A comparative study on citric acid production kinetics of two
*Yarrowia lipolytica* strains in two different media

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Microbial production of citric acid was performed by *Yarrowia lipolytica* NBRC 1658 and a domestic strain in the media containing glucose or fructose as substrates. The study was carried out in a batch system. The non-competitive substrate inhibition model was proposed for growth of the yeasts in both substrate media. Higher dry mass and lower Kₘ values were obtained in glucose media when compared to fructose media. The highest citric acid concentration (65.1 g/L) was obtained with the domestic strain in the medium containing 200 g/L fructose. In the media containing fructose, maximum citric acid concentration and productivity values determined with the domestic strain were approximately two-fold greater than those obtained with NBRC 1658 strain. The required initial concentration of glucose or fructose at which best citric acid production properties were observed changed between 100-200 g/L for both of the strains. The ratio of citric acid to isocitric acid was found to change between 11.70-16.62.

**Keywords:** Batch fermentation, citric acid, growth kinetics, production kinetics, *Yarrowia lipolytica*, yeast

**Introduction**

Citric acid is known as the most important organic acid produced in tonnage by fermentation¹ and is the most exploited biochemical product². The annual production of citric acid was reported as 1.6 million tons³. Citric acid is a commercially valuable microbial product, widely used in food, pharmaceutical and beverage industries⁴. It is the main additive used in the food industry. Citric acid is widely used to impart a pleasant, tart flavour to foods and beverages. It also contributes to the formulation of many foods as an acidulant, antioxidant, emulsifier or preservative⁵,⁶.

The supply of natural citric acid is very limited and the demand can only be satisfied by biotechnological fermentation processes⁷. A large number of microorganisms have been employed for citric acid production, but a few of them can produce citric acid in industrial scale⁸. It is reported that *Aspergillus niger* is almost exclusively used for industrial scale production of citric acid⁹, but during the last 30 years the interest of researchers has been attracted by the use of yeasts as citric acid producers⁴. Among the yeast species, *Yarrowia lipolytica* is known as a potential producer of citric acid⁴,⁷ and has been developed as a microbial cell factory for citric acid production in recent years³. The main advantages of using yeasts are mentioned as follows: Yeasts are characterized by greater resistance to high substrate concentrations than fungi, with comparable conversion rates, and have greater tolerance to metal ions that allows the use of less refined substrates. Using yeasts also gives a better process control due to unicellular nature of yeasts³,⁹. However, the major disadvantage of using yeasts is the simultaneous production of citric and isocitric acids⁸,¹⁰. It is reported that the ratio of citric:isocitric acid can vary between 1:1 to 20:1 according to the yeast strain, carbon source and micronutrient concentration⁸. Selection of a yeast strain with high citric acid production and giving high citric acid:isocitric acid ratios has been reported as the principal step of a citric acid production process.

Many yeasts that grow on carbohydrate substrates have the ability to accumulate high concentrations of citric acid during tricarboxylic acid cycle respiration. However, *Y. lipolytica* is the only species known for its capability of maximizing citric acid production¹¹. *Y. lipolytica* and other *Candida* strains are able to reduce citric acid from various substrates, whereby glucose has generated increasing interest¹². Other carbon sources,
such as, molasses, n-paraffins, hexadecanes, edible oils, starch hydrolysates and pure or raw glycerol, have been used to produce citric acid from \textit{Y. lipolytica}\textsuperscript{2,10,13}. The optimization of fermentation conditions are of primary importance in the development of any fermentation process owing to their impact on the economy and practability of the process\textsuperscript{2}. Work to improve fermentative production of citric acid is continually in progress. Different techniques of production, using a variety of substrates, strain selection and development are continuously studied.

The aim of the present study was to investigate growth and citric acid production kinetics of two different \textit{Y. lipolytica} strains, at different initial concentrations of glucose and fructose as substrates, in a batch system. This study was also planned to demonstrate the potential of citric acid production of a novel strain by comparing its production characteristics with \textit{Y. lipolytica} NBRC 1658, a citric acid producer.

