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Production of citric and isocitric acid by *Yarrowia lipolytica* strains grown on different carbon sources

[Farklı karbon kaynakları üzerinde büyüyen *Yarrowia lipolytica* suşları tarafından sitrik ve izositrik asidin üretimi]

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ABSTRACT

Objective: The goal of this study was to determine the influence of various carbon sources on the citric and isocitric acid production by various *Yarrowia lipolytica* strains.

Methods: The yeasts used in our study were first investigated for organic acid production using screening media. Then, the effect of several complex carbon sources on the citric and isocitric acid production of selected yeast strains was investigated. The amount of citric and isocitric acid production was determined via enzymatic reactions.

Results: In this study, 22 *Y. lipolytica* strains were investigated for the organic acid production. Among these strains, 2 strains (TEM YL 3 and TEM YL 20) were found to be highest organic-acid producer. Taken all results together, the highest amounts of citric acid (66.2 g/L for TEM YL 3, 50.0 g/L for TEM YL 20) were observed in the production medium containing sunflower oil.

Conclusion: Citric acid consumption, and thus, the need for it are constantly rising in our country, which imports citric acid. Therefore, in order to meet this need, further studies which will yield to the maximum citric acid production should be performed by utilizing waste carbon sources and by using new low-cost but high citric acid-producing strains.

Key Words: Organic acid production, citric acid, isocitric acid, *Yarrowia lipolytica*.

Conflict of Interest: Authors do not have any conflict of interest.

ÖZET

Amaç: Bu çalışmada, *Yarrowia lipolytica* suşları tarafından sitrik ve izositrik asit üretimi üzerine çeşitli karbon kaynaklarının etkisinin saptanması amaçlanmıştır.

Metod: İlk olarak çalışmamızda kullandığımız mayalar, organik asit üretimleri açısından tarama besiyerlerinde test edilmişlerdir. Daha sonra, bu maya strainleri tarafından üretilen sitrik ve izositrik asit üretimi üzerine farklı karbon kaynaklarının etkisi araştırılmıştır. Sitrik ve izositrik asit üretim miktarları enzimatik test kitleri kullanılarak saptanmıştır.

Bulgular: Bu çalışmada, organik asit üretimi açısından 22 Y. lipolytica suşu incelenmiştir. Bu 22 maya suşu arasından iki suşun (TEM YL 3 ve TEM YL 20), en yüksek organik asit üretici maya suşları olduğu bulunmuştur. Bütün sonuçlar değerlendirildiğinde, en yüksek sitrik asit üretimi (TEM YL 3 için 66.2 g/L, TEM YL 20 için 50.0 g/L) ayçiçek yağı içeren üretim ortamında saptanmıştır.

Sonuç: Özellikle sitrik asit ithalatı yapılan ülkemizde, sitrik asit tüketimi ve ona olan ihtiyaç sürekli olarak artmaktadır. Atık karbon kaynaklarının değerlendirilmesi ve maliyeti düşük ancak verimi yüksek sitrik asit üreten yeni suşların kullanılarak, maksimum sitrik asit verimini sağlayacak daha ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Organik asit üretimi, sitrik asit, izositrik asit, *Yarrowia lipolytica*.

Çıkar Çatışması: Yazarların çıkar çatışması bulunmamaktadır.

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Introduction

Considering the rising market of biotechnological processes for the production of various chemicals, the increasing interest in microbially produced organic acids as a key group among the building-block chemicals is not surprising [1]. With a global annual production exceeding 1.7 million tons, citric acid (CA) ranks first among the organic acid synthesized by microorganisms [2] and its production volume is second only to industrial ethanol as a fermentation product [3]. Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid), is a natural component and common metabolite of plants and animals and is the most versatile and widely used organic acid in foods, beverages, and pharmaceuticals. Citric acid and its salts (primarily sodium and potassium) are used in many industrial applications: as a pH adjustment, buffering, chelating, and derivatization [4]. Similarly, the requirements for isocitric acid (ICA) and its salts are also increasing. Monopotassium salt of threo-D-(S)-(+)-isocitric acid is used in several biochemical experiments (measurement of aconitate hydratase, NAD-isocitrate dehydrogenase, NADP-isocitrate dehydrogenase, isocitrate lyase) [5]. Additionally, there is obviously a growing interest in ICA as a useful pharmaceutical additive, food and beverage additive, in cosmetics and detergents, as well as a new chiral building block for chemical syntheses [1].

