

# Coproduction of Fructose and Ethanol from Dates by *S. cerevisiae* ATCC 36859

M. A. Zeinelabdeen, A. E. Abasaeed, M. H. Gaily, A. K. Sulieman, M. D. Putra

**Abstract**—Coproduction of fructose and ethanol from dates extract by a glucose-selective *S. cerevisiae* ATCC 36859 strain has been studied. Various initial sugar concentrations (i.e., 131.4, 315.3, 408.2, and 500.0 g/l) have been tested. The fermentation experiments were performed in a water shaker bath at 30°C and 120 rpm. The results showed that highest yields of fructose (95.0%) and ethanol (72.8%) were achieved for the 131.4 g/l concentration. Increasing the initial concentration to 315.3 g/l resulted in lower yields of fructose (82.2%) and ethanol (61.0%). However, further increase to 408.2 g/l increased the fructose yield (97.5%) at the expense of ethanol yield (42.0%) due to probable substrate inhibitions that resulted in lower glucose conversion. At 500 g initial sugar/l the growth rate of ATCC 36859 was highly inhibited.

**Keywords**—Dates, ethanol, fructose, fermentation, *S. cerevisiae*.

## I. INTRODUCTION

DATE PALM trees are grown in many parts of the world, including the Middle East, North Africa and South Asia [1], [2]. About 7.9 million tons of dates were produced in 2010 world-wide [3]. Egypt, Kingdom of Saudi Arabia and Iran are the top three dates producers in the world [4]. In 2010, production of date fruits in the Kingdom of Saudi Arabia exceeded one million tons; about 14% of world production [3]-[5]; however, dates export was less than 50000 tons [4]. Although the per capita consumption of dates in Saudi Arabia was the highest compared to the world, still large amounts of low quality dates (e.g., Ruzaiz and Sifri) are unconsumed [6], [7]. These huge amounts of unused dates could be utilized for the production of fructose, ethanol, acetic acid, lactic acid and other valuable products. Dates syrups are very rich in monosaccharides (fructose and glucose), minerals, vitamin and a small amount of sucrose [8]. Fructose and glucose constitute over 75% of the dry weight of pitted dates. Fructose is the sweetest natural sugar; 30% sweeter than sucrose, while glucose has only 70% of the sweetness of sucrose [9]. In its monomeric form, fructose is present in fruits such as raisins, apples, grapes and dates and polymeric form in fructan-rich plants such as dahlia, chicory and Jerusalem artichoke [10]. It is used in the food, beverage and confectionery industries as a natural sweetener [11]. Furthermore, fructose or high fructose syrups (HFS) have been widely used in diabetic and baby food

[10]. Commercial production of fructose was achieved mainly through saccharification of starch followed by glucose isomerization. Due to equilibrium limitations, such a process produces syrups with about 42% fructose that could be enhanced to 90% fructose though complex and rather expensive multistage chromatographic separation techniques [12]. The two fructose syrups (42% and 90%) are usually blended to produce the commonly marketed 55% HFS.

More recently, a process that uses ionic liquids to separate fructose and glucose from their mixtures has been patented [13]. Production of fructose and ethanol from synthetic sucrose media through selective fermentation by *S. cerevisiae* ATCC 36859 or ATCC 36859 has been also reported [14], [15]. It has been reported that carbohydrate or ethanol concentrations greater than 488 g/l or 62 g/l, respectively would inhibit the growth of ATCC 36859 [15]. *S. cerevisiae* ATCC 36858 has been employed for the production of fructose and ethanol from dates extract [7], [16]; while wild strain of *S. cerevisiae* were used for the production of ethanol from dates extract [17]. On the other hand, production of ethanol from wasted dates would provide a clean and sustainable alternative source of energy [18]-[20].

In this study, the coproduction of fructose and ethanol from dates' syrups through selective fermentation of the glucose component by *S. cerevisiae* ATCC 36859 will be investigated. The main focus of this study will be on the effect of initial substrate concentration on yields of fructose and ethanol. The results will be compared to a commercial wild strain of *S. cerevisiae*.

