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Ethanol and fructose are important materials for the fuel and food markets; high fructose syrup production processes are still costly. The effect of inoculum medium composition on the production of fructose and ethanol from dates by selective fermentation was investigated. The addition of minerals improved the fructose content in the produced syrup and ethanol productivity. The addition of malt extract to the medium increased fructose yield from 94% to 97%, but decreased ethanol yield from 78% to 69%. The presence of peptone increased ethanol productivity and fructose fraction in sugar, while iron increased fructose yield to 100% in syrups with 371 g initial sugar/L. Compared to the commonly marketed 55% fructose, selective fermentation of date extract produced sugar syrups with at least 82% fructose and significant amounts of ethanol. The excellent fit by the new expanded model prediction to the experimental data makes them valuable tools for further process development or industrial applications.

Keywords: Ethanol; High fructose syrup; Kinetics; *S. cerevisiae*; Selective fermentation

Introduction

Due to its high sweetness (about 1.7 times higher than sucrose), fructose is used worldwide in many applications, e.g., food, beverage, and pharmaceutical industries (Khosravanipour Mostafazadeh et al., 2011). High fructose corn syrup (HFCS) is commercially produced via saccharification of starch followed by enzymatic isomerization to obtain a 42% fructose syrup (Wu et al., 2010). Costly multistage chromatographic processes are employed to achieve 90% HFCS (Atiyeh and Duvnjak, 2001). On the other hand, a selective fermentation process could provide a very promising alternative for the production of fructose and ethanol from glucose/fructose mixtures (Di Luccio et al., 2002); the ethanol produced through glucose fermentation can easily be separated from the fructose (Atiyeh, 2003; Gaily, 2010).

Globally, the demand for ethanol, as a solvent and energy source, is steadily increasing (Rass-Hansen et al., 2007). Bioethanol could reduce the dependence on fossil fuels (Jargalsaikhan and Saraçoğlu, 2008). Compared to gasoline, bioethanol is renewable, safe to store, easy to handle, nontoxic, and sulfur free; in addition, it contributes less to air pollution and global warming (Labeckas and Slavinskas, 2009).

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Date palm trees are grown in many regions of the world, and about 8 million tons of dates were worldwide produced in 2010 (Jain, 2012). For example, the production of dates has been steadily increasing in USA from 23,700 tons in 2009 to 33,100 tons in 2011 (USA, Department of Agriculture). Unfortunately, about half of the date production is not utilized (Moshaf et al., 2011). Dates contain over 75% reduced sugars on a dry basis; nearly half of date sugar is fructose. This high sugar content makes date a natural and sustainable raw material for the production of fructose as well as ethanol.

In glucose–fructose mixture, selective fermentation of the glucose component into ethanol increases the percentage of fructose in the remaining sugar syrup; > 90% fructose is achievable. Processes using strains such as *Zymomonas mobilis, Tricholoma nudum, Fusarium* sp, *Pullularia pullulans* showed significant fructose consumption and formation of undesired by-products (Carvalho et al., 2008). On the other hand, fermentation processes using glucose-selective mutants of *Saccharomyces cerevisiae* have been shown to overcome the above-mentioned problems (Atiyeh and Duvnjak, 2001). Moreover, *S. cerevisiae* sustained high sugar concentrations in glucose–fructose mixtures, e.g., 470 g/L for ATCC 36858 (Putra et al., 2013) and 415 g/L for ATCC 36859 (Koren and Duvnjak, 1991)—an important factor that reduces downstream processing (i.e., ethanol separation) cost.

The addition of minerals in a yeast cultivation medium has been shown to play a positive role in yeast growth and subsequent fermentation (Youssef et al., 1999). The presence

of magnesium in the fermentation medium (FM) prolonged the exponential growth phase and increased the yeast cell mass concentration (Dombek and Ingram, 1986). Iron, as an enzyme cofactor, has been shown to contribute positively to cellular metabolism (Shakoury-Elizeh et al., 2010). Moreover, iron was shown to play numerous structural and functional roles in yeast cell physiology that are particularly significant in fermentation process (Walker, 2004). Cobalt was shown to increase the saccharification efficiency in the production of ethanol from cellulose (Kuhad et al., 2010). Manganese was identified as an essential element for metabolism and growth (Walker, 2004), and traces of nickel played significant roles in important metabolic reactions (Gikas, 2008). Zinc was essential for protein biosynthesis and carbohydrate metabolism that contributed to higher ethanol production (Vriesekoop et al., 2012). Urea, as an inexpensive source of nitrogen, was shown to increase ethanol production and fermentation efficiency (El-Refai et al., 1992).

Kinetic models that are capable of predicting the distribution of the products provide valuable tools for further process development such as large-scale or industrial application (Gorsek and Zajsek, 2010). Although, in some cases, the original Gompertz equation failed to model the growth of microorganisms (López et al., 2004; Zwietering et al., 1990), the modified Gompertz equation did (Zwietering et al., 1990). More recently, this modified model was applied successfully to predict ethanol production (Zajsek and Gorsek, 2010).

The performance of selective fermentation using date extract showed that at high initial sugar concentration (above 250 g/L), fructose and ethanol yields were below 90% and 62%, respectively (Putra et al., 2013). Sucrose hydrolysis was also low (about 15%). Thus, the main objective of this study was to improve the performance of *S. cerevisiae* in fermenting the date extract to produce high yields of fructose syrups and ethanol by reformulating the inoculum medium with the addition of various minerals. Also, to allow for future process development, a new modification to the modified Gompertz kinetic model was introduced here to predict the fructose fraction.