Currently, it is most commonly used worldwide for the microbial production of citric acid [6]. Commercially, citric acid is produced from molasses, sucrose, or glucose syrups by *Aspergillus* niger [7]. However, this process is accompanied by some environmental problems such as the accumulation of solid (gypsum) and heavy-metal-loaded liquid waste (sewage and ferrocyanide sludge). Because of the above-mentioned ecological problems of *Aspergillus* process, it is of interest to develop alternative processes using yeast as organism-producing agent [8]. A bioprocess for citric acid production using *Yarrowia lipolytica* would have several additional advantages compared to the *Aspergillus* process, including a larger substrate variety (using raw materials such as hydrocarbons, carbohydrates, plant oils and glycerol), higher maximal product formation rate, higher substrate concentrations and yield, a lower sensitivity to low dissolved oxygen concentrations and heavy metals, simple process control, and waste and sewage minimization [1, 7-12]. A disadvantage of using *Y. lipolytica* wild-type strains for the commercial production of citric acid is the simultaneous secretion of isocitric acid, which has an inferior buffer capacity and chelating ability compared to citric acid. Additionally, the crystallization of citric acid during the purification process is disturbed by isocitric acid contaminations >5%. The citric acid/isocitric acid ratio formed by *Y. lipolytica* mainly depends on the substrate and the strain used. Grown on carbohydrates or glycerol, wild type strains secrete 10-12% of isocitric acid, and on plant oils or n-alkanes, even 35-45% isocitric acid [1, 2, 13].

The aim of this study was to determine the influence of various carbon sources like glucose, glycerol and sunflower oil with various concentrations on the production of maximum citric acid and minimum isocitric acid by *Yarrowia lipolytica* TEM YL 3 and *Y. lipolytica* TEM YL 20.

Material and Methods

Microorganisms and growth conditions

Domestic *Y. lipolytica* strains TEM YL 3 and TEM YL 20 had been isolated from cheese [14] and identified with phenotypic and genotypic approach [14, 15]. Initially yeast cells were grown aerobically at 27°C in a liquid YPD medium including (1.0 % yeast extract, 2.0 % peptone, and 2.0 % glucose).

Screening of total organic acid production

The medium for screening of organic acid was modified as described by Finogenova et al. [16]. The two *Y. lipolytica* strains were grown on a medium of the following composition (g/L): $(NH_4)_2SO_4$ 1.0; KH_2PO_4 1.0; K_2HPO_4 • $3H_2O$ 0.16; $MgSO_4$ • $7H_2O$ 0.70; NaCl 0.50; $Ca(NO_3)$ • $4H_2O$ 0.40; bromocresol green 0.40; glucose 20.0; agar 20.0 and 1 L of bidistilled water. The yeast growth, which showed yellow color zones around them, was selected as the organic acid producers.

