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Utilization of Pitted Dates for the Production of Highly Concentrated Fructose Syrups by S. Cerevisiae

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Dates contain over 75 % reduced sugars in form of glucose and fructose. *Saccharomyces cerevisiae* strain ATCC 36858 has been used to selectively ferment the glucose component in the date syrup into ethanol. Separation of ethanol from the fermentation resulted in higher fructose concentrations. The selective fermentation was performed at three temperatures, i.e., 27, 30 and 33 °C at agitation speed of 120 rpm and initial syrup concentration of 15 % (150 g/L). The fructose fractions in the remaining sugar solution were 97.5, 99.2 and 97.8 % for fermentation temperatures of 27, 30 and 33 °C respectively. The corresponding fructose yields were 82.1, 95.1 and 85.7 %. However, for the wild non-selective strain all fructose was fermented simultaneously with the glucose component. The fructose fractions obtained here are much higher than those obtained with the classical isomerization processes that yield 55 % fructose syrups.

1. INTRODUCTION

In many parts of the world (e.g., Middle East, South Asia, North Africa and USA), date palm trees (*Phoenix dactylifera* L.) are grown (Selim et al., 2012). The worldwide production of dates exceeded 7.9 million tons of dates in 2010 (Jain, 2012). The Kingdom of Saudi Arabia produced over million tons of dates in 2010 - about 14 % of world production (Jain, 2012; Al-Shreed et al., 2012). Although Saudi Arabia donates some of its date production and the local consumption of dates in Saudi Arabia is high, still large amounts (30 – 45 % of the production) are unconsumed due to low quality of some varieties, such as Ruzaiz and Sifri (Moshaf et al., 2011). Many valuable products (e.g., ethanol, high fructose syrups, acetic acid…etc) can be produced from the unused dates. Date syrups are very rich in reduced sugars (in form of fructose and glucose), minerals, vitamin and a small amount of sucrose (Hasnaoui et al., 2010). Pitted date contains over 75 % fructose and glucose of its dry weight. Fructose is the sweetest natural sugar; 30 % sweeter than sucrose and about 90 % sweeter than glucose (Khosravanipour Mostafazadeh et al., 2011). As a monomer, fructose can be found in many fruits such as dates, apples and raisins. It is also found in a polymeric form in many fructan-rich plants, e.g., Jerusalem artichoke, chicory and dahlia (Lima et al., 2011). It is used as a natural sweetener in the food, confectionery and beverage industries and also in diabetic and baby food (Lima et al., 2011).

Commercially, fructose is produced by a process that involves two major steps: enzymatic saccharification of starch to form glucose and enzymatic isomerization of the produced glucose. The isomerization step is reversible; therefore, a typical isomerization process yields fructose syrups having about 42 % fructose (depending on isomerization temperature) due to equilibrium limitations. In order to enhance the fraction (concentration) of fructose in the final syrup, expensive separation techniques are often employed. Being isomers, fructose and glucose are very difficult to separate. The costly multistage chromatograph separation technique can produce fructose syrups with 90 % fructose fraction (Hanover and White, 1993). In order to produce the commonly marketed 55 % fructose syrups, the two fructose syrups (42 and 90 %) are usually blended. Fructose can also be produced by processes that involve enzymatic hydrolysis of inulin (Ricca et al., 2010). More recently, a process that uses ionic liquids to separate fructose and glucose from their mixtures has been patented (AlNashef et al., 2011). On the other hand, due to their high

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fructose content, the spoilage dates have a great industrial potential when used to produce, directly, fructose. Moreover, the ethanol produced from agricultural waste has been suggested to be a promising alternative to the fuel market (Lorenzo et al., 2010; Ceclan et al., 2012).
Production of fructose and ethanol from synthetic sucrose media through selective fermentation by *S. cerevisiae* ATCC 36858 or ATCC 36859 has been also reported (Atiyeh and Duvnjak, 2001; Koren and Duvnjak, 1992). *S. cerevisiae* ATCC 36858 has also been employed for the production of fructose and ethanol from dates extract (Putra et al., 2013; Gaily et al., 2011); while wild strain of *S. cerevisiae* were used for the production of ethanol from dates extract (Sulieman et al., 2013).
In this study, the effect of fermentation temperature on the production of fructose and ethanol from date syrups will be investigated. *S. cerevisiae* ATCC 36858 will be used in the fermentation. The results will be compared to a commercial wild strain of *S. cerevisiae*. The study aims at producing fructose syrups with high fructose fraction without significant fructose losses (i.e., high fructose yields).

