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Determination of growth and glycerol production kinetics of a wine yeast strain *Saccharomyces cerevisiae* Kalecik 1 in different substrate media

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Summary

Glycerol has been known as an important by-product of wine fermentations improving the sensory quality of wine. This study was carried out with an endogenic wine yeast strain *Saccharomyces cerevisiae* Kalecik 1. The kinetics of growth and glycerol biosynthesis were analysed at various initial concentrations of glucose, fructose, and sucrose in a batch system. Depending on the determined values of Monod constants, glucose (K_s = 28.09 g/l) was found as the most suitable substrate for the yeast growth. Initial glucose, fructose and sucrose concentrations necessary for maximum specific yeast growth rate were determined as 175 g, 100 l, and 200 g/l, respectively. The yeast produced glycerol at very high concentrations in fructose medium. Fructose was determined as the most suitable substrate for glycerol production while the strain showed low tendency to use it for growth. *S. cerevisiae* Kalecik 1 could not produce glycerol below 200 g/l initial sucrose concentration. When natural white grape juice was used as fermentation medium, maximum glycerol concentration and dry weight of the yeast were determined as 9.3 g/l and 11.8 g/l, respectively.

Introduction

Glycerol is a non-toxic triol with a slightly sweet taste which is soluble in water and other polar solvents, but insoluble in non-polar organic solvents. This polyalcohol is known to have many applications in the food, beverage, pharmaceutical and chemical industries (Agarwal 1990; Scanes *et al.* 1998; Kirk & Othmer 1999). It has been known that glycerol can be produced by chemical and biological synthesis. Yeasts have been the most widely used microorganisms for biological synthesis of glycerol. It is reported that the well-known wine, brewing and baking yeast *Saccharomyces cervisiae* is one of the most important glycerol-producing yeasts (Wang *et al.* 2001; Taherzadeh *et al.* 2002).

Glycerol is the most significant by-product of alcoholic fermentation after ethanol and carbon dioxide. It is synthesized in the cytosol of the yeast *S. cerevisiae* from the glycolytic intermediate dihydroxyacetone phosphate in two steps that are catalysed by glycerol-3-phosphate dehydrogenase (Gpd) and glycerol-3-phosphatase (Gpp), respectively, (Scanes *et al.* 1998; Remize *et al.* 2000; Dequin 2001).

The concentration of glycerol usually formed by *S. cerevisiae* in wine varies between 1–15 g/l, with average values approximately 7 g/l (Scanes *et al.* 1998). Glycerol is an important constituent of wine which does not contribute directly to wine aroma due to its non-volatile nature, but significantly contributes to wine quality by providing sweetness, fullness, and smoothness (Eustace & Thornton 1987; Wang *et al.* 2001).

It is suggested that the level of glycerol production by *S. cerevisiae* is very important for wine quality in the beverage industry. Due to its positive effects on wine's sensory properties, many attemps have been made to increase the glycerol yield during fermentation (Omori *et al.* 1996; Dequin 2001; Taherzadeh *et al.* 2002).

Glycerol production by yeasts is influenced by many growth and environmental factors. Substrate type, initial substrate concentration, pH, temperature, nitrogen source, aeration rate, and inoculation rate are among these factors (Spencer 1968).

For wine fermentations, the sugar concentration of the must is an important factor for growth and glycerol production of wine yeast *S. cerevisiae* (Remize *et al.* 2000). Typically, the sugar concentration of grape must is given as 200 g/l or more at maturity, which consists of equal proportions of glucose and fructose. Sucrose in grape juice only occurs in trace amounts. It is known that the water activity (a_w) of grape must slightly inhibits the growth of *S. cerevisiae* but can also lead to other physiological responses (Scanes *et al.* 1998; Wang *et al.* 2001). Increased synthesis and accumulation of glycerol is the most important among these responses. In

studies on *S. cerevisiae*, it is reported that the glycerol yield in continuous culture was three-to-four fold greater at 0.971 a_w than 0.994 a_w (Wang *et al.* 2001).

Glycerol production in wine could be optimized by controlling cultivation conditions or selecting appropriate strains. This study was organised to compare the effectiveness of glucose, fructose, and sucrose on growth and glycerol production kinetics of an endogenic wine yeast strain, *S. cerevisiae* Kalecik 1. A further objective was to evaluate that effect of grape juice medium by determining the maximum dry weight of the strain and the maximum concentration of glycerol produced.

Materials and methods

Yeast strain

An endogenic wine yeast strain *Saccharomyces cerevisiae* Kalecik 1 was used in the study. This strain has been isolated from Kalecik Karası grapes which are commercially used for wine production and was kindly provided by Prof. Dr. F. Özçelik (University of Ankara, Department of Food Engineering, Turkey). The yeast was kept as stock culture at 4 °C on Yeast Extract Malt Extract Glucose (YMG) agar which consists of 10 g yeast extract (Lab M, U.K), 10 g malt extract (Lab M, U.K), 20 g glucose (Merck, Germany), and 15 g agar (Lab M, U.K) per liter.