## II. MATERIALS AND METHODS

Syrups with various initial sugar concentrations (ISC) were prepared from pitted dates (Ruzaiz variety) dips (a locally common name for a concentrated dates' syrup) in 1000ml conical flasks using deionized water. The substrates were sterilized for 15 minutes at 121°C.

The glucose selective yeast, *S. cerevisiae* ATCC 36859 that was obtained from American Type Culture Collection, USA in a pellet form, was activated and transferred according to the standard procedure of the ATCC. The strain was inoculated in a Yeast Broth that contained 10.0g glucose, 30.0g yeast extract, 3.50g peptone, 2.0g  $\text{KH}_2\text{PO}_4$ , 1.0g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 1.0g  $(\text{NH}_4)_2\text{SO}_4$  dissolved in 1.0 liter deionized water. The broth was sterilized in sterilizer for 15 minutes at 121°C.

Two grams of the wild yeast were added to 200ml of the above sterilized Yeast Broth and was propagated in a temperature controlled water bath shaker at 30°C and 120rpm for 2 days.

M. A. Zeinelabdeen, M. H. Gaily, A. K. Sulieman, and M. D. Putra are with the Chemical Engineering Department, King Saud University, PO Box 800, Riyadh 11421, Saudi Arabia (e-mail: mazaam@yahoo.com, mgaily@ksu.edu.sa, ashraf08@ksu.edu.sa, mputra@ksu.edu.sa).

A. E. Abasaeed (corresponding author) is with the Chemical Engineering Department, King Saud University, PO Box 800, Riyadh 11421, Saudi Arabia (abasaeed@ksu.edu.sa).

The volume ratio of yeast medium to the substrate was 3:17 in the sterilized 500ml conical flasks (working volume 100 ml). After inoculating with the yeast broth, the final sugar concentrations were 131.4, 315.3, 408.2, and 500.0g/l. The fermentation experiments were conducted in a controlled temperature water bath shaker at 30°C and 120rpm without controlling pH. Samples were taken using a sterilized disposable pipette. A portion of the sample was centrifuged at 15000rpm for 1min to separate the cells from the solution and then the clear solution was transferred to a small vial for sugars and ethanol analysis. The other portion was transferred to sterile test tubes for cell count determination.

The sugars (glucose, fructose and sucrose) and ethanol were determined quantitatively using high performance liquid chromatography (HPLC; Agilent 1200 Infinitely series) equipped with an RID detector and Aminex® column. Cell count was determined by NucleoCounter® YC-100TM system cell counter.

### III. RESULTS AND DISCUSSION

The results of the fermentation are presented in Figs. 1-4. The total in the figures indicates the total sugar concentrations (i.e., the summation of glucose, fructose and sucrose at any time). The fructose fraction (FF) is defined as the amount of fructose at any time to the amount of total sugars. The fructose loss (FL) is the amount of fructose that had been fermented to the initial amount of fructose. The ethanol yield (EY) is the amount of ethanol that had been produced to the amount of theoretical ethanol that would have been produced based on consumption of total sugars. All FF, FL and EY are given as percentages in the figures.

Fig. 1 shows the kinetic profiles of the various sugars and ethanol resulting from the fermentation of the dates' syrup having an initial concentration of 131.7g/l by the commercial *S. cerevisiae* strain. As evident from Fig. 1 (a), both glucose and fructose have been fermented by the commercial strain; though the initial consumption of glucose was much higher than that of fructose. After 24h of fermentation time, all glucose and fructose were consumed. The fructose fraction (FF) was zero and of course the fructose loss (FL) was 100% as shown in Fig. 1 (b). The ethanol yield (EY) after complete fermentation was 77.6% of its theoretical value. These results indicate clearly the nonselective nature of this strain.

The kinetic profiles of the sugars and ethanol are shown in Fig. 2 (a), whereas the fructose fraction, fructose loss and ethanol yields are shown in Fig. 2 (b) for the fermentation of dates' syrup by using *S. cerevisiae* ATCC 36859. The initial sugar concentration was 131.4 g/l. The high selectivity of ATCC 36859 towards glucose is evident from Fig. 2 (a). While glucose was completely consumed, fructose was almost unfermented. The fructose fraction was 93.4% with minimum fructose loss (5.0%). These fructose losses were much less than those reported in literature [15] where ATCC 36859 was used to ferment Jerusalem artichoke extract (20%). The ethanol yield was less than that obtained with the commercial strain.

