

## Selective fermentation of pitted dates by *S. cerevisiae* for the production of concentrated fructose syrups and ethanol

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**Abstract.** About half of worldwide production of dates is unconsumed. Dates contain over 75 % reduced sugars (mostly glucose and fructose with nearly equal amount). Compared to the commercial *Saccharomyces cerevisiae* wild strain, the strains ATCC 36858 and 36859 could produce high concentration fructose syrups. The fructose fractions obtained were 95.9 and 97.4% for ATCC 36858 and 86.5 and 91.4% for ATCC 36859 at 30 and 33°C, respectively. Fructose yields higher than 90% were obtained using ATCC 36858 compared to those obtained using ATCC 36859 which were 87.3 and 66.1% at 30 and 33°C, respectively. The ethanol yield using ATCC 36858 was higher than that using ATCC 36859 by 16 and 9% at 30 and 33°C, respectively. Through this finding, the production of fructose and ethanol from date extract is a promising process. Moreover, the fructose fractions obtained here (about 90%) are much higher than those obtained with the commercial process, i.e. 55 % fructose syrups.

### 1. Introduction

Date palm trees are one of the most important agricultures grown in many regions of the world, i.e., Arabian Peninsula, North Africa and Middle East. Countries such as Australia, India, Pakistan, Southern Africa, Mexico, South America, and United States have started growing date trees [1]. The Kingdom of Saudi Arabia produced over million tons of dates in 2010 [2] compared to about 8 million tons of dates was worldwide produced in 2010 [3]. Though date is one of the staple foods, about half of dates are still unconsumed [4]. Date syrups are very rich in sugars (fructose and glucose), a small amount of sucrose, minerals and vitamin [5]. Pitted date contains over 75 % sugars of its dry weight. Half of date sugar is fructose. Fructose is the sweetest natural sugar that is 30 % sweeter than sucrose [6]; thus it is used as sweetener (nowadays marketed as high fructose corn syrup) and also used in the food, confectionery and beverage industry [6].

Commercial fructose is produced via enzymatic saccharification of starch to glucose and a subsequent enzymatic isomerization of the glucose. About 42 % fructose syrup is obtained due to equilibrium limitations. Because glucose and fructose are isomer, the separation of them is difficult. To concentrate the fructose fraction in the syrup, costly multistage chromatograph separation is employed to achieve 90 % fructose fraction [7]. The commonly marketed 55 % fructose syrups are obtained by blending 42 and 90% fructose syrups. Recently, a method using ionic liquids to separate fructose and glucose has been invented [8]. On the other hand, the spoilage dates have great industrial potential when used to produce fructose. Moreover, the ethanol produced from agricultural waste has



been suggested to be a promising alternative in the fuel market [9,10]; it will therefore be a green solution in the sustainable energy [11].

A selective fermentation process has been suggested to produce fructose and ethanol. These productions has been also studied either using sucrose media or artichoke juice by *S. cerevisiae* ATCC 36858 or ATCC 36859 [12,13]. More recently, *S. cerevisiae* ATCC 36858 has been utilized for the production of fructose and ethanol from dates extract [14,15].

Underutilized dates provide a suitable opportunity for the production of fructose and ethanol. In this paper, the performance of *S. cerevisiae* ATCC 36858 and 36859 on date extract media will be studied. The commercial wild strain of *S. cerevisiae* to produce ethanol will be also compared to the ATCC strains. The purpose of this study is to produce the fructose syrups from date extract with high fructose fraction without significant fructose losses.

## 2. Materials and methods

### 2.1. Substrate extraction and preparation

A low quality variety of dates, locally called Ruzai, were used in this investigation. The substrate was prepared by using 1 L of deionized water to extract the sugar content from 400 grams of pitted date in a temperature controlled shaker water bath at 40°C for 2 hours according to the procedure developed by Gaily et al. [16]. The extract was twice-filtered to remove fibers. Sugar content of dates was in the range 75-80% (w/w) and was composed mainly of equal amounts of fructose and glucoses. The initial total sugar concentration was adjusted as required by adding deionized water. The substrate was sterilized in an autoclave sterilizer at 121°C for 15 minutes.