Organic acid producing media and analytical methods

The citric acid and isocitric acid produting medium consisted of the following (g/L): $(NH_4)_2SO_4$ 1.0; KH_2PO_4 1.0; K_2HPO_4 • $3H_2O$ 0.16; $MgSO_4$ • $7H_2O$ 0.70; NaCl 0.50; $Ca(NO_3)$ • $4H_2O$ 0.40; H_3BO_3 0.5; $CuSO_4$ • $5H_2O$ 0.04; KI 0.1; $MnSO_4$ • $4H_2O$ 0.4; Na_2MoO_4 • $2H_2O$ 0.2; $ZnSO_4$ • $2H_2O$ 0.4; $FeCl_3$ • $6H_2O$ 0.6; thiamine 0.0003. Also the medium including 50 and 100 (g/L) concentrations of glucose, glycerol and sunflower oil as a carbon sources was investigated. Initial pH of this medium was adjusted to 5.5. For cultivation, the yeast was grown at 27°C for 408 hours and shaken at 150 rpm in 50 ml production medium in 250 ml flasks [17]. The cells were harvested by centrifugation at 13000 rpm for 5 min at 72 h, 120 h, 192 h, 216 h, 264 h, 336 h and 408 h in the given order. After harvesting, optical density of culture supernatants (OD660 nm) was measured as described by Mauersberger et al. [17]. In addition, the pH was determined for each flask at regular intervals. All experiments were performed at least in triplicate.

While citric acid was determined using K-CITR enzymatic test kit (Megazyme, Wicklow, Ireland), isocitric acid was determined using K-ISOC enzymatic test kit (Megazyme, Wicklow, Ireland) according to the manufacturer's recommendations [11].

Results

Screening of total organic acid production

Since zone diameters in 20–50 g/L glucose concentrations

gradually increase from 6 mm to 20 mm at the 24th and 48th hours of the production in the *Y. lipolytica* TEM YL 3 strain, and from 4 mm to 15 mm in the *Y. lipolytica* TEM YL 20 strain, these two strains were considered as the highest organic-acid producing yeast strains among the 22 *Y. lipolytica* strains screened in terms of organic acid production (Figure 1).

Determination of the amounts of CA and ICA with the enzymatic methods

While the yeast cells grown on 50 g/L and 100 g/L glucose, 50 g/L glycerol and 50 g/L sunflower oil transitioned to stationary phase at end of the 48th hour of incubation, the yeast cells grown on 100 g/L glycerol and sunflower oil transitioned to stationary phase at end of the 72nd hour. Therefore, in this study, CA and ICA values were measured at the 72nd, 120th, 192nd, 216th, 264th, 336th and 408th hours of the production.

It was determined that while the *Y. lipolytica* TEM YL 3 strain produced 1.01–18.24 g/L CA and 0.22–2.27 g/L ICA in the 50 g/L glucose concentration, the *Y. lipolytica* TEM YL 20 strain produced 0.33–17.36 g/L CA and 0.15–3.00 g/L ICA under the same conditions vary at over time (Table 1).

While 0.61–33.30 g/L CA and 0.21- 4.40 g/L ICA were produced by the *Y. lipolytica* TEM YL 3 strain in the 100 g/L glucose concentration, 1.26–36.30 g/L CA and 0.10–4.43 g/L ICA were produced by the *Y. lipolytica* TEM YL 20 strain under the same conditions (Table 2).

The *Y. lipolytica* TEM YL 3 strain produced 0.35–6.30 g/L CA and 0.042–1.09 g/L ICA in the 50 g/L glycerol concentration. However, the *Y. lipolytica* TEM YL 20 strain produced 0.28–6.74 g/L CA and 0.018–1.96 g/L ICA under the same conditions (Table 3).

In the 100 g/L glycerol concentration, 0.26–35.60 g/L CA and 0.00–4.50 g/L ICA were obtained with the *Y. lipolytica* TEM YL 3 strain, and 0.46–37.50 g/L CA and 0.07–4.86 g/L ICA were obtained with the *Y. lipolytica* TEM YL 20 strain (Table 4).

In the 50 g/L sunflower oil concentration, CA and ICA values for the *Y. lipolytica* TEM YL 3 strain were 2.16–22.30 g/L and 2.02–15.40 g/L respectively and for the *Y. lipolytica* TEM YL 20 strain, they were 0.17–31.41 g/L and ICA 0.97–17.51 g/L respectively (Table 5).

