 50 mg of sulphur dioxide was added per litre of the must. The must was settled at 10 °C overnight (24 h), and then racked. The must was then pasteurised at 70 °C for 15 min in a water bath. The experiments were carried out in 10⁻¹ glass jars fitted with air locks, at 18 and 25 °C. The inoculation ratio was 0.2% (w/v). All fermentation was performed in duplicate without stirring and the results are presented as means ± SD. Samples were taken periodically during fermentation for analysis

of biomass, cell number, and ethanol and sugar concentration. At the time of sampling, the glass jars were shaken in order to homogenise the contents. After fermentation, the wines were racked and 30 mg of sulphur dioxide was added per litre. After storing the wines for 3 months at 15 °C they were clarified and bottled.

Analyses

During fermentation 250 ml of each sample were removed at 12-h intervals and analysed for viable yeast count (Bakker, 1991), biomass (Bakker, 1991; Cruz et al., 2002), reducing sugar (Martin, 1987), and ethanol (Ough and Amerine, 1988). Viable yeast count was carried out immediately as soon as the samples were removed. Biomass, reducing sugar, and ethanol analyses were carried out after the fermentation was stopped by the addition of 2.5% formaldehyde (40%). The samples for reducing sugar and ethanol analyses were stored at -25 °C. All samples were analysed in triplicate.

Calculation of Kinetic and Yield Parameters

Specific growth rates were calculated during the exponential phase from the slope of natural ln viable cell vs. time (denoted as μ^*) and ln biomass values vs. time (denoted as μ) graphs. Generation times (g) and doubling times (t_d) were calculated by incorporating the specific growth rates, calculated as described above, into the following formulae: $g = 0.693/\mu^*$ and $t_d = 0.693/\mu$, respectively. Growth-associated ethanol production constants (β) were calculated during the exponential phase from the slope of alcohol concentration vs. biomass concentration graphs. Growth yield coefficients ($Y_{X/S}$) and product yield coefficients ($Y_{P/S}$) were calculated during exponential growth from the slope of biomass vs. sugar concentration, and alcohol concentration vs. sugar concentration graphs, respectively (Shuler and Kargi, 1992).

Sensory Evaluation

The wines were subjected to the triangle test to assess the differences between the wines fermented by different yeast species at different temperatures (Barillere and Benard, 1986). Ten experienced tasters from the Department of Food Engineering of the University of Çukurova were asked to detect differences. The wines were presented in coded, covered, tulip-shaped black glasses to mask colour differences. The significance of the

test ($P < 0.05$ and $P < 0.01$) was determined from statistical tables (Larmond, 1969). Calculations were carried out using SPSS v.10.

Results and Discussions

Effect of Fermentation Temperature on Yeast Growth and Fermentation Profile

The effect of fermentation temperature on growth kinetics of *S. cerevisiae* (Zymaflore VL1) and *S. cerevisiae* (Uvaferm CM) is shown in Figure 1. According to Figure 1 it can cautiously be stated that no lag phase was observed during the growth of either species at either temperature. According to Jackson (2000), active dry yeast used as inoculum in wineries comes from cultures grown exponentially in aerated media and short or apparent absence of a lag phase in yeast growth may be the result of the pre-adapted state of the cells used as inoculum. However, it should be considered that the samples in the present study were withdrawn at 12-h intervals and the lag phase could have already taken place before the sampling. At both temperatures studied, a distinct stationary phase was observed following the exponential phase. Growth rate and maximum biomass concentration obtained were higher with Uvaferm CM. As expected, the growth of both species varied according to the temperature, i.e. a higher growth rate was observed with higher temperature.

Substrate consumption and ethanol formation by Zymaflore VL1 and Uvaferm CM are shown in Figures 2 and 3, respectively. Fermentation profiles of both yeast species were similar. When all the sugar was used up and the ethanol concentration rose to the maximum level, the yeast growth stopped and the stationary phase started at

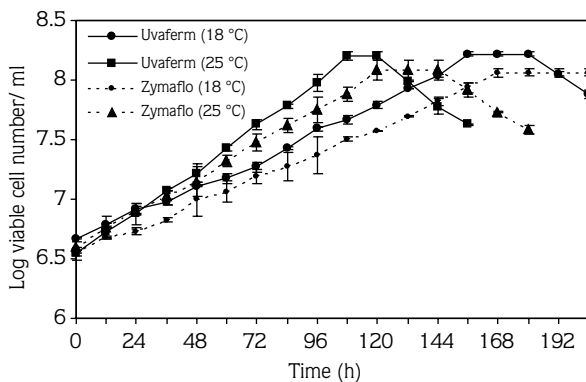


Figure 1. Variation in log cell number at different temperatures.

both temperatures. It was reported that ethanol accumulation in fermenters inhibits specific growth rate, specific ethanol production rate, cell viability, and substrate consumption (Özilgen et al., 1991). The yeasts were able to utilise sugar completely at both temperatures. As expected, fermentation was shorter at 25 °C compared to 18 °C. Fermentation was completed in a shorter time by Uvaferm CM. Both sugar consumption and ethanol formation rates were higher at 25 °C compared to those at 18 °C. The rate of yeast growth and alcoholic fermentation increases as temperature increases, with maximum rates generally occurring at temperatures between 20 and 25 °C (Fleet and Heard, 1993).