### 2.2. Yeast propagation and media

In this contribution, three strains of *Saccharomyces cerevisiae* yeast were used: a commercial over-the-shelf strain that is used in bakeries (hereafter called Wild strain) and two glucose selective strains supplied by the American Type Culture Collection (ATCC, Manassas, VA, USA ), namely: ATCC 36858 and ATCC 36859. The inoculation medium for the Wild *S. cerevisiae* strain was composed of 3g yeast extract, 3g malt extract, 5g peptone and 10g dextrose dissolved in 1L of deionized water. 200 ml of the inoculum medium were transferred to a 500 ml conical flask and autoclaved at 121°C for 15 minutes before adding 2g of the Wild yeast to it. The content was allowed to propagate for two days in a temperature controlled water bath shaker at 120 rpm and 30°C. The two selective strains (ATCC 36858 and ATCC 36859) were removed from their ampoules and revived according to ATCC procedure and were allowed to grow in a suitable liquid medium at 30°C. The composition of the inoculum medium for ATCC 36858 was similar to that of the Wild strain. The composition of the medium for ATCC 36859 was composed of 10g glucose, 30g yeast extract, 3.5g peptone, 2g  $\text{KH}_2\text{PO}_4$ , 1 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 1g  $(\text{NH}_4)_2\text{SO}_4$  dissolved in 1L of deionized water. The inoculum media were then transferred to 500 ml conical flasks and autoclaved at 121 °C for 15 minutes. The selective strain was then added to its sterilized inoculum medium. The mixture was propagated in a temperature controlled shaker water bath at 120 rpm and 30°C for two days to be ready for fermentation experiments.

### 2.3. Fermentation experiment

The fermentation experiments were conducted in a 1 liter batch fermentor (Lambda Minifor Laboratory Fermentor, Sihlbruggstrasse, Switzerland) using the three strains (Wild, ATCC 36858 and ATCC 36859). The fermentations were performed at a constant agitation of 120 rpm and two temperatures i.e., 30 and 33°C. In all fermentation experiments, 400 ml of the mixture of the substrate (340 ml) and the inoculation medium containing yeast (60 ml) was fed to the fermentor. The substrate was fed initially into the fermentor vessel and the whole system was sterilized for 15 minutes at 121°C before adding medium containing yeast. The fermentor was then turned on and the temperature, agitation and pH were monitored during fermentation. Samples were aseptically collected for analysis.

## 2.4. Sample analysis

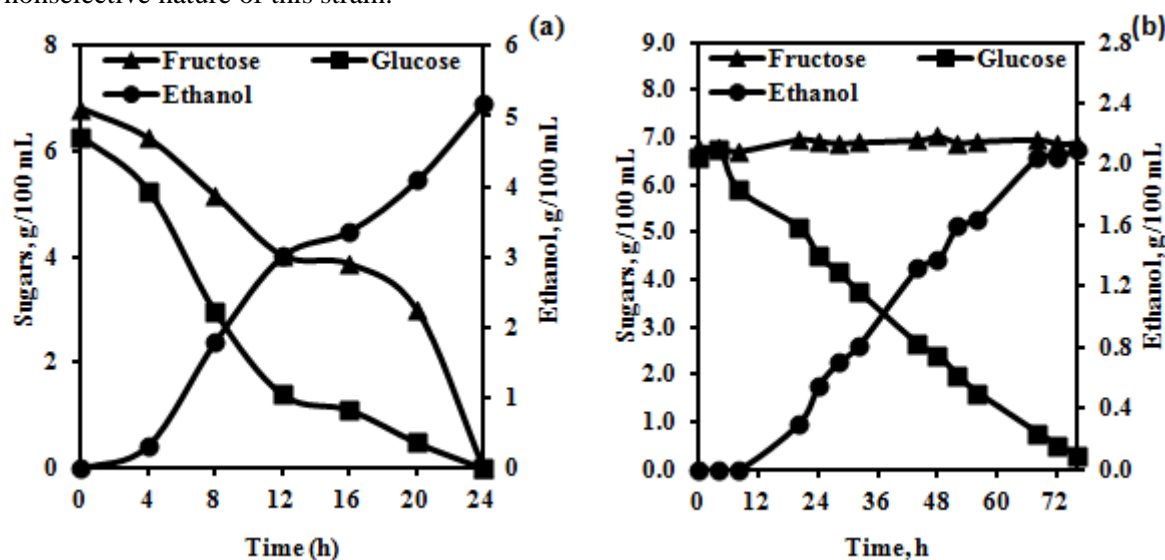
Samples were analyzed using High Performance Liquid Chromatography (HPLC). Samples were micro-centrifuged before injection to the HPLC to separate cells and fibers. The HPLC, (Agilent 1200 Infinitely series) was equipped with RID detector. Aminex® column (Cat. #125-0115; 150 x 7.8mm from BIO-RAD) was used for detection of sugars (i.e., fructose, glucose and sucrose) and ethanol. The column was maintained at 40°C. 1 mM sulfuric acid solution was used as a mobile phase at a flow rate of 0.8 ml/min.