Preparation of inocula and fermentation medium

Cultures which were stored in YMG agar, were activated in the same medium by maintaining consecutive transfers. The inoculum used in the experiments was prepared by incubation at 30 °C for 24 h in a medium of the same composition as the fermentation medium, namely 20 g glucose (Merck, Germany), 1 g yeast extract (Lab M, U.K), 1 g KH_2PO_4 (Carlo Erba, Italy), and 0.5 g $MgSO_4{\cdot}7H_2O$ (Pancreac, Spain) per liter. Medium was sterilized by autoclaving at 121°C for 10 min, and the initial pH of the medium was adjusted to 4.00 by using 0.1 M HCl (Riedel-De Häen, 37% extra pure, Germany). Determining the effects of different substrates was accomplished by replacing the glucose in the fermentation medium by the substrate to be tested over a range of concentrations. Concentrations of other ingredients were kept constant during the experiments.

Grape juice

In a part of this study, pasteurized white grape juices of a known company (Kavaklýdere Şarapları A.Ş) were also used as fermentation medium, and variation in glycerol production and dry weight of the yeast during fermentation time was determined. The grape juice originated from Sultaniye white grapes of the Aegean Region. The composition of the grape juice is defined as follows; 4 g/l total acidity (equivalent in sulphuric acid), 150 g/l total sugar, SO_2 of less than 0.5 mg/l, and no alcohol. The pH of grape juice was determined as 3.5. In the experiments, firstly juices were transferred into 300-ml flasks with a 200 ml working volume. Samples were then pasteurized to eliminate backround flora in a water bath at 80 °C for 10 min and cooled immediately before inoculation. Grape juice experiments were carried out in duplicate.

Equipment

Experiments were carried out in water bath shakers with temperature and shaker rate control systems, with 250 ml working volume in suitable flasks. Temperature was held constant at 30 °C and the shaking rate at 70 strokes/min during the experiments.

Biomass determination

Firstly, wet weight-absorbance and wet weight-dry weight calibration curves were prepared. Fermentation medium was inoculated at 5% (v/v) with the yeast culture and samples (10 ml) were taken at specific time intervals for centrifugation (5000 rev/min, 25 min). The precipitate was used for determination of wet weight and so was suspended in a constant volume of distilled water for attenuance measurement at 500 nm (UV 210PC Shimadzu and Bausch & Lomb Spectronic 20). A wet weight vs. absorbance curve was then prepared by using these data. For constructing the dry weight curve, samples were prepared as stated above, and the precipitate was dried at 100 ± 2°C for 2 h after determining wet weight. Relationship between wet weight and dry weight was represented as a calibration curve. During the experiments, dry weight of the yeast was calculated by using these two curves.

In the experiments, samples were taken from fermentation media and centrifuged as stated above. The precipitate was suspended in a constant volume of distilled water so as to determine dry weight spectrophotometrically at 500 nm. After centrifugation of the sample, the supernatant was used for glycerol analysis as described below.

Glycerol analysis

Glycerol concentrations in the fermentation media were determined by periodate-chromotropic acidmethod (Lambert & Neish 1950; Zhuge *et al.* 2001). In this spectrophotometric method, glycerol is quantitatively oxidized to formaldehyde by periodic acid. Formaldehyde is then determined directly in the oxidation mixture, by colour reaction with chromotropic acid. Supernatants of centrifuged samples were used for glycerol analysis spectrophotometrically at 570 nm.

pH measurement

Measurement of pH of the fermentation media was done by using Jenway 3010 model pH meter.

Determining the effects of glucose, fructose, and sucrose on growth and glycerol production kinetics of it S. cerevisiae Kalecik 1

In shake-flask experiments, the effects of initial concentrations of glucose (technical grade, Balmumcu, Turkey), fructose (food grade, Takita, Turkey) and sucrose (food grade, Gima, Turkey) were examined. The initial concentrations were 20–350 g/l for glucose and fructose; and 50–400 g/l for sucrose. A range of substrate concentrations were chosen according to the natural composition of grape must and higher concentrations were not experimented. Since the pH of grape must is 3.5–4.2, the initial pH of fermentation medium was adjusted to a value of 4.00. The yeast was inoculated into the fermentation medium at an inoculation ratio of 5%. For each substrate, specific microbial growth rates (μ), specific glycerol production rates (v_g), maximum glycerol concentrations (P_m), maximum dry weights (x_m), glycerol yields ($Y_{P/S0}$), and cell yields ($Y_{x/S0}$) were calculated. Specific microbial growth rates were determined from the graphs of the changes of dry weight with fermentation time. Specific growth rate values were calculated from the logarithmic plots of the dry weight data vs. time. Specific glycerol production rates were calculated from the following relationship by using the changes in glycerol and dry weight concentrations with time.

$$v_g = \frac{1}{x}\frac{dp}{dt} \tag{1}$$

Maximumvalues of the specific glycerol production rates (v) were also determined in the experiments. In the last stage, natural grape juice was used as fermentation medium. Maximum glycerol concentration and dry weight were investigated during the fermentation.