\textbf{Materials and Methods}

\textbf{Yeast Strains}

\textit{Y. lipolytica} NBRC 1658 was obtained from National Institute of Technology and Evaluation (NITE), Biological Resource Center, Japan. The strain NBRC 1658 was defined as a “citric acid producer” by the culture collection. \textit{Y. lipolytica} IFO 1195 was obtained from the same culture collection in Japan. The domestic strain \textit{Y. lipolytica} 57 was obtained from the culture collection of Food Engineering Department, Faculty of Engineering, Ankara University, Turkey. The selection of this strain was performed with a preliminary study in which citric acid production abilities of 30 domestic strains belonging to different species, such as, \textit{Candida guillermondii} (7), \textit{C. pelliculosa} (4), \textit{C. intermedia} (2), \textit{C. parapsilosi} (4), \textit{Rhodotorula glutinis} (8), \textit{Saccharomyces cerevisiae} (3), \textit{Pichia anomala} (1) and \textit{Y. lipolytica} (1), as well as the strain \textit{Y. lipolytica} IFO 1195 were investigated. The domestic yeast strains belonging to the culture collection of Food Engineering Department, Hacettepe University, Turkey, had been isolated from some high-sugar foods (honey, fruit yoghurt, dried apricot, dried fig, date, glucose syrup, etc.) in a previous study performed by Senses-Ergul and Ozbas\textsuperscript{14}. Screening of citric acid production capabilities of the strains were performed in a fermentation medium containing (in g/L): glucose, 100; NH\textsubscript{4}Cl, 2; KH\textsubscript{2}PO\textsubscript{4}, 1; MgSO\textsubscript{4}.7H\textsubscript{2}O, 1; yeast extract, 1; and CaCO\textsubscript{3}, 40\textsuperscript{15}. Of the tested strains, the highest citric acid production was obtained by the domestic \textit{Y. lipolytica} 57 which was chosen for further studies. At the beginning of the present study, identification of \textit{Y. lipolytica} 57 and \textit{Y. lipolytica} NBRC 1658 was confirmed with the use of rapid API ID 32C (bioMérieux, France) test system and also molecular methods (data not shown). The yeast strains were kept as stock cultures at 4°C on yeast extract malt extract (YM) agar consisting of (in g/L): yeast extract 3, malt extract 3, peptone 5, glucose 10, and agar 15. Cultures stored in YM agar were activated in the same medium by maintaining consecutive transfers.

\textbf{Growth and Fermentation Media}

The inocula used in the experiments were prepared by incubation of the cultures at 28°C for 24 h in a modified growth medium containing (in g/L): glucose, 30; yeast extract, 2; NH\textsubscript{4}Cl, 2; KH\textsubscript{2}PO\textsubscript{4}, 0.5; and MgSO\textsubscript{4}.7H\textsubscript{2}O, 1\textsuperscript{16}. The experiments were carried out in a fermentation medium containing (in g/L): glucose, 0-242 or fructose, 0-200; NH\textsubscript{4}Cl, 2; KH\textsubscript{2}PO\textsubscript{4}, 1; MgSO\textsubscript{4}.7H\textsubscript{2}O, 1; yeast extract, 1; CaCO\textsubscript{3}, 40\textsuperscript{15}. CaCO\textsubscript{3} was used in the fermentation medium as a buffering agent. The media were sterilized in an autoclave at 121°C for 15 min. Initial pH of the fermentation medium was adjusted to 5.2 by using 1 N HCl.

\textbf{Equipment and Fermentation Conditions}

Fermentations were carried out in water bath shakers using 300 mL cotton-plugged flasks containing 100 mL of fermentation medium. The yeasts were inoculated separately to fermentation media at an inoculum volume percentage of 5%. Experiments were carried out at constant temperature of 30°C, with a shaking rate of 100 strokes/min. All of the runs were performed in duplicate. Mean values of the results were plotted on the graphs.