## 3. Results and discussion

Figure 1 show comparison between selective fermentation and non-selective fermentation of date extract at 30°C as presented in section 3.1. The comparison between *S. cerevisiae* strain ATCC 36858 and ATCC 36859 are presented in Figures 2-4. The fructose yield, fructose fraction in sugar and ethanol yield are shown in Figure 5. The fructose yield is calculated based on the amount of fructose present (g) at the end of fermentation to its initial amount (g). Fructose fraction denotes the amount of fructose (g) to the amount of total sugars (g) at the end of process. The theoretical ethanol yield is defined as the produced ethanol (g) to the consumed total sugars (g) at the end of fermentation. These parameters are given as percentage (%) in the figures.

### 3.1. Selective and non-selective fermentations of date extract

The kinetic profiles resulting from the non-selective fermentation of the date syrup using a commercial *S. cerevisiae* strain, i.e. wild strain at 30°C are shown in Figure 1a. It is clear from the figure that the wild strain fermented both glucose and fructose, with a slightly lower initial rate of fructose fermentation. The two sugars were consumed after a fermentation time of about 24 h. The concentration of ethanol is high due to the consumption of the two sugars. These results reveal the nonselective nature of this strain.



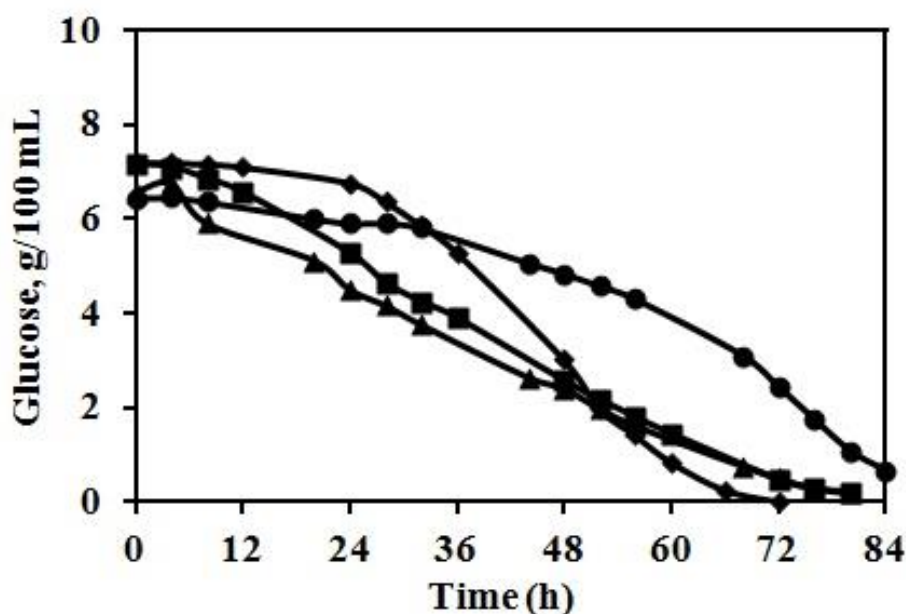
**Figure 1.** Kinetic profiles at 30°C in dates extract: (a) non-selective fermentation by wild *S. cerevisiae*; (b) selective fermentation by *S. cerevisiae* ATCC 36858

On the other hand, at the same temperature compared to the wild strain, the *S. cerevisiae* strain ATCC 36858 was highly selective towards fermentation of glucose (Figure 1b). When glucose has been completely fermented, the fructose component was almost unaffected. The ethanol yield was less than that obtained with the wild strain as shown in Figure 1b due to probably the higher total sugar concentration (unconsumed fructose) during fermentation that relates to osmotic phenomena [17]. This

is also reasonable since the ethanol started to be produced after 20 h for selective fermentation, while it was produced at 4 h for non-selective one. Thus, a slower fermentation occurred for the selective process that continued to 72 h.

### 3.2. Selective fermentation by *S. cerevisiae* ATCC 36858 and 36859

Figure 2 presents the kinetic profiles of glucose for both ATCC 36858 and 36859 at 30 and 33°C. At the beginning of process, the glucose was consumed slowly, then after 12 h it dropped at higher rates. There are no major differences of trend between 30 and 33°C for ATCC 36858; in contrast, for ATCC 36859 at 33°C, the glucose quickly decreased even it is completely consumed before 72 h compared to at 30°C.