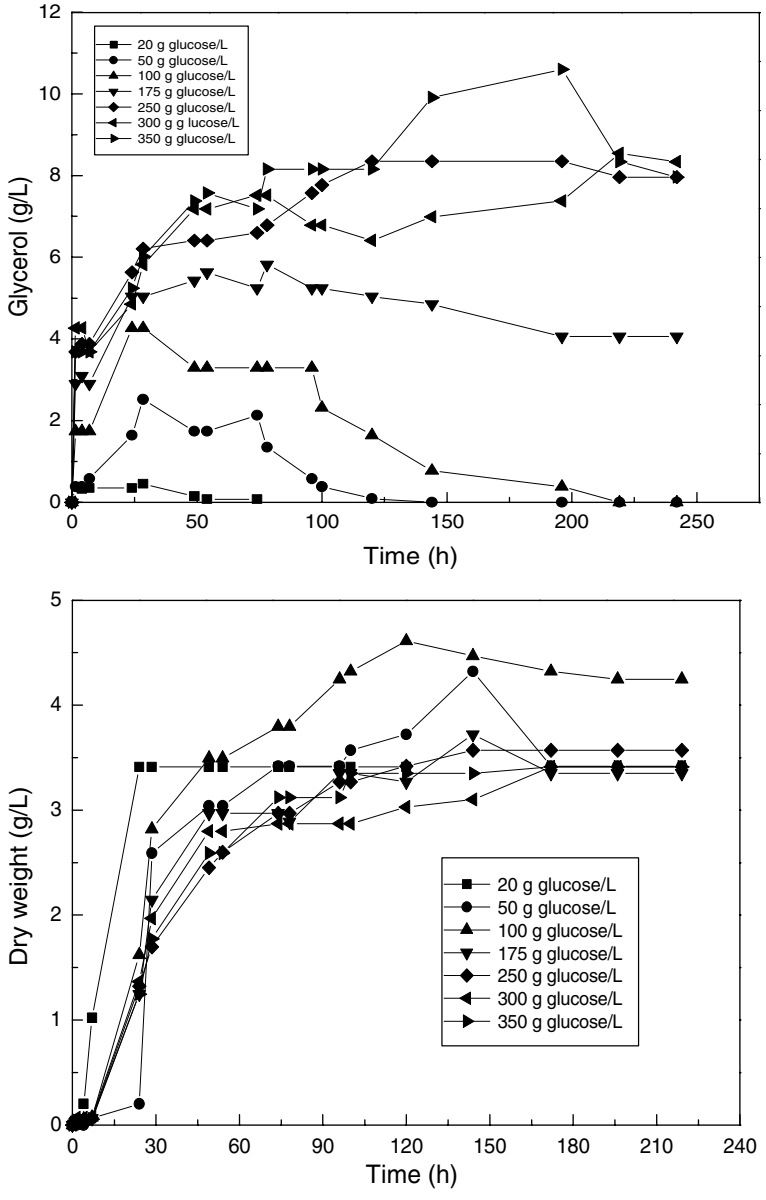

Figure 1. Variation in glycerol production and dry weight of *S. cerevisiae* Kalecik 1 during fermentation time in medium containing glucose.

Statistical analysis

Non-linear regression analysis was carried out by using the SPSS 10 statistical package program, and equations were derived and kinetic constants were calculated (Özdamar 1999).

Results

During the incubation time, changes in glycerol concentrations and dry weights were determined at specific time intervals for each substrate. Specific growth rates (μ) and glycerol production rates (v_g) were calculated. Initial glucose, fructose, and sucrose concentrations of the medium were chosen as independent variables for the specific growth rate and glycerol production rate of the culture. The non-competative substrate inhibition model represented the growth data reasonably well.

$$\mu = \frac{\mu_{max} S_0}{K_s + S_0 + \frac{S_0^2}{K_I}} \quad (2)$$

Here, μ_{max} is the maximum specific growth rate, S_0 is initial substrate concentration, K_s is saturation constant of the Monod model, and K_I is the substrate dissociation constant.

Effects of initial glucose concentration

Effects of initial glucose concentrations (S_{G0}) were investigated in the range of 0–350 g/l. Variations in glycerol concentration and dry weight of the strain during fermentation time is shown in Figure 1. It was found that *S. cerevisiae* Kalecik 1 reached maximum specific growth rate at initial glucose concentration of 175 g/l where maximum specific glycerol production rate was obtained at 300 g/l initial glucose concentration (Figure 2). Both the specific cell growth and glycerol production rate tended to decrease for higher values of S_{G0}, due to inhibitory effect of glucose.