\textbf{Biomass Determination}

Dry mass (\(x\)) of the yeast was determined spectrophotometrically by using wet mass-absorbance and wet mass-dry mass calibration curves which had been prepared before. During the experiments, samples were taken out from the fermentation media at a time interval of 24-48 h. 6 N HCl was added into the samples in order to dissolve CaCO\textsubscript{3}\textsuperscript{15}. Samples were centrifuged at 5000 rpm for 25 min. Precipitate was used for determination of dry mass spectrophotometrically at 660 nm (UV 210PC Shimadzu and Bausch & Lomb Spectronic 20). Supernatant was used for citric acid, isocitric acid and substrate analysis.
Analytical Methods
Concentration of citric acid was measured spectrophotometrically by pyridine-acetic anhydride method\textsuperscript{17,18}. Glucose and isocitric acid concentrations were determined enzymatically (Boehringer Mannheim, Germany). Concentration of fructose was determined by measuring total sugar concentration by Lane-Eynon method\textsuperscript{19}.

Determining Kinetic Characteristics of Cell Growth and Citric Acid Production
Initial concentrations of glucose and fructose \((S_{G0}\text{ and } S_{F0})\) in the fermentation media were changed between 0-242 g/L and 0-200 g/L, respectively. For both of the strains, specific microbial growth rates \((\mu)\), citric acid productivities \((r_s)\), maximum specific citric acid production rates \((\nu_m)\), glucose and fructose consumption rates \((-r_G\text{ & } -r_F)\), maximum citric acid concentrations \((C_{Cm})\), maximum dry mass \((x_m)\), citric acid yields \((Y_{P/S0})\) and cell yields \((Y_{X/S0})\) were calculated. Specific microbial growth rate for the exponential growth phase was calculated from the semi-logarithmic plot of the dry mass data versus time. Specific citric acid production rates \((q_p)\) were calculated from the following relationship by using the changes in citric acid concentrations and dry mass with time\textsuperscript{20}.
\[
q_p = \frac{1}{x} \frac{dP}{dt} \quad \quad \ldots (1)
\]

Maximum values of the specific citric acid production rates \((\nu_m)\) were also determined in the experiments.

Citric acid yields were calculated according to Eq. 2\textsuperscript{20}:
\[
Y_{P/S0} = \frac{C_{Cm}}{S_{0}} \cdot 100 \quad \quad \ldots (2)
\]

Statistical Analysis
Calculations of kinetic constants and rates were performed by using Origin 6.0 program package (Microcal Software, Inc., Northampton, MA01060, USA). Equations expressing the changes of specific growth and citric acid production rates with initial glucose or fructose concentration were derived from non-linear regression analysis by using the same package program.

Results
During the fermentation period, variations in citric acid concentrations and dry mass of the strains were determined at specific time intervals for both of the substrates. Calculated specific growth rates and maximum specific citric acid production rates were expressed as a function of initial glucose or fructose concentration. It was found that the non-competitive substrate inhibition model was suitable for definition of the growth data (Eq. 3)\textsuperscript{21}.
\[
\mu = \frac{\mu_{max} S_o}{K_s + S_o + \left(\frac{S_o^2}{K_I}\right)} \quad \quad \ldots (3)
\]

In Eq. 3, \(\mu_{max}\) is the maximum specific growth rate, \(S_o\) is initial substrate concentration, \(K_s\) is saturation constant of the Monod model, and \(K_I\) is the substrate dissociation constant.

For \textit{Y. lipolytica} NBRC 1658 and the domestic \textit{Y. lipolytica} 57, changes of microorganism dry mass \((dm)\) and citric acid concentration with time at different initial glucose concentrations are represented in Fig. 1. A very low cell growth and no citric acid production was observed when substrate was not added to the medium. The highest value for maximum dry mass was obtained at 200 g/L initial glucose concentration for both of the strains. Citric acid

\begin{figure}
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\includegraphics[width=\textwidth]{fig1.png}
\caption{Variations in dry mass and citric acid production of \textit{Y. lipolytica} NBRC 1658 (a, c) and \textit{Y. lipolytica} 57 (b, d) at different initial glucose concentrations during fermentation time.}
\end{figure}
production of the yeasts reached to a maximum level in approximately 300 h. Maximum citric acid concentration was obtained as 34.15 g/L for the strain NBRC 1658, and 44.39 g/L for the domestic strain at 150 g/L initial glucose concentration. There was a decrease in maximum citric acid concentrations above 150 g/L initial glucose concentration. For both of the strains, changes in citric acid productivity with time and calculated specific growth and citric acid production rates at different initial glucose concentrations were shown in Fig. 2. Higher productivity values were obtained for the novel endogenic strain when compared with NBRC 1658.