2. Materials and Methods

2.1 Substrate preparation

The sugars were extracted from a low quality date variety (local name is Ruzaiz variety) using deionized water at a weight ratio of 2:5 (pitted date to water) at 50 °C. The concentration of the syrup was adjusted to 150 g sugars/L by dilution. The substrate was sterilized for 15 minutes at 121 °C.

2.2 Microorganism and media

The glucose selective yeast, *S. cerevisiae* ATCC 36858, obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) in a pellet form, was activated and transferred according to the standard procedure of the ATCC. The strain was inoculated in a sterilized Yeast Broth that contained 10.0 g glucose, 3.0 g yeast extract, 5.0 g peptone and 3.0 malt extract dissolved in 1.0 liter deionized water. The broth was sterilized in an autoclave sterilizer (Astell AMB230N, Sidcup, Kent, UK) for 15 minutes at 121 °C. Two grams of the wild yeast were added to 200 ml of the above sterilized Yeast Broth and was propagated in a temperature controlled water bath shaker (Julabo SW23, Marcon Boulevard, Allentwon, USA) at 30 °C and 120 rpm for 2 days.

2.3 Fermentation experiment

The fermentation medium contained the substrate (sterilized date extract) and the inoculated medium at a ratio of 17:3 by volume. The fermentation experiments were conducted in 500 ml conical flasks (working volume 100 ml) placed in a controlled temperature water bath shaker (Julabo SW23, Marcon Boulevard, Allentown, USA) operated at 120 rpm and different fermentation temperatures, namely 27, 30 and 33 °C without controlling the pH. Duplicate runs of each experiment were performed and the concentrations reported in the figures are the average values whereas error bars show the highest and lowest values.

2.4 Sample analysis

Samples were aseptically withdrawn from the fermentation flasks using sterilized disposable pipettes. The drawn sample was centrifuged at 15000 rpm for 1 min to separate the cells from the solution and then the clear solution was transferred to a small vial for sugars and ethanol analysis.
The sugars (glucose, fructose and sucrose) and ethanol were determined quantitatively using high performance liquid chromatography (HPLC-Agilent 1200 Infinitely series, Wilmington, DE, USA) equipped with an RID detector and Aminex® column (150 x 7.8mm, from BIO-RAD®, Foster City, California, USA).

3. Results and Discussion

The results of the fermentation experiments are presented in Figures 1-7, while Table 1 presents the initial concentrations of sugars in the extract. It also presents the fructose yield and fructose fraction as well as the ethanol yield at the end of fermentation. The fructose yield is defined as the amount of fructose present at any time to its initial amount; whereas, fructose fraction denotes the amount of fructose at any time to the amount of total sugars. Fructose yield and fraction as well as ethanol are calculated based on average values of concentrations and are given as percentage (%) in the figures and Table 1. The initial concentration of the total sugars was 128.8$\pm$4.2 g/L in all experiments; the sucrose component was 1.8$\pm$0.1 g/L.The kinetic profiles resulting from the fermentation of the date syrup using a commercial *S. cerevisiae* strain (hereafter called wild strain) at 30 °C are shown in Figure 1. It is clear from the figure that the wild strain fermented both glucose and fructose, with a slightly higher initial rate of glucose 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.

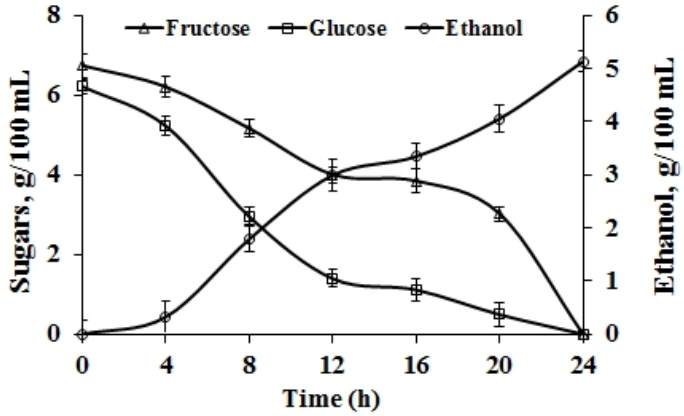

Figure 1: Kinetic profiles of the fermentation of dates extract by wild S. cerevisiae at 30℃

Figure 2 shows the kinetic profiles of the sugars and ethanol whereas Figure 3 shows the profiles of fructose yield and fraction during the course of the fermentation at 27 °C. The strain ATCC 36858 was highly selective towards fermentation of glucose (Figure 2). When glucose has been completely fermented, the fructose component was almost unaffected. The fructose yield was about 82 % and its fraction in the syrup was 97.5 % as shown in Figure 3. The losses in fructose (2.5 %) were much less than those reported in literature (Koren and Duvnjak, 1992). The ethanol yield was less than that obtained with the commercial strain as shown in Table 1 due to probably the higher total sugar concentration and unconsumed fructose during fermentation that relates to osmotic phenomena (Jones et al., 1981).