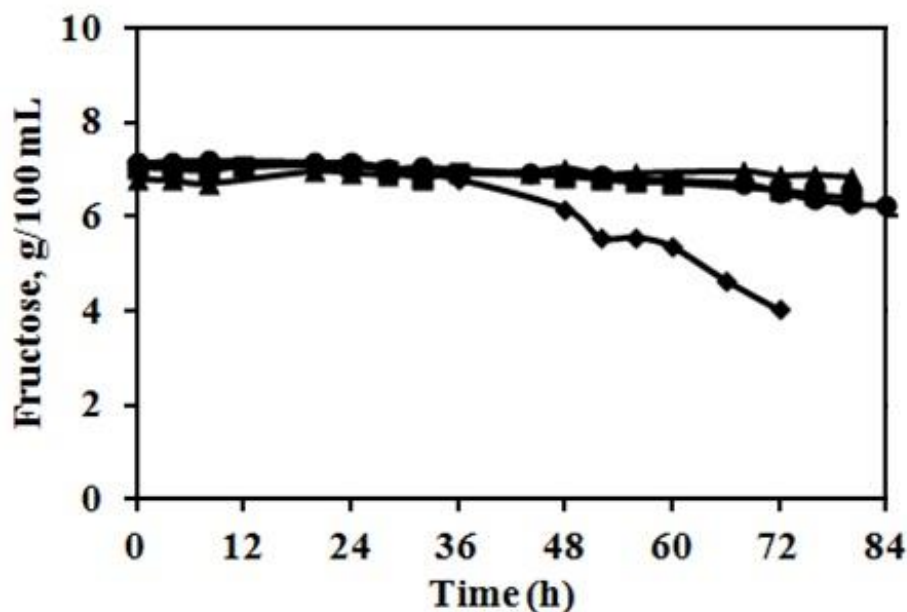


**Figure 2.** Kinetic profiles of glucose: (ATCC 36858-30°C (closed triangles)); (ATCC 36858-33°C (closed squares)); (ATCC 36859-30°C (closed circles)); (ATCC 36859-33°C (closed rhombuses)).

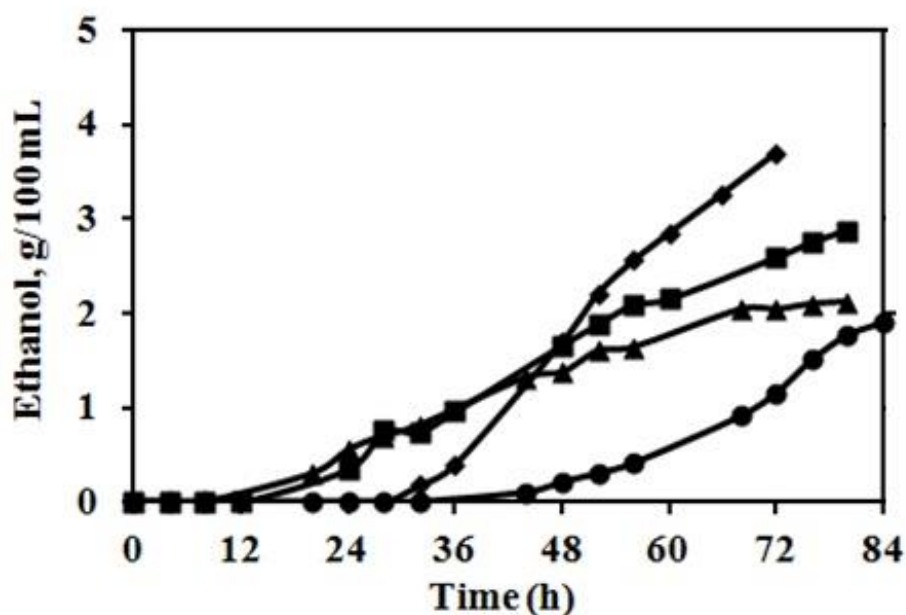
The kinetic profiles of fructose for both ATCC 36858 and 36859 at 30 and 33°C are shown in Figure 3. The fructose was slightly consumed by the both strain; the exception was for ATCC 36859 at 33°C which showed significant fructose consumption. The fructose dropped up to 4 g/100 ml at 72 h which is corresponding to 42% loss of fructose. It seems that the selectivity of the yeast ATCC 36859 significantly declined at 33°C as indicated by fast consumption of fructose.