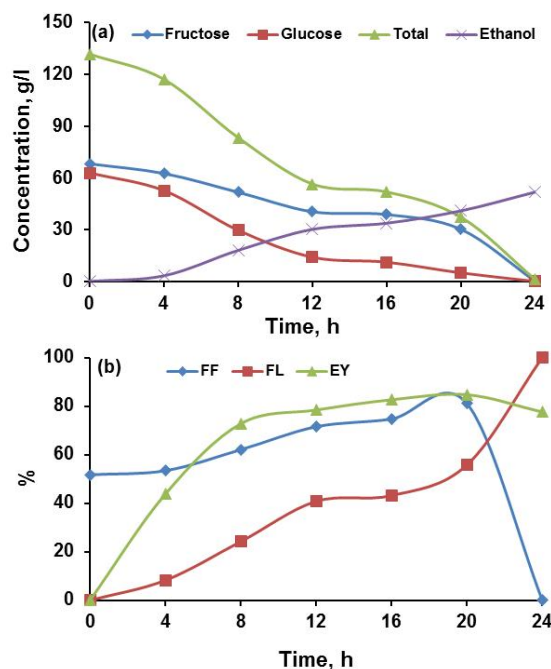


Fig. 1 Fermentation of dates extract by wild *S. cerevisiae* at 30°C and initial sugar concentration 131.7g/l. (a) concentration profiles of sugars and ethanol; (b) fructose fraction (FF), fructose loss (FL) and ethanol yield (EY) profiles

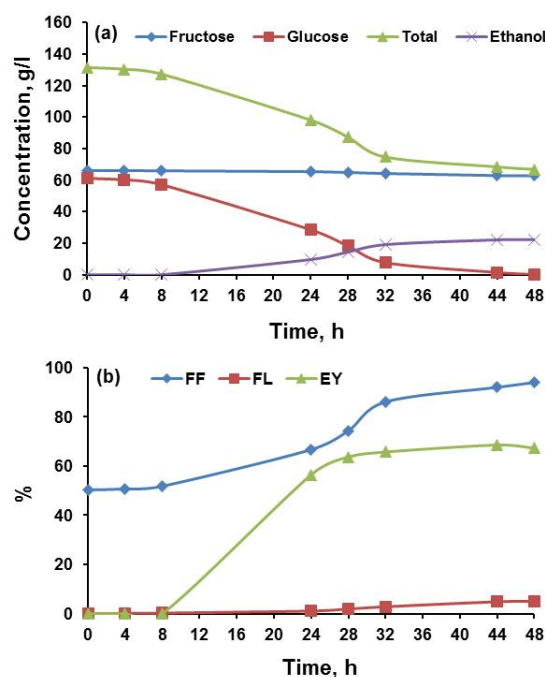


Fig. 2 Fermentation of dates extract by *S. cerevisiae* ATCC 36859 at 30°C and initial sugar concentration 131.4 g/l. (a) concentration profiles of sugars and ethanol; (b) fructose fraction (FF), fructose loss (FL) and ethanol yield (EY) profiles

Increasing the initial sugar concentration to 315.3 g/l resulted in the kinetic profiles shown in Fig. 3 (a) for the selective fermentation using ATCC 36859. As shown in Fig. 3 (b), the fructose fraction was still high; however, fructose losses increased to 17.2% which close to the 20% fructose loss that was obtained when the same strain was used to ferment a mixture of glucose and fructose [15]. Also, the ethanol yield dropped to 41.1% compared to that obtained at 131.4 g ISC/l. at 99% fermentation of glucose (about 74h), fructose losses were less than 12%.