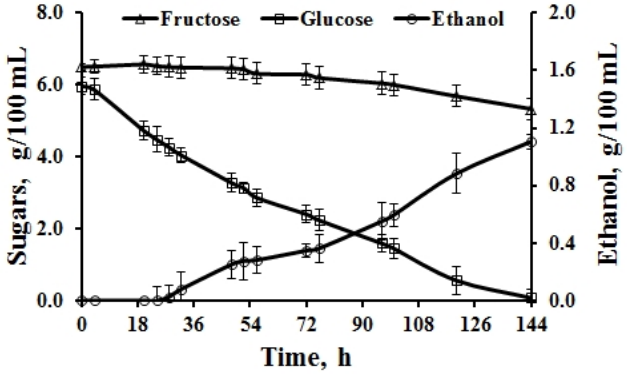

Figure 2: Kinetic profiles of the fermentation of dates extract by S. cerevisiae ATCC 36858 at 27 ℃

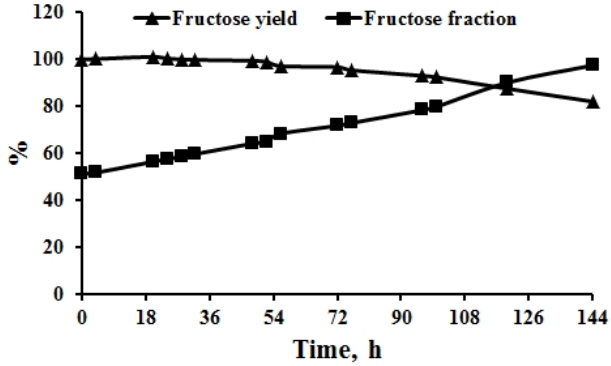

Figure 3: Profiles of percentage fructose yield and fructose fraction for ATCC 36858 at 27 ℃

Table 1: Comparison of yeast strains at various fermentation temperatures

Strain	Temperature (℃)	Initial concentrations (g/L)			Ethanol yield (%)	Fructose yield (%)	Fructose fraction (%)
		Glucose	Fructose	Sucrose			
Wild	*30*	*62.8±3.5*	*68.1±4.1*	*1.7±0.1*	*75*	*0.0*	*--*
ATCC 36858	*27*	*58.6±3.2*	*65.1±2.4*	*1.9±0.1*	*32*	*82.1*	*97.5*
ATCC 36858	*30*	*63.1±1.5*	*67.8±1.4*	*1.7±0.1*	*49*	*95.1*	*99.2*
ATCC 36858	*33*	*57.6±1.4*	*64.9±1.5*	*1.9±0.1*	*40*	*85.7*	*97.8*

Increasing the fermentation temperature to 30 °C resulted in the kinetic profiles shown in Figure 4 for the selective fermentation using ATCC 36858. It is clear from the figure that an insignificant amount of fructose has been fermented at complete conversion of glucose. The ethanol yield was higher than that obtained at 27 °C (Table 1) due to the lower activity of the yeast at 27 °C as optimum temperature for the growth of *S. cerevisiae* strains was in the range of 30-35 °C (Salvado et al., 2011). As shown in Figure 5, the fructose yield was higher than that for 27 °C and the fructose fraction was much higher (99.2 %); giving an almost pure fructose syrup.

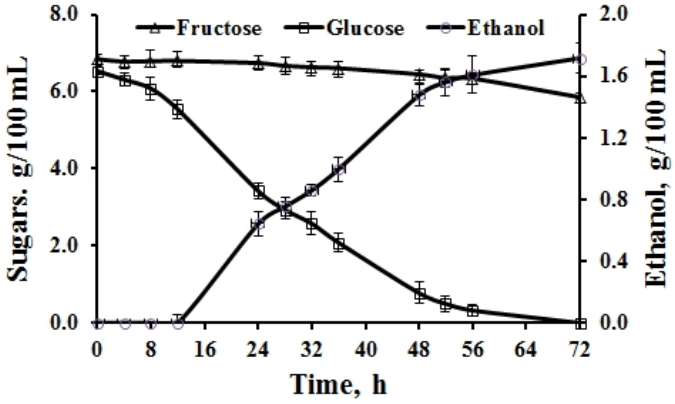

Figure 4: Kinetic profiles of the fermentation of dates extract by S. cerevisiae ATCC 36858 at 30 ℃