While in the 100 g/L initial sunflower oil concentration, 0.10–66.20 g/L CA and 0.04–46.8 g/L ICA were produced by the *Y. lipolytica* TEM YL 3 strain, 1.12–50 g/L CA and 0.74–53.76 ICA were produced by the *Y. lipolytica* TEM YL 20 strain under the same conditions (Table 6).

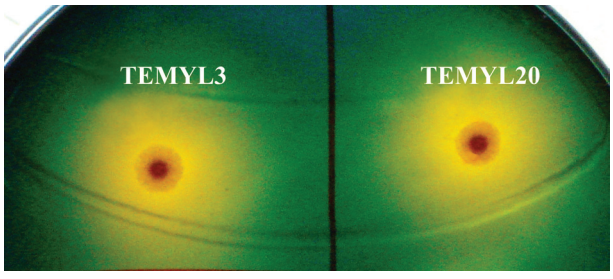

Figure 1. Screening of organic acid production from two yeast strains. Yellow zones around the yeast colonies are positive.

Discussion

In this study, the amounts of CA and ICA produced by the *Y. lipolytica* TEM YL 3 and TEM YL 20 strains were determined with the enzymatic kits. When the studies in the literature are reviewed, it is seen that in general, HPLC is used for determining the amount of organic acids [5, 6, 18, 19]. However, the amounts of CA and ICA cannot be determined separately with chromatographic methods; thus, the amount of ICA is determined with the enzymatic kit. Therefore, after considering the sources [11, 17, 20] stating that enzymatic method is more useful in the determination of the amounts of CA and ICA secreted into the production medium, we preferred this method in this study.

In our country, there are a very limited number of studies on the subject [21, 22]. Karasu [21] investigated various parameters (carbon source, pH, temperature, some minerals) that affect the production of citric acid with *Yarrowia lipolytica* and evaluated the citric acid production capacity of some of the endogenous yeast strains. It has been observed that studies in the world mostly concentrate on the improvement of current strains as higher productive agents through mutations or on the realization of higher citric acid production by supporting the production with different methods rather than on the screening of higher citric acid-producing strains [19, 23–25]. In Turkey, however, screening of high citric acid producing strains and the development of these strains still are of importance.

In recent years, glycerol has been successfully used as a carbon source in the production of citric acid with *Y. lipolytica* [6, 18, 19, 26, 27]. In the literature, it has been reported that in addition to studies in which pure glycerol is used as a substrate, there are other studies in which crude glycerol is used as a byproduct in the production of biodiesel and soap [2, 3, 19, 22, 28].

In the literature, the number of studies in which sunflower oil are used as a substrate is limited. When these studies are analyzed, in one of them conducted with *Yarrowia lipolytica* UOFS Y-1701 through the batch system, it was determined that citric acid production increased from 0.5 g/L to 7.18 g/L in the presence of 30 g/L sunflower oil and 1% acetate in the medium [29]. In another study, in the 10-liter fermenter continuous feed system, in the concentration of the VKM *Y. lipolytica* Y-2373 wild type strain and 20 g/L sunflower oil, the amounts of ICA and CA at pH 6.0 were determined as 70.0 g/L and 50.5 g/L respectively and at pH 4.5, the amounts of CA and ICA were 68 g/L and 55 g/L respectively. In the same study, while the

Table 1. CA and ICA values for the *Y. lipolytica* TEM YL 3 and *Y. lipolytica* TEM YL 20 strain at the initial 50 g/L glucose concentration

Time (hours)		72	120	168	216	264	336	408
Y. lipolytica TEMYL3	CA (g/L)	1.01	4.15	8.48	11.06	14.30	17	18.24
	ICA (g/L)	0.22	0.64	1.13	1.38	1.72	2.26	2.27
Y. lipolytica TEMYL20	CA (g/L)	0.33	4.22	9.22	13.20	16	17	17.36
	ICA (g/L)	0.15	0.82	1.66	2.20	2.92	2.98	3.00

Table 2. CA and ICA values for the *Y. lipolytica* TEM YL 3 and *Y. lipolytica* TEM YL 20 strain at the initial 100 g/L glucose concentration