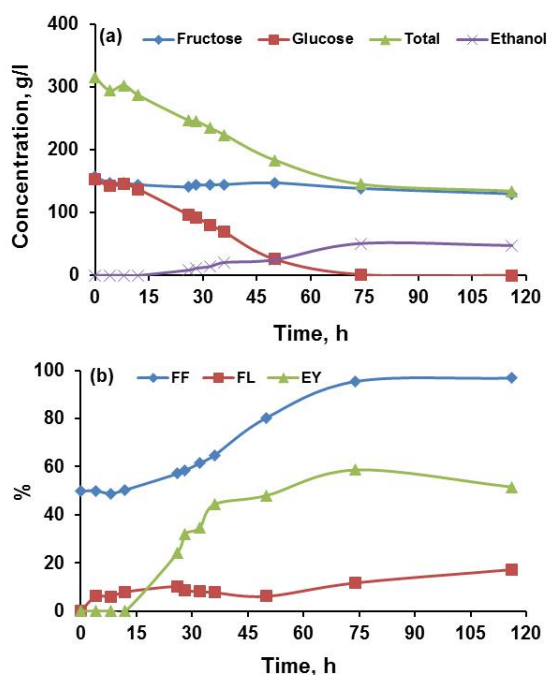


Fig. 3 Fermentation of dates extract by *S. cerevisiae* ATCC 36859 at 30°C and initial sugar concentration 315.3g/l. (a) concentration profiles of sugars and ethanol; (b) fructose fraction (FF), fructose loss (FL) and ethanol yield (EY)

Further increase of initial sugar concentration to 408.2g/l elongated the fermentation and only 2/3 of the glucose that was initially present was fermented after 280h as shown in Fig. 4 (a). This confirms the findings of Koren and Duvnjak [15] that higher sugar or ethanol concentration will inhibit the growth of ATCC 36859. Although, fructose losses were low, the fructose fraction was also low due to unconverted glucose as shown in Fig. 4 (b). Table I shows percentage fructose fraction (FF), fructose losses (FL), ethanol yield (EY) and fermented glucose (FG) for the two *S. cerevisiae* strains at various initial sugar concentrations (ISC). The extreme inhibition to yeast growth [15] is quite evident from the results at 500 g ISC/l given in the table.

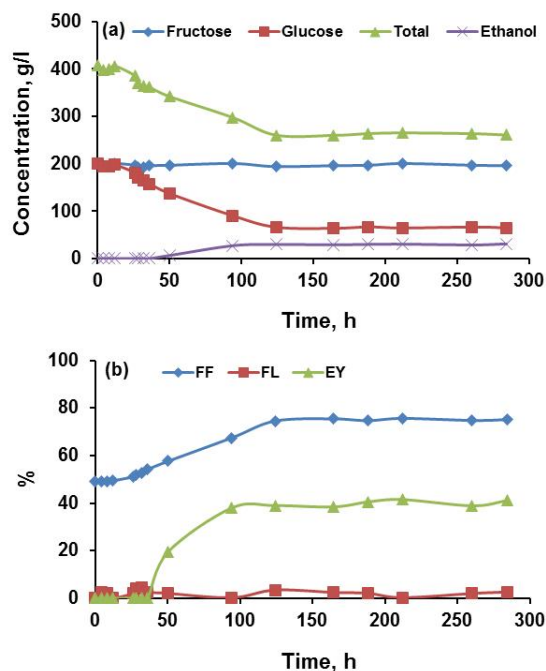


Fig. 4 Fermentation of dates extract by *S. cerevisiae* ATCC 36859 at 30°C and initial sugar concentration 408.2 g/l. (a) concentration profiles of sugars and ethanol; (b) fructose fraction (FF), fructose loss (FL) and ethanol yield (EY) profiles

TABLE I  
COMPARISON OF FERMENTATIONS AT VARIOUS INITIAL SUGAR CONCENTRATIONS (ISC)

Strain	ISC	FF	FL	EY	FG
Wild	131.7	0.0	100.0	77.6	100.0
ATCC 36859	131.4	93.9	5.0	67.2	100.0
ATCC 36859	315.3	96.8	17.2	51.6	100.0
ATCC 36859	408.2	75.1	2.7	41.1	67.5
ATCC 36859	500.0	51.6	0.3	0	6.0

#### IV. CONCLUSION

Dates provide sustainable substrates for the coproduction of fructose and ethanol. Compared to the commonly marketed 55% fructose syrups, selective fermentation of dates extract provides syrups having more than 90% fructose. ATCC 36859 is very effective in selective fermentation. Compared to wild *S. cerevisiae*, the mutant strain ATCC 36859 showed very little fructose losses at complete fermentation of glucose.