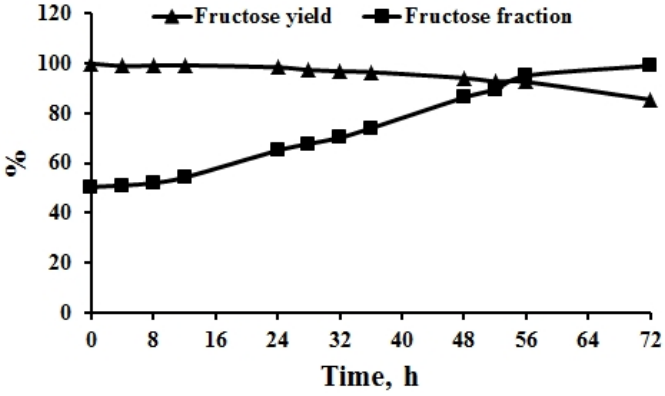

Figure 5: Profiles of percentage fructose yield and fructose fraction for ATCC 36858 at 30 ℃

Figure 6 shows the kinetic profiles of fructose, glucose and ethanol during the ATCC 36858 fermentation of date syrup at 33 °C. At a near complete fermentation of the glucose component, the fructose was minimally affected. Though the ethanol concentration was a bit higher than the yield at 30 ºC, the optimum ethanol yield was at 30 ºC due to the ethanol inhibition at higher temperature (Dombek and Ingram, 1986). For the fermentation temperature of 33 °C, the fructose yield (85.7 %) dropped compared to that obtained at 30 °C but still higher than that at 27 °C. On the other hand the fructose fraction remained high (97.8 %) as seen from Table 1.

It is worthy to note that the ethanol yeild reported here is lower than those reported in the literature (Atiyeh and Duvnjak, 2001; Koren and Duvnjak, 1992). These differences could be attributed to the higher amounts of minerals and yeast extract as well as differences in substrates in their studies (Da cruz et al., 2003; Youssef, 1999).

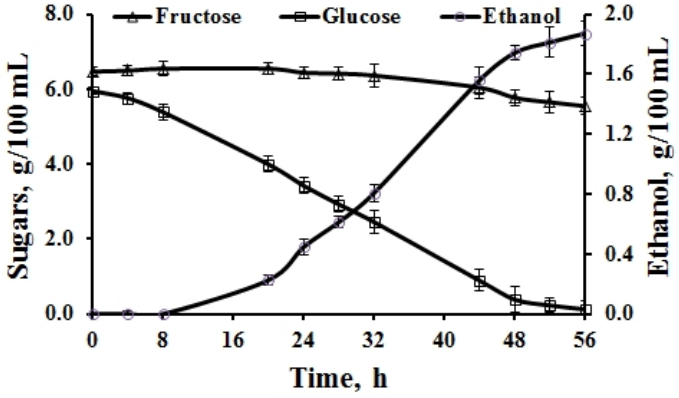

Figure 6: Kinetic profiles of the fermentation of dates extract by S. cerevisiae ATCC 36858 at 33 ℃

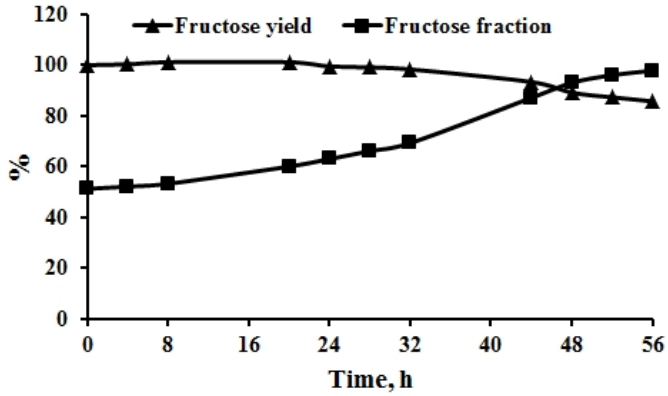

Figure 7: Profiles of percentage fructose yield and fructose fraction for ATCC 36858 at 33 ℃

4. Conclusion

Fructose and glucose constitute over 75 % of the dry weight of pitted dates. Selective fermentation of the glucose component in date extract could provide a very promising route for the production of highly concentrated fructose syrups. *S. cerevisiae* strain ATCC 36858 was very effective in performing the selective fermentation. Fermentation temperature of 30 °C was shown to provide the highest fructose yield (95.1 %) and fructose fraction (99.2 %) in the final syrup. Compared to the commonly marketed 55 % fructose syrups, selective fermentation of dates extract provides syrups having more than 95 % fructose.

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