The specific growth and maximum specific glycerol production rates were correlated by using the non-linear regression method as follows:

$$\mu = \frac{0.160 \times S_{G0}}{28.09 + S_{G0} + \left(\frac{S_{G0}^2}{776.72}\right)}, \quad R^2 = 0.891 \quad (3)$$

$$v = \frac{0.256 \times S_{Go}}{195.77 + S_{Go}}, \quad R^2 = 0.937 \quad (4)$$

In the glucose medium, μ_{max} was found as 0.16 h^{-1}, where K_s and K_I were determined as 28.09 g/l and 776.72 g/l, respectively. Dependence of maximum glycerol concentrations (P_m), maximum dry weights (X_m), and glycerol yields ($Y_{P/S0}$) to initial glucose concentration is shown in Table 1. Maximum glycerol concentration was obtained at initial glucose concentration of 350 g/l while glycerol yield was maximum at 125 g/l. Maximum dry weight of the yeast was 4.40 g/l at 100 g/l of initial glucose concentration. Cell yield ($Y_{x/S0}$) was calculated as 2.74×10^{-3} (g yeast cells/g glucose) in glucose medium.

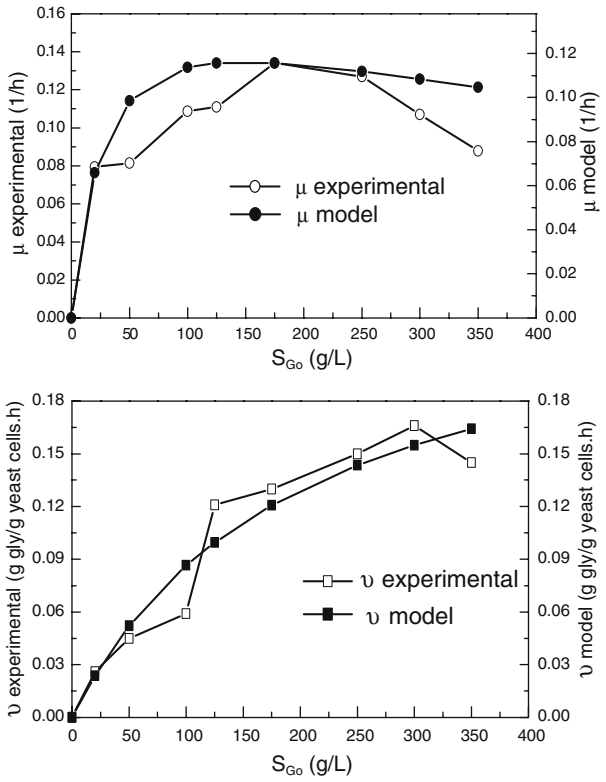

Figure 2. Variation in specific growth and glycerol production rates related to the initial glucose concentration for *S. cerevisiae* Kalecik 1 (μ: specific growth rate, 1/h; *v*: specific glycerol production rate, g glycerol/(g yeast cells/hour); S_{G0}: initial glucose concentration, g/l).

Table 1. Effects of initial glucose concentration on maximum glycerol concentration, maximum dry weight, and glycerol yield for *S. cerevisiae* Kalecik 1.

S_{G0} (g/l)	P_m (g/l)	X_m (g/l)	$Y_{P/S0}$ (%)
0	0.00	0.00	0.00
20	0.70	3.70	3.50
50	2.50	4.30	5.00
100	4.30	4.40	4.30
125	6.60	4.00	5.28
175	5.60	3.70	3.20
250	8.20	3.60	3.28
300	8.50	3.40	2.83
350	10.40	3.40	2.97

S_{G0}: initial glucose concentration; P_m: maximum glycerol concentration; X_m: maximum dry weight; $Y_{P/S0}$: glycerol yield (%).

Effects of initial fructose concentration

The effects of initial fructose concentration (S_{F0}) on the specific cell growth and glycerol production rates were investigated in the range 0–350 g/l of fructose.

Changes in glycerol concentrations and dry weights by fermentation time were shown in Figure 3. Maximum specific growth rate was obtained at 100 g/l, and inhibition on cell growth was seen above this concentration (Figure 4). Optimum glycerol production was obtained at 350 g/l initial fructose concentration in this range.

The equations which represent the variation of specific growth rate and maximum specific glycerol production rate relative to the initial fructose concentration were obtained using non-linear regression analysis and shown below:

$$\mu = \frac{0.83 \times S_{F0}}{122.06 + S_{Fo} + \left(\frac{S_{F0}^2}{57.53}\right)}, \quad R^2 = 0.983 \qquad (5)$$

$$v = \frac{1.82 \times S_{F0}}{272.48 + S_{F0}}, \quad R^2 = 0.994 \qquad (6)$$

In the experiments where fructose was used as substrate, μ_{max} was found as 0.83 h^{-1} where K_s and K_I were determined as 122.06 g/l and 57.53 g/l, respectively. Table 2 shows effects of initial fructose concentration on maximum glycerol concentrations, maximum dry weights, and glycerol yields. Relatively high glycerol concentrations were obtained in fructose medium that reached 75 g/l at 350 g/l initial fructose concentration. Decrease in dry weight was seen in the increasing initial fructose concentration. The same effect was observed in glycerol yield which has a maximum value at 20 g/l initial fructose concentration. Cell yield ($Y_{x/S0}$) was calculated as 1.68×10^{-3} (g yeast cells/g fructose) in fructose medium.