The Effect of Fermentation Temperature on Kinetic and Yield Parameters of Zymaflore VL1

Kinetic and yield parameters of Zymaflore VL1 were calculated and the results are presented in Table 1. As can be seen, all parameter values were dependent on the fermentation temperature. The increase in temperature

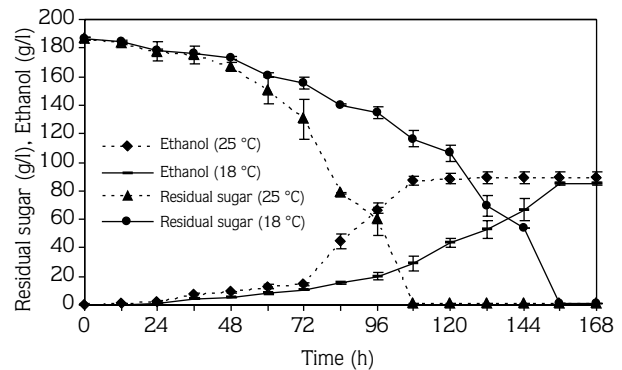


Figure 2. The effect of temperature on sugar consumption and ethanol formation by Zymaflore VL1.

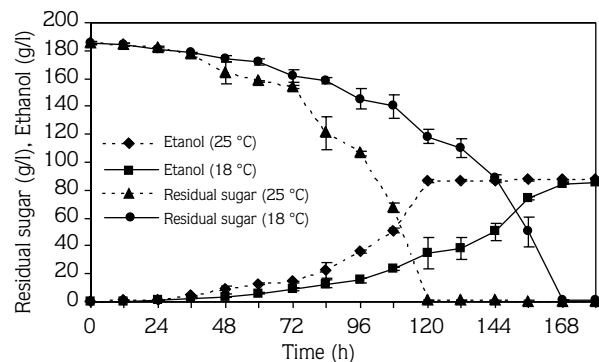


Figure 3. The effect of temperature on sugar consumption and ethanol formation by Uvaferm CM.

resulted in an increase in μ and $Y_{X/S}$ values, and a decrease in g , t_d , β , and $Y_{P/S}$ values. In other words, the microbial growth rate and conversion of carbon source to biomass increased with increasing temperature, while ethanol yield ($Y_{P/S}$) decreased. The minimum and maximum temperatures of growth were altered by the presence of ethanol, organic acids, and fatty acids. The minimum temperature supporting the growth of *Saccharomyces* is quite ethanol sensitive and may be elevated to as high as 27 °C, depending upon conditions. Tolerance to both ethanol and temperature is very strain dependent (Bisson, 1999).

In a study carried out by Ciani and Picciotti (1995) using modified grape juice without added sulphur dioxide at 25 °C, specific growth rates for *S. cerevisiae* calculated by cell number and dry weight were 0.262 and 0.085 h⁻¹, respectively. Giovanelli et al. (1996) investigated the kinetics of grape juice fermentation under aerobic and anaerobic conditions between 17 and 18 °C with the addition of sulphur dioxide at a total concentration of 22.72 mg/l. They reported the following specific growth rates for *S. cerevisiae* calculated by cell number: 0.13 h⁻¹ under aerobic conditions and 0.07 h⁻¹ under anaerobic conditions. The specific growth rate of *S. cerevisiae* grown on synthetic medium containing 1% maltose at 30 °C was 0.40 h⁻¹ (Mwesigye and Barfod, 1996). The specific growth rates obtained in the present study were lower than the reported values. As a result, low specific growth rates led to low t_d and g values; however, the differences in experimental conditions should be taken into account when comparing kinetic parameters because environmental factors, including the availability of nutrients and oxygen, the presence of inhibitors, and physical parameters, such as temperature, pH, and

pressure, strongly influence the growth kinetics of micro-organisms (Dawes and Sutherland, 1992).

Temperature affects fermentation in many ways. At low temperatures yeasts tend to be less sensitive to the toxic effects of high alcohol concentration. The growth rate of yeast cells is strongly influenced by fermentation temperature. This is particularly evident during the exponential phase. At warmer temperatures (> 20 °C), yeast cells experience a rapid decline in viability at the end of fermentation. At cooler temperatures, cell growth is retarded, but viability is enhanced. Cool temperatures prolong the lag phase of fermentation and slow the rate of fermentation. Excessively high temperatures may disrupt enzyme and membrane functions, resulting in stuck fermentation. Although quick onset and completion of fermentation have advantages, the preferred temperature for vinification is often less than the optimum for ethanol production or yeast growth. Because yeast strains differ in response to temperature, the optimum temperature for vinification can vary widely (Jackson, 2000).