Time (hours)		72	120	168	216	264	336	408
Y. lipolytica TEMYL3	CA (g/L)	0.69	3.20	8.40	12	18.17	29.03	33.30
	ICA (g/L)	0.21	0.64	1.03	1.41	1.80	2.67	4.40
Y. lipolytica TEMYL20	CA (g/L)	1.26	3.27	9.36	15.45	21.25	31.46	36.30
	ICA (g/L)	0.10	0.62	1.04	2.13	2.74	4.23	4.43

Table 3. CA and ICA values for the *Y. lipolytica* TEM YL 3 and *Y. lipolytica* TEM YL 20 strain at the initial 50 g/L glycerol concentration

Time (hours)		72	120	168	216	264	336	408
Y. lipolytica TEMYL3	CA (g/L)	0.042	0.57	1.05	1.06	1.08	1.09	1.09
	ICA (g/L)	0.042	0.57	1.05	1.06	1.08	1.09	1.09
Y. lipolytica TEMYL20	CA (g/L)	0.28	0.80	6.49	6.49	6.49	6.50	6.74
	ICA (g/L)	0.018	0.09	1.85	1.90	1.91	1.91	1.96

Table 4. CA and ICA values for the *Y. lipolytica* TEM YL 3 and *Y. lipolytica* TEM YL 20 strain at the initial 100 g/L glycerol concentration

Time (hours)		72	120	168	216	264	336	408
Y. lipolytica TEMYL3	CA (g/L)	0.26	0.70	4.01	9.30	18.12	32.01	35.60
	ICA (g/L)	0.00	0.13	0.90	1.73	2.96	3.93	4.50
Y. lipolytica TEMYL20	CA (g/L)	0.46	1.56	4.86	10.28	19.76	34.20	37.50
	ICA (g/L)	0.07	0.6	1.25	2.32	2.66	4.63	4.86

Table 5. CA and ICA values for the *Y. lipolytica* TEM YL 3 and *Y. lipolytica* TEM YL 20 strain at the initial 50 g/L sunflower oil concentration

Time (hours)		72	120	168	216	264	336	408
Y. lipolytica TEMYL3	CA (g/L)	2.16	5.41	10	16	19	20.24	22.30
	ICA (g/L)	2.02	3.53	10.05	12.43	14.9	15.22	15.40
Y. lipolytica TEMYL20	CA (g/L)	0.17	5	9.50	22.30	24.60	30.23	31.41
	ICA (g/L)	0.97	4.74	10.91	17.48	17.494	17.50	17.51

Table 6. CA and ICA values for the *Y. lipolytica* TEM YL 3 and *Y. lipolytica* TEM YL 20 strain at the initial 100 g/L sunflower oil concentration

Time (hours)		72	120	168	216	264	336	408
Y. lipolytica TEMYL3	CA (g/L)	0.10	8.25	11.66	31.41	33	53.56	66.20
	ICA (g/L)	0.04	11.10	22.32	33	32.35	44.57	46.8
Y. lipolytica TEMYL20	CA (g/L)	1.12	4	5.16	19.50	33.15	42	50
	ICA (g/L)	0.74	6.66	19.08	23.90	26.28	45.17	53.76

amount of CA reached 150.0 g/L at pH 4.5 with the mutant *Y. lipolytica* N15 strain, the amount of the ICA was insignificant [5].

In our study, when all the results related to glucose, glycerol, and sunflower oil were evaluated, the highest amounts of CA (66.2 g/L for TEM YL 3, 50.0 g/L for TEM YL 20) were determined in the production medium containing sunflower oil. However, the highest amounts of ICA (46.8 g/L for TEM YL 3, 53.76 g/L for TEM YL 20) were also obtained under the same conditions. It is thought that high amounts of citric acid can be attained in the production medium where sunflower oil is used if additional studies are conducted to reduce the amount of isocitric acid or mutant organisms are used in terms of aconitase enzyme. Therefore, when sunflower oil is used as a carbon source, the disadvantage resulting from the high amount of ICA production can be turned into an advantage. Due to above mentioned reasons; it is obvious that sunflower oil can be used as an alternative carbon source in the production of both CA and ICA.