Figure 4 shows the kinetic profiles of ethanol for both ATCC 36858 and 36859 at 30 and 33°C. It is clear from the figure that increasing the temperature increased the ethanol production. The production of ethanol for ATCC 36858 was higher than ATCC 36859 at 30°C. The significant production for ATCC 36859 at 33°C was related to the higher consumption of fructose. However, the earlier (before 24 h) ethanol produced for ATCC 36858 indicates that the yeast has short lag phase of growth [14] as manifested by earlier glucose consumption (before 12 h) compared to ATCC 36859.

The fermentation performances, measured in terms of fructose yield, fructose fraction and ethanol yield, can be observed from Figure 5. It is clear that the higher temperature led to the lower fructose yield. For all temperatures, the fructose yield for ATCC 36858 (> 90%) were much higher than ATCC 36859 which are less than 88%. 100.6% fructose yield for ATCC 36858 at 30°C was due to the sucrose hydrolysis to glucose and fructose; this finding shows the ability of this strain to hydrolyze the sucrose [12]. The fructose fraction in sugar and the ethanol yield for ATCC 36858 were also higher than those for ATCC 36859.



**Figure 3.** Kinetic profiles of fructose: (ATCC 36858-30°C (closed triangles)); (ATCC 36858-33°C (closed squares)); (ATCC 36859-30°C (closed circles)); (ATCC 36859-33°C (closed rhombuses)).



**Figure 4.** Kinetic profiles of ethanol: (ATCC 36858-30°C (closed triangles)); (ATCC 36858-33°C (closed squares)); (ATCC 36859-30°C (closed circles)); (ATCC 36859-33°C (closed rhombuses)).

Furthermore, increasing temperature 36858 increased ethanol yield for ATCC and ATCC 36859 by 12% and 23%, respectively. It has been reported that the optimum growth of yeast at around 30°C [18]; here the highest fructose yield was obtained at 30°C. On the other hand, the optimum ethanol was achieved at some temperatures slightly above those required for optimum yeast growth [19].

These findings also show that the wild yeast is favored when production of ethanol is the target (i.e., highest ethanol yield), while the selective strains ATCC 36858 and 36859 are favored for the simultaneous production of fructose and ethanol.

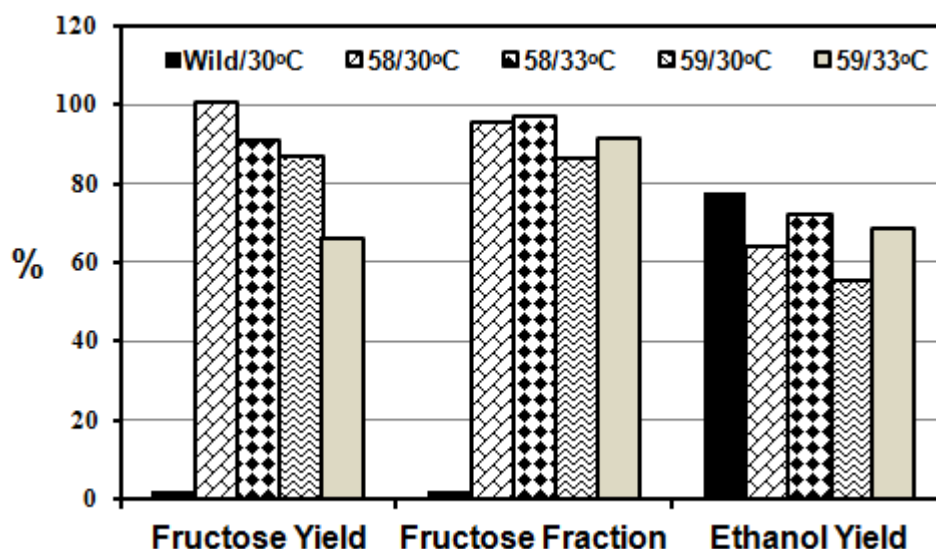


Figure 5. Performance of fermentation with various yeast strains

#### 4. Conclusion

Simultaneous production of fructose and ethanol from date extract was achieved by using two glucose-selective strains of *S. cerevisiae*, namely: ATCC 36858 and 36859 from date extract. On the other hand, the production of ethanol only was achieved by commercial yeast which fermented fructose. Fructose syrups with about 90% fructose fraction in sugars were obtained using the glucose-selective strains. The higher fermentation temperature led to the higher fructose yield. The process using *S. cerevisiae* ATCC 36858 showed better performances, i.e., higher fructose yield, fructose fraction and ethanol yield than using *S. cerevisiae* ATCC 36859. The higher ethanol yield was obtained in higher temperature. Therefore, the selective fermentation of glucose in the date extract is a very promising way for the production of high concentration fructose syrup.

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