The Effect of Fermentation Temperature on Kinetic and Yield Parameters of Uvaferm CM.

Kinetic and yield parameters of Uvaferm CM are presented in Table 2. The effect of temperature on growth parameters was more distinct. The specific growth rate at 25 °C was much higher than that at 18 °C; however, the effect of temperature on yield parameters was not distinct because the β , $Y_{P/S}$, and $Y_{X/S}$ values at both temperatures were very similar. Temperature is an important factor affecting the performance of cells. The yield coefficient is also affected by temperature (Shuler and Kargi, 1992).

Table 1. Kinetic and yield parameters of Zymaflore VL1.

Kinetic and yield parameters	18 °C	25 °C
μ (h ⁻¹)	0.0245 ± 0.0007	0.0355 ± 0.0007
μ^* (h ⁻¹)	0.0205 ± 0.0008	0.0275 ± 0.0003
g (h)	33.8 ± 1.16	25.2 ± 0.64
t_d (h)	28.3 ± 0.81	19.5 ± 0.38
β (g ethanol/g biomass)	9.40 ± 0.14	8.50 ± 0.57
$Y_{X/S}$ (g/g)	0.0525 ± 0.0007	0.0535 ± 0.0007
$Y_{P/S}$ (g/g)	0.499 ± 0.011	0.455 ± 0.015

Table 2. Kinetic and yield parameters of Uvaferm CM.

Kinetic and yield parameters	18 °C	25 °C
μ (h ⁻¹)	0.0265 ± 0.0007	0.0410 ± 0.0014
μ^* (h ⁻¹)	0.0225 ± 0.0008	0.0350 ± 0.0009
g (h)	30.8 ± 0.96	19.8 ± 0.79
t_d (h)	26.2 ± 0.7	16.9 ± 0.57
β (g ethanol/g biomass)	8.15 ± 0.07	8.10 ± 0.28
$Y_{X/S}$ (g/g)	0.0575 ± 0.0007	0.0580 ± 0.0007
$Y_{P/S}$ (g/g)	0.483 ± 0.0045	0.474 ± 0.008

Table 3. Summary of triangle difference tests comparing the wines.

	Number of correct species or temperature/totals	Number of taster preferred Zymoflore VL1	Number of tasters preferred Uvaferm CM
Zymaflore VL1 (18 °C) Uvaferm CM (18 °C)	8/10**	3	7
Zymaflore VL1 (25 °C) Uvaferm CM (25 °C)	7/10*	3	7
Zymaflore VL1 (18 °C) Zymaflore VL1 (25 °C)	7/10**	3	7
Uvaferm CM (18 °C) Uvaferm CM (25 °C)	7/10*	3	7

The difference is significant at 5% (*) and 1% (**).

In a study carried out by Aldiguier et al. (2004) on synergistic temperature and ethanol effect on *Saccharomyces cerevisiae*, it was found that increasing the temperature from 30 to 39 °C resulted in a drastic decrease in biomass, but an increase in ethanol production. They obtained the following values at 27 °C: μ_m : 0.31 (h⁻¹); $Y_{X/S}$: 0.079 (g/g); $Y_{P/S}$: 0.43 (g/g). Specific growth rates and $Y_{X/S}$ (biomass yield on glucose) of 4 different *S. cerevisiae* strains on glucose were between 0.34 and 0.44 h⁻¹, and 0.09-0.10 (Dijken et al., 2000). Phisalaphong et al. (2006) reported that a high temperature led to a decrease in ethanol and cell yields, but an increase in the inhibitory effect of ethanol and sugar on cell growth and ethanol production.

Sensory Evaluation

The taste panel data is summarised in Table 3. The results showed that the panel was able to discern the wines fermented by Uvaferm CM and Zymaflore VL1 at different temperatures. The panel preferred the wines fermented by Uvaferm CM at 18 °C. The temperature at which alcoholic fermentation is conducted not only affects the rate of yeast growth, but also the biochemical reactions of the yeast, which ultimately determines the chemical composition and sensory quality of the wine (Fleet and Heard, 1993). Fruitiness in white wine is a highly valued characteristic. Important in this regard is the increased synthesis of fruit esters, such as isoamyl,

isobutyl, and hexyl acetates. These esters are both synthesised and retained to a greater degree at cool temperatures (Jackson, 2000).

Conclusions

The following conclusions can be drawn from this study:

- There seemed to be no lag phase during the growth of both yeast species at either temperature, which is a desired trait of wine yeast.
- At both temperatures tested, the growth rate of Uvaferm CM was faster than that of Zymaflore VL1 and Uvaferm CM completed fermentation in a shorter time.
- Increasing temperature resulted in a decrease in the ethanol yield of both yeast species.
- The quality of Uvaferm CM-fermented wine seems to be better than that of Zymaflore VL1-fermented wine since the wines produced by Uvaferm CM were preferred by the taste panel.

Acknowledgement

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