A substrate inhibition effect on cell growth was observed for both of the strains at high initial glucose concentrations. Maximum specific growth rate was obtained at 50 g/L initial glucose concentration for both the strains. The non-competitive substrate inhibition model estimated for growth of \textit{Y. lipolytica} NBRC 1658 in glucose medium and derived from non-linear regression analysis is given in Eq. 4. It is obvious from high $R^2$ value that non-competitive substrate inhibition model represented the growth data reasonably well.

\[
\mu = \frac{0.070 \, S_{Go}}{18.81 + S_{Go} + \left(\frac{S_{Go}^2}{110.07}\right)} \quad \ldots \text{(4)}
\]

$R^2 = 0.903$

According to Eq. 4; maximum specific growth rate ($\mu_{\text{max}}$) was found as 0.070 h$^{-1}$, while saturation constant ($K_s$) and substrate dissociation constant ($K_i$) were determined as 18.81 g/L and 110.07 g/L, respectively.

The highest experimental value for maximum specific citric acid production rate was obtained at 150 g/L initial glucose concentration (Fig. 2). The variation of maximum specific citric acid production rate with initial glucose concentration for the strain NBRC 1658 is represented in Eq. 5. It can be observed that the derived model adequately fit the experimental data.

\[
\nu_m = \frac{0.007}{1 + 3609.88 \times e^{-0.339S_{Go}}} \quad \ldots \text{(5)}
\]

$R^2 = 0.967$

The estimated model that gives the relationship between specific growth rate and initial glucose concentration for the novel domestic \textit{Y. lipolytica} strain is shown in Eq. 6. From the non-competitive inhibition model, $\mu_{\text{max}}$ was found as 0.120 h$^{-1}$. $K_s$ and $K_i$ values obtained in glucose medium were determined as 57.55 g/L and 71.40 g/L, respectively.

\[
\mu = \frac{0.120 \, S_{Go}}{57.55 + S_{Go} + \left(\frac{S_{Go}^2}{71.40}\right)} \quad \ldots \text{(6)}
\]

$R^2 = 0.799$

Maximum value of $\nu_m$ was obtained at 100 g/L initial glucose concentration for the novel endogenic strain. Eq. 7 shows the variation of specific citric acid production rate with initial glucose concentration for this strain.

\[
\nu_m = -0.01863 + 0.00113S_{Go} - 8.958 \times 10^4 \, S_{Go}^2 + 1.999 \times 10^4 \, S_{Go}^3 \quad \ldots \text{(7)}
\]

$R^2 = 0.929$
Glucose concentrations determined at specific time intervals are shown on Fig. 3. Calculated values of glucose consumption rates and conversion ratios ($x_G$) are also represented on the figure. It was found that in the medium with 20 g/L initial glucose concentration, all of the glucose was consumed in 94 h for the strain NBRC 1658. When initial concentration of glucose was 50 g/L, no glucose was determined in the fermentation medium after 112 h. In the media containing 100 and 150 g/L glucose, no residual glucose was determined at the end of the fermentation, so that obtained glucose conversion ratios were 1 in those media. Unconsumed glucose concentrations were found as 83.3 g/L and 128.8 g/L in the media containing 200 and 242 g/L glucose, respectively. In the experiments performed with the novel domestic strain, whole glucose was consumed in the media with 20, 50, 100 and 150 g/L initial glucose concentrations. When initial glucose concentration was 200 and 235 g/L, residual glucose concentration was determined as 92.4 and 131.2 g/L, respectively.