#### ACKNOWLEDGMENT

The authors extend their appreciation to the National Science, Technology and Innovation Plan (NSTIP) at King Saud University for their generous support and funding of this study as a part of project # 08-ADV391-02.

#### REFERENCES

- [1] K. Selim, M. AbdeLe-Bary, and O. Ismael, "Effect of irradiation and heat treatments on the quality characteristics of siwi date fruit (*Phoenix dactylifera*)," *AgroLife Scientific J.*, vol. 1, pp. 103-111, 2012.

- [2] A. A. Al-Qarawi, B. H. Ali, S. A. Al-Mougy, and H. M. Mousa, "Gastrointestinal transit in mice treated with various extracts of date (*Phoenix dactylifera* L.)," *Food Chem Toxicol.*, vol. 41, no. 1, pp. 37-39, Jan. 2003.
- [3] S. M. Jain, "In vitro mutagenesis for improving date palm (*Phoenix dactylifera* L.)," *Emir. J. Food Agric.*, vol. 24, no. 5, pp. 400-407, 2012.
- [4] F. Al-shreed, et al, "A study on the export of Saudi Arabian dates in the global markets," *JDAE.*, vol. 4, no. 9, pp. 268-274, May. 2012.
- [5] N. A. Al-Wusaibai, A. Ben Abdallah, M. S. Al-Husainai, H. Al-Salman and M. Elballaj, "A comparative study between mechanical and manual pollination in two premier Saudi Arabian date palm cultivars," *INDJST.*, vol. 5, no. 4, pp. 2487-2490, Apr. 2012.
- [6] S. Moshaf, Z. H. Esfahani, and M. H. Aziz, "Optimization of conditions for Xanthan gum production from waste date in submerged fermentation," *WASET.*, vol. 57, pp. 521-524, 2011.
- [7] M. D. Putra, et al, "Production of fructose from highly concentrated dates extract (Periodical style—Accepted for publication)," *Biotech. Lett.*, to be published.
- [8] A. Hasnaoui, et al, "Chemical composition and microbial quality of dates grown in Figuig oasis of Morocco," *IJAB.*, vol. 12, no.2, pp. 311-314, Mar. 2010.
- [9] A. K. Mostafazadeh, M. Sarshar, Sh. Javadian, M. R. Zarefard, and Z. A. Haghighi, "Separation of fructose and glucose from date syrup using resin chromatographic method: experimental data and mathematical modeling," *Sep Purif Technol.*, vol. 79, no. 1, pp. 72-78, May. 2011.
- [10] D. M. Lima, et al, "Fructose syrup: a biotechnology asset," *Food Technol. Biotechnol.*, vol. 49, no. 4, pp. 424-434, Oct. 2011.
- [11] S. E. Fleming, and J. W. Grootwasink, "Preparation of high-fructose syrup from the tubers of the Jerusalem artichoke (*Helianthus tuberosus* L.)," *Crit Rev Food Sc.*, vol. 12, no. 1, pp. 1-28, Nov. 1979.
- [12] L. M. Hanover, and J. S. White, "Manufacturing, composition, and applications of fructose 1'2," *AJCN.*, vol. 58, no. 5, pp. 724S-732S, Dec. 1993.
- [13] I. M. Al-Nashef, M. H. Gaily, S. M. Al-Zahrani, and A. E. Abasaheed, "Method for separating fructose and glucose," *US 7942972 B2*, May. 2011.
- [14] H. Atiyeh, and Z. Duvnjak, "Study of the production of fructose and ethanol from sucrose media by *Saccharomyces cerevisiae*," *Appl Microbiol Biotechnol.*, vol. 57, no. 3, pp. 407-411, Oct. 2001.
- [15] D. W. Koren, and Z. Duvnjak, "Fed-batch production of concentrated fructose syrup and ethanol using *Saccharomyces cerevisiae* ATCC 36859," *Acta Biotechnol.*, vol. 12, no. 6, pp. 489-496, 1992.
- [16] M. H. Gaily, et al, "The effect of activation time on the production of fructose and bioethanol from date extract," *AJB.*, vol. 11, no. 33, pp. 8212-8217, Apr. 2012.
- [17] A. K. Suliman, M. H. Gaily, M. A. Zeinelabdeen, M. D. Putra, and A. E. Abasaheed, "Production of bioethanol fuel from low-grade-date extract," *IJCEA.*, vol. 4, no. 3, pp. 140-143, Jun. 2013.
- [18] S. Govindaswamy, and L. M. Vane, "Kinetics of growth and ethanol production on different carbon substrates using genetically engineered xylose-fermenting yeast," *Bioresource Technol.*, vol. 98, no. 3, pp. 677-685, Feb. 2007.
- [19] M. Balat, H. Balat, and C. Oz, "Progress in bioethanol processing," *Prog Energ Combust.*, vol. 34, no. 5, pp. 551-573, Oct. 2008.
- [20] M. E. E. Abashar, and S. S. E. E. Elnashaie, "Dynamic and chaotic behavior of periodically forced fermentors for bioethanol production," *Chem Eng Sci.*, vol. 65, no. 16, pp. 4894-4905, Aug. 2010.