In conclusion, CA consumption, and thus, the need for it are constantly rising in our country which imports CA. Therefore, in order to meet this need, further studies which will yield to the maximum CA production should be performed by utilizing waste carbon sources, by using new low-cost but high citric acid-producing strains, and if necessary, by reducing the amount of ICA production by mutating these strains. We also hope that this study and other similar studies will bring a new and useful perspective to the production of citric acid which has not been produced in our country since 1999.

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References

[1] Holz M, Förster A, Mauerberger S, Barth G. Aconitase overexpression changes the product ratio of citric acid production by *Yarrowia lipolytica*. Appl Microbiol Biotechnol 2009; 81: 1887-1096.

[2] Rywińska A, Juszcyk P, Wojtatowicz M, Rymowicz W. Chemostat study of citric acid production from glycerol by *Yarrowia lipolytica*. J Biotechnol 2011; 152: 54-57.

[3] Levinson WE, Kurtzman CP, Kuo TM. Characterization of *Yarrowia lipolytica* and related species for citric acid production from glycerol. Enzyme Microbiol Technol 2007; 41: 292-295.

[4] Crolla A, Kennedy KJ. Optimization of citric acid production from *Candida lipolytica* Y-1095 using n-paraffin. J Biotechnol 2001; 89: 27-40.

[5] Kamzolova SV, Finogenova TV, Morgunov IG. Microbiological production of citric and isocitric acids from sunflower oil. Food Technol Biotechnol 2008; 46(1):51-59.

[6] Rywińska A, Rymowicz W. High-yield production of citric acid by *Yarrowia lipolytica* on glycerol in repeated-batch bioreactors. J Ind Microbiol Biotechnol 2010; 37:431-435.

[7] Pazouki M, Felse PA, Sinha J, Panda T. Comparative studies on citric acid production by Aspergillus niger and *Candida lipolytica* using molasses and glucose. Bioprocess Eng 2000; 22:353-361.

[8] Förster A, Aurich A, Mauerberger S, Barth G. Citric acid production from sucrose using a recombinant strain of the yeast *Yarrowia lipolytica*. Appl Microbial Cell Physiol 2007; 75:1409-1417.

[9] Moeller L, Strehlitz B, Aurich A, Zehnsdorf A, Bley T. Optimization of citric acid production from glucose by *Yarrowia lipolytica*. Eng Life Science 2007; 5:504-511.

[10] Papanikolaou S, Chatzifragkou A, Fakas S, Galiotou-Panayotou M, Komaitis M, et al. Biosynthesis of lipids and organic acids by *Yarrowia lipolytica* strains cultivatedon glucose. Eur J Lipid Sci Technol 2009; 111:1221-1232.

[11] Makafer L, Mirbaheri M, Darvishi F, Nahvi I, Zarkesh-Esfahani H, et al. Isolation of lipase and citric acid producing yeasts from agro-industrial wastewater. New Biotechnol 2010; 27(4):337-340.

[12] Antonucci S, Bravi M, Bubbico R, Di Michele A, Verdone N. Selectivity in citric acid production by *Yarrowia lipolytica*. Enzyme Microbiol Technol 2001; 28:189-195.

[13] Förster A, Jacobs K, Juretzek T, Mauerberger S, Barth G. Overexpression of the ICL1 gene changes the product ratio of citric acid production by *Yarrowia lipolytica*. Appl Microbiol Biotechnol 2007; 77:861-869.

[14] Akpınar O, Ucar F, Yalcın HT. Screening and regulation of alkaline extracellular protease and ribonuclease production of *Yarrowia lipolytica* strains isolated and identified from different cheeses in Turkey. Annals of Microbiology 2011; 61:907–915.