The effects of initial glucose concentration on maximum citric acid concentration, maximum dry mass, product yield and maximum productivity are shown on Table 1. For the strain NBRC 1658, maximum values of citric acid concentration and productivity were obtained at 150 g/L initial glucose concentration, while maximum citric acid yield was obtained in the medium with 100 g/L glucose. Similar results were obtained for the domestic strain that the highest citric acid concentration was determined at 150 g/L initial glucose concentration. Maximum values for product yield and productivity were obtained in the medium containing 100 g/L glucose. NBRC 1658 and the domestic strain reached maximum productivity in 160 and 280 h, respectively. Cell yield of NBRC 1658 strain was calculated as 0.162 g dm/g glucose, while it was 0.134 g dm/g glucose for the domestic strain.

The effects of initial fructose concentration on cell growth and citric acid production is represented in Fig. 4. High values for maximum dry mass were obtained at initial concentrations of 150 and 200 g/L for both of the strains. Maximum citric acid concentration was obtained as 33.70 g/L at 150 g/L initial fructose concentration for the strain NBRC 1658. Citric acid production was very low at low initial fructose concentrations. For the domestic strain, maximum citric acid concentration was obtained as 65.10 g/L at 200 g/L initial fructose concentration. Between 50-200 g/L initial fructose concentration, citric acid production of this strain was found higher than that of NBRC 1658 strain. The same comment can be made for productivity and

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<th>$S_{Go}$ (g/L)</th>
<th>$C_{cm}$ (g/L)</th>
<th>$X_m$ (g/L)</th>
<th>$Y_{PSSO}$ (%)</th>
<th>$r_{cm}$ [g citric acid/(L.h)]</th>
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maximum specific citric acid production rate, which is shown in Fig. 5. For both of the strains, maximum specific growth rate was obtained at 50 g/L initial fructose concentration and a substrate inhibition effect was observed above this concentration. The relationship between initial fructose concentration and specific growth rate of the strain NBRC 1658 was defined with non-competitive substrate inhibition model and represented in Eq. 8. From the equation; $\mu_{\text{max}}$ was found as 0.251 h$^{-1}$ while $K_s$ and $K_I$ were obtained as 24.42 g/L and 71.56 g/L, respectively.

$$
\mu = \frac{0.251 S_{Fo}}{24.42 + S_{Fo} + \left(\frac{S_{Fo}^2}{71.56}\right)}
$$

R$^2$ = 0.955

Maximum value for the specific citric acid production rate was obtained at 150 g/L initial fructose concentration for the strain NBRC 1658. The equation derived from non-linear regression analysis for definition of the change in maximum specific citric acid production rate with initial fructose concentration is given in Eq. 9.

$$
v_m = -2.17 \times 10^{-4} + 6.597 \times 10^5 S_{Fo} - 2.01 \times 10^7 S_{Fo}^2
$$

R$^2$ = 0.989

In the experiments performed with the domestic strain, the curve representing specific growth rate as a function of initial fructose concentration in Fig. 5 was formed by using the relationship in Eq. 10. The model equation gives $\mu_{\text{max}}$ value as 0.069 h$^{-1}$; and kinetic constants $K_s$ and $K_I$ were determined as 69.25 g/L and 120.03 g/L, respectively.

$$
\mu = \frac{0.069 S_{Fo}}{69.25 + S_{Fo} + \left(\frac{S_{Fo}^2}{120.03}\right)}
$$

R$^2$ = 0.904

The highest experimental value for maximum specific citric acid production rate was obtained at 100 g/L initial fructose concentration for the domestic strain. The change of maximum specific citric acid production rate with initial fructose concentration was expressed by Eq. 11 with a high R$^2$ value and shown below:

$$
\mu = \frac{0.069 S_{Fo}}{100 + S_{Fo} + \left(\frac{S_{Fo}^2}{120.03}\right)}
$$

R$^2$ = 0.904

Fig. 4—Variations in dry mass and citric acid production of Y. lipolytica NBRC 1658 (a, c) and Y. lipolytica 57 (b, d) at different initial fructose concentrations during fermentation time.