Effects of initial sucrose concentration

The effects of initial sucrose concentration on the growth and glycerol production of the yeast were investigated in the range 0–400 g/l of sucrose. Changes

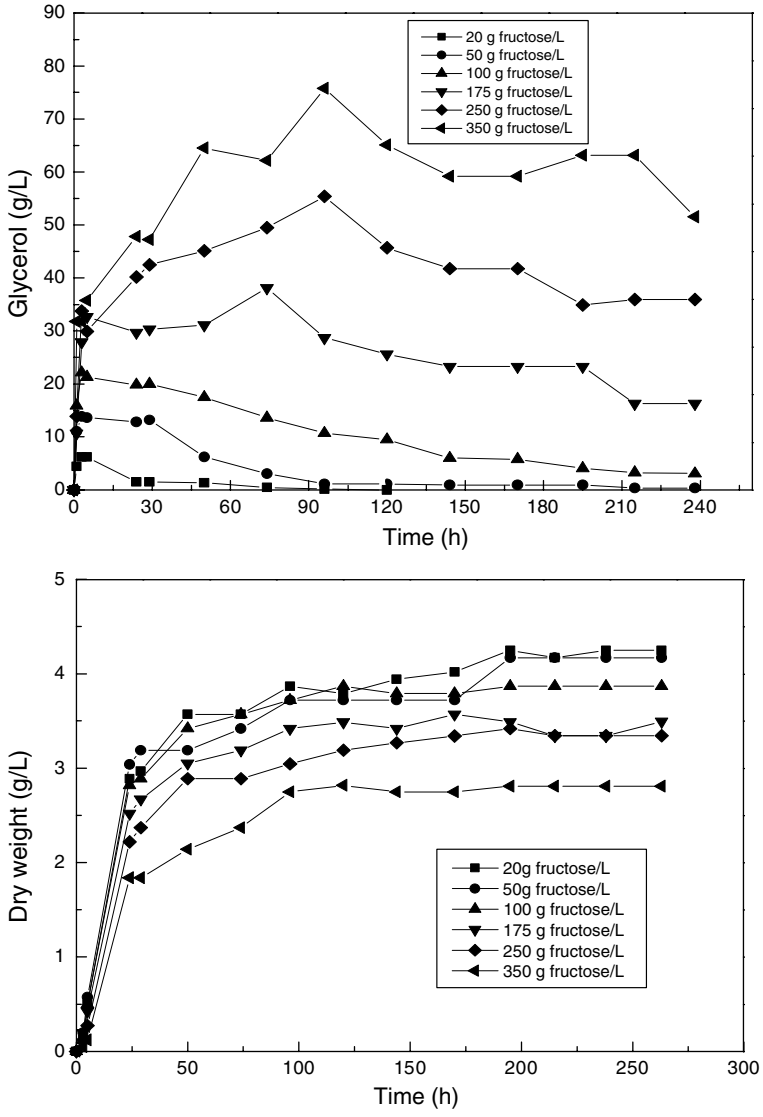

Figure 3. Variation in glycerol production and dry weight of *S. cerevisiae* Kalecik 1 during fermentation time in medium containing fructose.

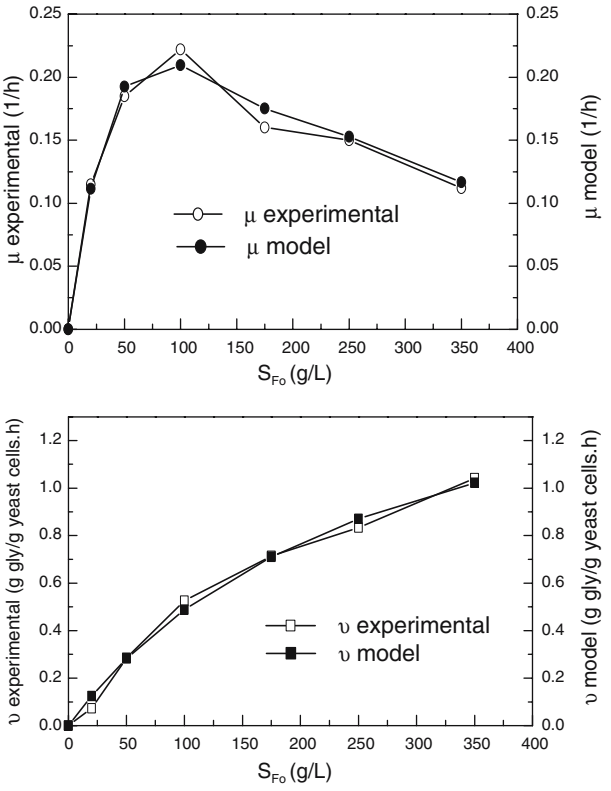

Figure 4. Variation in specific growth and glycerol production rates related to the initial fructose concentration for *S. cerevisiae* Kalecik 1 (μ: specific growth rate, 1/h; v: specific glycerol production rate, g glycerol/(g yeast cells/hour); S_{F0}: initial fructose concentration, g/l).