[15] Akpınar O, Ucar F. Molecular characterization of *Yarrowia lipolytica* strains isolated from different environments and lipase profiling. Turk J Biol 2013; 37:249-258.

[16] Finogenova TV, Shishkanova NV, Ermakova T, Kataeva IA. Properties of *Candida lipolytica* mutants with the modified glyoxylate cycle and their ability to produce citric acid and icocitric acid. Appl Microbiol Biothecnol 1986; 23:378-383.

[17] Mauersberger S, Krause K, Barth G. Induction of Citric Acid/ Isocitric Acid and α-Ketoglutaric Acid Production in the Yeast *Yarrowia lipolytica*. In (Eds. Wolf K, Breunig K, Barth G, Anastassiadis S and Rehm JH) Non-Conventional Yeast in Genetics, Biochemistry and Biotechnology 2003; pp. 395-400, Springer-Verlag Berlin Heidelberg, Germany.

[18] Papanikolaou S, Muniglia L, Chevalot I, Aggelis G, and Marc I. *Yarrowia lipolytica* as a potential producer of citric acid from raw glycerol. J Appl Microbiol 2002; 92:737-744.

[19] Rywinska A, Rymowicz W, Marcinkiewicz M. Valorization of raw glycerol for citric acid production by *Yarrowia lipolytica* yeast, Electr J Biotechnol 2010; 13(4):1-9.

[20] Mirbagheri M, Iraj N, Giti E, Mafakher L, Darvishi F. Taxonomic characterization and potential biotechnological applications of *Yarrowia lipolytica* isolated from meat and meat products, Jundishapur J Microbiol 2012; 1:346-351.

[21] Karasu, YS. *Yarrowia lipolytica* ile sitrik asit üretimine etki eden parametrelerin incelenmesi ve bazı endojen maya suşlarinin sitrik asit üretim kapasitelerinin değerlendirilmesi, Doktora Tezi, Hacettepe Üniversitesi 2007; 273s.

[22] Karasu YS, Bozdemir MT, Özbağ ZY. Comparative study of the effects of glycerol and mannitol on citric acid production by two *Yarrowia lipolytica* strains. Rom Biotech Lett 2009; 14(6):4870-4881.

[23] Wojtatowicz M, Rymowicz W, Kautola H. Comparison of different strains of the yeast *Yarrowia Lipolytica* for citric acid production from glucose hydrol. Appl Biochem Biotechnol 1991; 31:165-174.

[24] Mirbagheri M, Iraj N, Giti E, Darvishi F. Enhanced production of citric acid in *Yarrowia lipolytica* by Triton X-100. Appl Biochem Biotechnol 2011; 165:1068-1074.

[25] Kamzolova VS, Fatykhova RA, Dedyukhina GE, Anastassiadis GS, Golovchenko PN et al. Citric acid production by yeast grown on glycerol-containing waste from biodiesel industry, Food Technol Biotechnol 2011; 49:65–74.

[26] Imandi SB, Bandaru VVR, Somalanka SR, Bandaru SR, Garapati HR. Application of statistical experimental designs for the optimization of medium constituents for the production of citric acid from pineapple waste, Bioresour Technol 2008; 99:4445-4450.

[27] Rymowicz W, Fatykhova AR, Kamzolova SV, Rywinska A, Morgunov IG. Citric acid production from glycerol containing waste of biodiesel industry by *Yarrowia lipolytica* in batch, repeated batch, and cell recycle regimes. Appl Microbiol Biotechnol 2010; 87:971–979.

[28] Papanikolaou S, Aggelis G. Biotechnological valorization of biodiesel derived glycerol waste through production of single cell oil and citric acid by *Yarrowia lipolytica*. Lipid Technol 2009; 21:83-87.

[29] Venter T, Kock FLJ, Botes JP, Smit SM, Hugo A, et al. Acetate enhances citric acid production by *Yarrowia lipolytica* when grown on sunflower oil. System Appl Microbiol 2004; 27:135–138.