Fig. 5—Variations in productivity with time at different initial fructose concentrations and changes of maximum specific citric acid production rate and specific growth rate with initial fructose concentration for Y. lipolytica NBRC 1658 (a) and Y. lipolytica 57 (b).
\[ u_m = \frac{0.01659}{1 + 16371.14 \times e^{-0.14836S_{fo}}} \]  ... (11)

\[ R^2 = 0.991 \]

In the experiments performed with fructose media, the best results for citric acid production were obtained at 150 g/L initial fructose concentration for the strain NBRC 1658, and at 200 g/L for the domestic strain. There was a decrease in citric acid concentration at 200 g/L initial fructose concentration for NBRC 1658. With this strain, in order to determine the amount of residual sugar, changes in fructose concentrations with time were determined at the experiments concerning 150 and 200 g/L initial fructose concentrations. Fructose consumption of the domestic strain during fermentation time was investigated in the medium with 200 g/L initial fructose concentration. Variations in fructose concentration, fructose consumption rate and conversion ratios with time in the mentioned experiments are represented in Fig. 6. When initial fructose concentration was used as 150 g/L, 77 g/L residual sugar was determined after 87 h, and all of the sugar was consumed at the end of the fermentation for the strain NBRC 1658. At 200 g/L initial fructose concentration, 40 g/L residual sugar was determined at the end of the fermentation. Substrate conversion ratio was calculated as 0.80 in that medium. In the experiments performed with the domestic strain at 200 g/L initial fructose concentration, 26.6 g/L sugar was determined in the medium at the end of the fermentation, leading to a substrate conversion ratio of 0.87.

The effects of initial fructose concentration on maximum citric acid concentration, maximum dry mass, product yield and maximum productivity are shown on Table 2. The best results for citric acid production and dry mass were obtained at 150 g/L initial fructose concentration for NBRC 1658 strain. It can be observed from Table 2 that highest values for citric acid concentration, dry mass, product yield and productivity were obtained in the medium containing 200 g/L fructose for the domestic strain. Cell yields of NBRC 1658 and the domestic strain in fructose medium were calculated as 0.113 and 0.089 g dm/g fructose, respectively.

In Fig. 7, variations in isocitric acid concentrations with time are represented. Isocitric acid production of the strains were investigated at the conditions which gave the best results for citric acid production. For the

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<th>( S_{fo} ) (g/L)</th>
<th>( C_{cm} ) (g/L)</th>
<th>( x_m ) (g/L)</th>
<th>( Y_{P/SO} ) (%)</th>
<th>( r_{cm} ) [g citric acid/(L.h)]</th>
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domestic strain, the highest citric acid concentrations were obtained in the media with initial concentration of 150 g/L glucose and 200 g/L fructose. For the strain NBRC 1658, the best results for citric acid production were obtained at 150 g/L initial substrate concentration in both glucose and fructose media. In our other experiments (data not shown), optimal citric acid production was obtained at pH 7 for the strain NBRC 1658. For this reason, the experiment for determining the variation of isocitric acid with time was performed at initial pH of 7 when working with this strain. In the experiments with the domestic strain, initial pH of the medium were kept as 5.2. Maximum isocitric acid concentrations in glucose and fructose medium were found as 4.15 and 5.58 g/L for the domestic strain, giving citric:isocitric acid ratio of 11.7 and 11.87, respectively. In the experiments carried out with NBRC 1658, maximum concentration of isocitric acid was found as 1.88 g/L in glucose media and 2.61 g/L in the medium containing fructose. For this strain, the ratios of citric:isocitric acid were found as 12.47 and 16.62 in glucose and fructose media, respectively.

**Discussion**

In this study, growth and citric acid production characteristics of two *Y. lipolytica* strains were compared at two different media. Both the strains grew better in glucose media than in fructose media; since higher dry mass and lower $K_s$ values were obtained in glucose media. The yeasts showed high tendency to use glucose for growth. In all the experiments, higher values of dry mass were obtained with NBRC strain than with the domestic strain. Substrate inhibition effect was observed on the growth of yeasts at high substrate concentrations above the initial concentration that maximum specific growth rate was obtained.