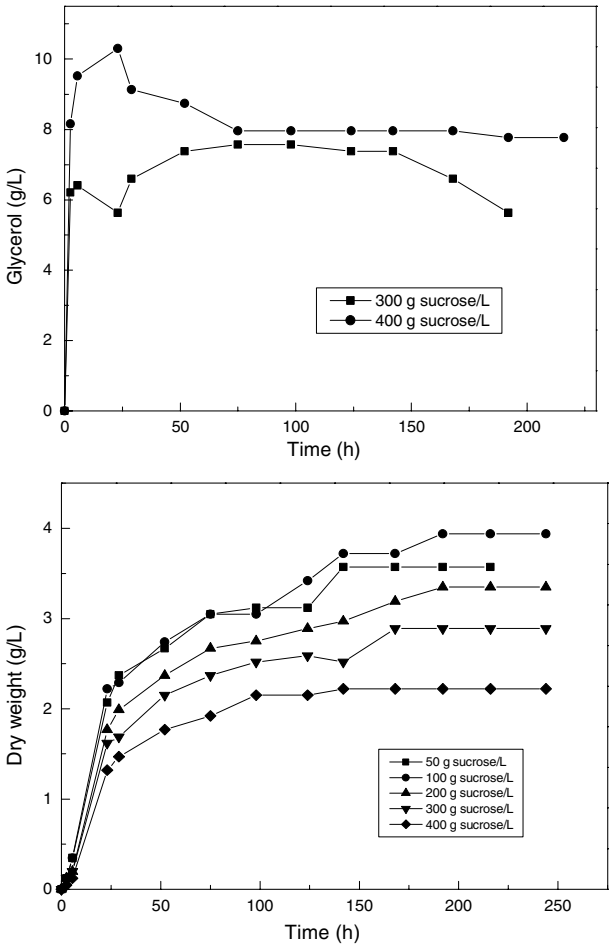

Figure 5. Variation in glycerol production and dry weight of *S. cerevisiae* Kalecik 1 during fermentation time in medium containing sucrose.

Table 2. Effects of initial fructose concentration on maximum glycerol concentration, maximum dry weight, and glycerol yield for *S. cerevisiae* Kalecik 1.

S_{F0}(g/l)	P_m(g/l)	X_m(g/l)	$Y_{P/S0}$(%)
0	0.0	0.00	0.0
20	6.2	4.20	31.0
50	14.0	4.20	28.0
100	22.5	3.90	22.5
175	38.0	3.55	21.7
250	55.0	3.40	22.0
350	75.0	2.85	21.4

S_{F0}: initial fructose concentration; P_m: maximum glycerol concentration; X_m: maximum dry weight; $Y_{P/S0}$: glycerol yield (%).

in glycerol concentrations and dry weights with fermentation time are represented in Figure 5. Optimal growth was observed at 200 g/l initial concentration of sucrose, above which an inhibitory effect was seen. It was found that *S. cerevisiae* Kalecik 1 could not produce glycerol below 200 g/l initial concentration of sucrose. Glycerol production began above 200 g/l and reached maximum rate at 400 g/l (Figure 6). When sucrose was used as substrate, low levels of specific glycerol production rates were obtained when compared to the other substrates.

The equations which represent the variation of specific growth rate and maximum specific glycerol production rate related to initial sucrose concentration were derived by using non-linear regression method and shown below:

$$\mu = \frac{0.299 \times S_{S0}}{80.83 + S_{So} + \left(\frac{S_{S0}^2}{215.28}\right)}, \quad R^2 = 0.961 \tag{7}$$

$$v = \frac{-0.110465}{1 + e^{\left(\frac{S_{S0} - 229.41}{33.152}\right)}} + 0.07824, \quad R^2 = 1.000 \tag{8}$$

The equation belonging to v was derived by using data which was obtained between 200 and 400 g/l initial sucrose concentration.

In sucrose medium, maximum specific growth rate of the strain was found as 0.299 h^{-1} where K_s and K_I were determined as 80.83 g/l and 215.28 g/l, respectively. Maximum glycerol concentrations, maximum dry weights, and glycerol yields were calculated for each initial sucrose concentration, as in the experiments with glucose- and fructose-containing media. Maximum dry weight of 4.35 g/l was obtained at 100 g/l initial sucrose concentration. A decrease in dry weight was observed above this concentration. At 300 and 400 g/l initial