**Mohamed A. Zeinelabdeen:** Born in Sudan. He got his BSc in Chemical Engineering from Karary Academy of Technology in Sudan (now Karary University) 2003. Worked at Karary University as a teaching assistant from 2003-2010. Eng. Zeinelabdeen Joined King Saud University (Kingdom of Saudi Arabia) as a researcher since 2010. He is currently pursuing an MS degree in Biochemical Engineering at the Chemical Engineering department, King Saud University.



**Ahmed E. Abasaheed:** Born June, 28, 1954 in Sudan where he got his BS from University of Khartoum, 1978. He got his MS from University of Florida, USA, 1982 and PhD from Auburn University, USA, 1987. All degrees are in Chemical Engineering with minors in Mathematics (MS) and Environmental Engineering (PhD). He currently serves as a Professor of Chemical

Engineering at King Saud University. He previously worked as an Associate Research Professor at the Renewable Energy research Institute in Sudan. His current research interest include selective fermentation, dry reforming of methane and oxidative dehydrogenation of alkanes. Professor Abasaheed has authored over 140 publications, 3 books and 5 US and EU Patents. He has received many awards for his academic and research achievements.



**Mohamed H. Gaily:** Born in Sudan, on 21<sup>st</sup> November, 1967. He has obtained his Ph. D. degree in Chemical Engineering in the year 2010 from University of Khartoum, Sudan. M. Sc. Degree in Agricultural Process Engineering in the year 1999 from Universiti Putra Malaysia, Malaysia and B. Sc. in Chemical Engineering in 1992 from University of Khartoum, Sudan. He is working as a researcher in Chemical Engineering Department, King Saud University in Saudi Arabia. He also worked as a lecturer at University of Khartoum. He has published several research articles in the field of biochemical engineering. His research interests include selective fermentation and production of biofuels. He registered, with others, a US patent # 7942972 describing a method of separating fructose and glucose. Dr. Gaily is a member for Saudi Chemical Engineering Society and Saudi Chemical Society. He worked for academic accreditation committee and strategic plan committee for Engineering College at King Saud University, Saudi Arabia.



**Ashraf K. Suliman:** Born in Sudan in 1975. He got his BS and MS from University of Khartoum in 1999 and 2003 respectively. His BS is in Chemical Engineering and his MS in computational Mathematics and its application in Chemical Engineering. He currently serves as a lecturer in the Chemical Engineering Department His current research interests include selective fermentation and mathematical modeling of reactive systems.



**Meilana Dharma Putra:** Born 1982 in Indonesia. He obtained a BSc degree from GadjahMada University in 2005, Indonesia. He got his MSc from King Saud University, KSA in 2010 and is now pursuing a PhD degree in the same University. All degrees are in Chemical Engineering. Since 2006, he has been an official lecturer at LambungMangkurat University, the oldest University in Borneo Island. His research interests include selective fermentation, oxidative dehydrogenation of alkanes and recycling of used lubricating oil. He has contributed over 9 publications.