When results are examined in the mean of citric acid production, substrate preference of the two yeasts are exactly different. The highest citric acid concentration (65.1 g/L) was obtained with the domestic strain in the medium containing 200 g fructose/L. Citric acid concentrations obtained with this strain in glucose media were lower than those obtained in fructose media. There was not a significant difference between maximum citric acid concentrations obtained in glucose and fructose media with the strain NBRC 1658. At almost all initial glucose or fructose concentrations, citric acid production level of the domestic strain was higher than that of the strain NBRC 1658. The difference between citric acid production abilities of the both yeasts has been definitely appeared with the results in fructose media. In the media containing fructose, maximum citric acid concentrations and productivities determined with the domestic strain were approximately two-fold greater than those obtained with NBRC 1658.

Determination of substrate type and initial substrate concentration has a significant role for optimizing culture conditions of a citric acid production process. High substrate concentration (120-250 g/L) is given as a factor enhancing microbial citric acid production. In a study performed by Antonucci *et al.*, it is reported that specific rate of product formation and substrate consumption rate increases at high substrate concentrations. Being in agreement with the studies in the literature, initial glucose or fructose concentration at which best results were obtained for citric acid production were 100 g/L or above concentrations in the present study. In a study carried out with *C. lipolytica* Y 1095, it is reported that maximum citric acid concentrations obtained at 100 and 150 g/L initial glucose concentrations were 32.3 and 50 g/L, respectively. In another study performed by Wojtatowicz *et al.*, glucose hydrol was used as a substrate and 80 g/L citric acid was obtained at 100 g/L initial glucose concentration with a mutant strain of *Y. lipolytica*. Fructose is reported to take place among the substrate profile of yeasts for citric acid production. The number of studies concerning...
citric acid production with this alternative substrate is limited. Hamissa and Abou-Zeid\textsuperscript{15} reported that 23.75 g/L citric acid was obtained with a *Y. lipolytica* strain in a medium containing 100 g/L fructose. In the present study, determined citric acid concentrations, especially, in fructose media are relatively high when compared to those reported in the literature. Since glucose is a cheaper substrate, its usage may be advantageous in a citric acid production process in the mean of scale up. This can be valid for the strain NBRC 1658, since similar results were obtained in glucose and fructose media. But the domestic strain surprisingly produced very high amounts of citric acid in the media containing fructose and higher substrate conversion rates were obtained in that medium. Therefore, fructose seems to be an alternative substrate for citric acid production with this strain. These results may lead to further investigations concerning the use of several wastes or natural substrates containing fructose, such as, fruit juice wastes. Another promising result of this study was low isocitric acid production of the yeasts in both substrates; the ratio of citric:isocitric acid was changed between 11.70-16.62 in the examined media. Isocitric acid production is the main disadvantage of use of yeasts, and its amount is one of the most important selection criteria for a citric acid producing yeast\textsuperscript{22,24}. In several studies, citric:isocitric acid ratio is reported to change according to the strain, carbon source and other media components, ranging between 1-20. In a study carried out with *C. lipolytica* and *Y. lipolytica* strains, it is reported that one strain was selected as a high citric acid producer and gave a citric:isocitric acid ratio of 5.25. It is stated in the same study that the highest ratio was obtained as 13 when different media were used\textsuperscript{5}.

Besides determination of the effects of different substrates, one aim of this study was the comparison of citric acid production capabilities of two *Y. lipolytica* strains. When domestic strain was used, similar or higher citric acid concentrations and citric:isocitric acid ratios were determined when compared to the results obtained with NBRC 1658 strain. There is no reported previous study performed with the domestic strain used in the present study. The strain NBRC 1658 is presented as a “citric acid producer” by the culture collection. By pointing out better citric acid production properties of the domestic strain than NBRC 1658 strain, the results in the present study give valuable data for generating a novel “citric acid producer”. Demonstrating citric acid production amount at different media with this novel strain, the results are also informative in determining culture conditions for scale-up in a commercial production process.

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**References**