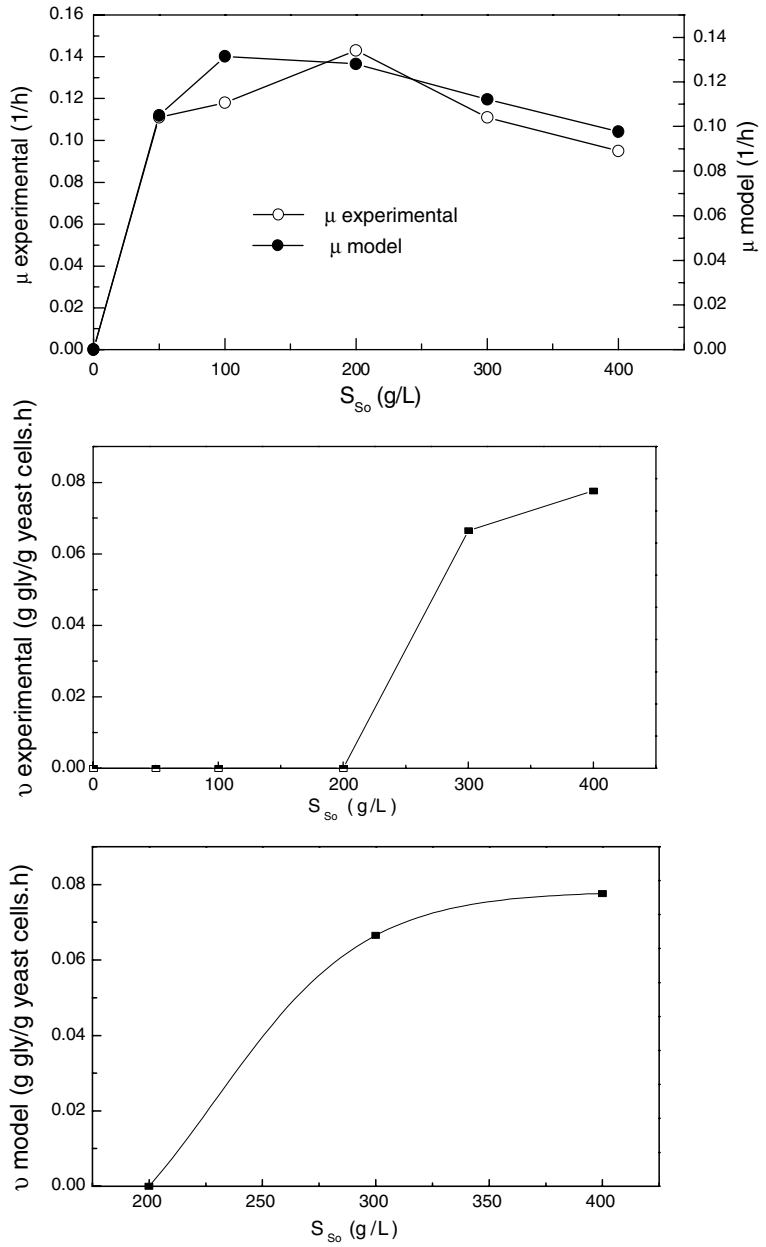

Figure 6. Variation in specific growth and glycerol production rates related to initial sucrose concentration for *S. cerevisiae* Kalecik 1 (μ: specific growth rate, 1/h; v: specific glycerol production rate, g glycerol/(g yeast cells/hour); S_{S0}: initial sucrose concentration, g/l).

sucrose concentrations, obtained P_{max} values were 7.5 and 10.3 g/l, and $Y_{P/S0}$ values 2.50 and 2.58%, respectively. P_{max} and $Y_{P/S0}$ values were reasonably less than those in the other substrates. Cell yield ($Y_{x/S0}$) was calculated as 1.47×10^{-3} (g yeast cells/g sucrose) in sucrose medium.

Effects of grape juice medium

Pasteurized natural white grape juice was inoculated at ratio of 5% culture for investigation of growth and glycerol production of the yeast. Variations in glycerol concentration and dry weight of the yeast with fermentation time were determined. *S. cerevisiae* Kalecik 1 produced the maximum glycerol concentration of 11.8 g/l and reached a maximum dry weight of 9.3 g/l in grape juice. The stationary phase began after 46 h where decrease in glycerol concentration started. Altough there is no exact data about the full composition of the grape juice, it is clear that this medium supports growth of the yeast and is suitable for glycerol production.

Discussion

In this study, in which effects of initial concentrations of glucose, fructose and sucrose on growth and glycerol production of *S. cerevisiae* Kalecik 1 were investigated, an inhibition effect was observed at initial concentrations of all substrates which were above the optimum substrate concentrations necessary for yeast growth. Glucose was found as the substrate which *S. cerevisiae*

Kalecik 1 showed most tendency to use for growth, as the lowest K_s value was obtained in the medium containing glucose. In the medium containing fructose, it was determined that the strain showed a low tendency for growth, but very high glycerol production levels were obtained when compared to the other substrates. It has been known that xerotolerance character is affected by type of solute, temperature, and other ecological factors. The minimum a_w for *S. cerevisiae* is given as 0.89 when the solute is glucose or sucrose, and 0.91 when the solute is fructose (Deak & Beuchat 1996; Viljoen & Heard 2000). As the yeast needs higher a_w values for growth in fructose medium, it is thought that this creates the conditions which supress cell growth but induce glycerol production because of osmotic stress.

Very high glycerol concentrations and specific glycerol production rates were obtained in the medium containing fructose. Maximum glycerol concentrations in fructose medium were approximately seven-fold that in glucose and sucrose media. As *S. cerevisiae* Kalecik 1 was unable to produce glycerol in medium with initial sucrose concentration below 200 g/l, and had very low specific glycerol production rates and yields above this concentration, we can say that sucrose is not a suitable substrate for glycerol production by this strain. From this point of view, the most suitable substrate for glycerol production was found to be fructose.

It is clear that high concentrations of sucrose are needed for glycerol production by this strain. Knowing that sucrose occurs in trace amounts in grape must, it is thought that there will be no effectiveness of sucrose in must for glycerol production during fermentation by *S. cerevisiae* Kalecik 1. In contrast, glucose and especially fructose present in must were found to have important roles in glycerol production during wine fermentation.

In the last stage of the experiment, when grape juice was used as fermentation medium, a significantly high level of maximum dry weight was obtained when compared to the other substrates. Glycerol production was also induced and the highest concentration was obtained in the medium containing fructose. It is reported that use of must, which is obtained from mature grapes, as a substrate increases glycerol synthesis and significantly affects the final cell population (Scanes *et al.* 1998; Remize *et al.* 2000).

Various experiments may be planned in the future for using grape juice as fermentation medium which will determine the characteristics of yeast growth and glycerol production in the fermentation stage of wine production.

References

Agarwal, G.P. 1990 Microbial Bioproducts. In *Advances in Biochemical Engineering Biotechnology*, ed. Fiechter, A. pp. 96–128. Germany: Springer. ISBN 0387525696.

Deak, T. & Beuchat, L.R. 1996 *Handbook of Food Spoilage Yeasts*, 209 pp. USA: CRC Press. ISBN 0-8493-2703-2.

Dequin, S. 2001 The potential of genetic engineering for improving brewing, wine-making and baking yeasts. *Applied Microbiology and Biotechnology* **56**, 577–588.

Eustace, R. & Thornton, R.J. 1987 Selective hybridization of wine yeasts for higher yields of glycerol. *Canadian Journal of Microbiology* **33**, 112–117.

Kirk R.E. & Othmer D.F. 1999 Glycerol. In *Concise Encyclopedia of Chemical Technology*, ed. Kroschwitz, J. pp. 1003–1004, New York: Interscience. ISBN 0-471-29698-8.

Lambert, M. & Neish, A.C. 1950 Rapid method for estimation of glycerol in fermentation solution. *Canadian Journal of Research* **28**, 83–89.

Omori, T., Ogawa, K., Umemoto, Y., Yuki, K., Kajihara, Y., Shimoda, M. & Wada, H. 1996 Enhancement of glycerol production by brewing yeast *(Saccharomyces cerevisiae)* with heat shock treatment. *Journal of Fermentation and Bioengineering* **82**, 187–190.

Özdamar, K. 1999 *Paket programlar ve istatistiksel veri analizi 1*, 535 pp. Turkey: Kaan Kitabevi. ISBN 9756787007.

Remize, F., Sablayrolles,, J., M. & Dequin, S. 2000 Re-assessment of the influence of yeast strain and environmental factors on glycerol production in wine. *Journal of Applied Microbiology* **88**, 371–378.

Scanes, K.T., Hohmann, S. & Prior, B.A. 1998 Glycerol production by the yeast *Saccharomyces cerevisiae* and its relevance to wine: a review. *South African Journal of Enology and Viticulture* **19**, 17–24.

Spencer J.F.T. 1968 Production of polyhydric alcohols by yeasts. In *Progress in Industrial Microbiology*, ed. Hockenhull D.J.D. pp. 2–42, Edinburgh: Churchill Livingstone. ISBN 0700013652.

Taherzadeh, M.J., Adler, L. & Lidén, G. 2002 Strategies for enhancing fermentative production of glycerol – a review. *Enzyme and Microbial Technology* **31**, 53–66.

Viljoen B.J. & Heard G.M. 2000 *Saccharomyces cerevisiae*. In *Encyclopedia of Food Microbiology*, eds. Robinson R.K., Batt C. A. & Patel, P.D. pp. 1918–1924. USA: Academic Press. ISBN 0-12-227070-3.

Wang, Z.X., Zhuge, J., Fang, H. & Prior, B.A. 2001 Glycerol production by microbial fermentation: a review. *Biotechnology Advances* **19**, 201–223.

Zhuge, J., Fang, H.Y., Wang, Z.X., Chen, D.Z., Jin, H.R. & Gu, H.L. 2001 Glycerol production by a novel osmotolerant yeast *Candida glycerinogenes*. *Applied Microbiology and Biotechnology* **55**, 686–692.