Experimental Section

Raw Materials

Sugars were extracted from dates using deionized water; the weight ratio of pitted dates to water was 2:5. The extraction experiments were performed at different temperatures and time. The extract was centrifuged at 6500 rpm for 6 min to remove suspended solids. The final date syrup (without the addition of any supplement) was then sterilized (Astell AMB230 N, Sidcup, Kent, UK) at 121°C for 15 min.

Microorganism and Inoculum Media

Saccharomyces cerevisiae ATCC 36858 (Lobo and Maitra, 1977) was revived according to ATCC procedure and was further incubated on agar slants. It was then transferred to 5 mL of a sterilized liquid medium (LM) and annealed for 36 h at 30°C in an incubator (Jeio-tech Model ON-12 G, Seoul, Korea). The culture was further propagated in a 500-mL flask that contained 100 mL of LM. It was placed in a rotary shaker incubator (Innova 43 Incubator Shaker Series, Enfield, CT, USA) at 30°C and 120 rpm for 36 h. Various inoculum liquid media were prepared; the composition of each medium is listed in Table I. The inoculum media were produced for each run. The composition of LM1 was according to ATCC recommendation, and it was used as a reference point.

Fermentation Experiments

The fermentation experiments were carried out in 500-mL conical flasks (100-mL working volume) that were placed in a rotary shaker (Innova 43 Incubator Shaker Series, Enfield, CT, USA) at 30°C and 120 rpm. The FM was composed of 80% date extract as substrate and 20% inoculum medium (LM) of *S. cerevisiae*. The fermentation media are denoted FM1, FM2, and so on, corresponding to inoculum media LM1, LM2, and so on, as given in Table I, which also shows the initial sugar concentration for each fermentation media.

Analytical Procedures

The samples were periodically taken and then centrifuged at 15,000 rpm to separate the cell mass. The cell mass concentration was measured using the dry weight method. The yeast was washed twice by deionized water and then re-centrifuged before drying overnight at 105°C. Sucrose, glucose, fructose, ethanol, and glycerol in the fermentation broth were analyzed using high-performance liquid chromatography (HPLC-Agilent 1200 Infinitely series, Wilmington, DE, USA) equipped with an RI detector and Aminex® column (150 × 7.8 mm Cat. #125-0115, BIO-RAD®, Foster City, CA, USA). The column was maintained at 40°C, and 1 mM sulfuric acid solution was used as a mobile phase at a flow rate of 0.8 mL/min. The conductivity of the extract was measured using a sensitive conductivity meter (Omega CDH-420, Stamford, CT, USA).

Definitions

The following definitions were used in the calculation:

1. Fructose yield

$$Y_F(\%) = \frac{F_L}{F_0} \times 100\% \quad (1)$$

2. Fructose fraction

$$\eta = \frac{F_T}{S_T} \times 100\% \quad (2)$$

3. Biomass yield

$$Y_{X/S} = \frac{X_L - X_0}{S_0 - S_L} \quad (3)$$

Table I. Composition of inoculum liquid media (LM)

Content	LM1	LM2	LM3	LM4	LM5	LM6	LM7	LM8	LM9	LM10	LM11	LM12	LM13	LM14	LM15
Glucose (g)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Yeast extract (g)	3	3	10	10	10	10	10	10	10	10	10	10	10	10	10
Malt extract (g)	3	3	—	—	—	—	5	—	—	—	—	—	—	—	—
Peptone (g)	5	5	3.5	3.5	3.5	3.5	3.5	—	3.5	3.5	3.5	3.5	3.5	3.5	3.5
KH_2PO_4 (g)	—	2	—	2	2	2	2	2	2	2	2	2	2	2	2
$MgSO_4 \cdot 7H_2O$ (g)	—	1	—	1	1	1	1	1	1	1	1	1	1	1	1
$(NH_4)_2SO_4$ (g)	—	1	—	1	1	1	1	1	1	1	1	1	1	1	1
$ZnSO_4 \cdot H_2O$ (g)	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
$NiSO_4 \cdot 6H_2O$ (g)	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
$CoCl_2$ (g)	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
$MnSO_4 \cdot H_2O$ (g)	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
$FeSO_4 \cdot 7H_2O$ (g)	—	—	—	—	—	—	—	—	—	—	—	—	1	—	0.5
Urea (g)	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
H_2O (L)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Initial sugar* (g/L)	284	281	279	279	299	365	306	358	358	358	373	367	371	370	360
Fermentation medium**	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	FM9	FM10	FM11	FM12	FM13	FM14	FM15

*The initial sugar concentration in the fermentation medium (FM) after mixing date extract with LM of *S. cerevisiae* ATCC 36858.
**The FM is composed of date extract (80%) and inoculum medium (20%) of LM.

4. Specific growth rate of cell mass

$$\mu = \frac{\ln(X_L) - \ln(X_0)}{t_{fL} - t_{f0}} \quad (4)$$

5. Ethanol yield

$$Y_{E/S} = \frac{E_L - E_0}{(S_0 - S_L) \times 0.51} \times 100\% \quad (5)$$

The value of 0.51 (92/180) is the theoretical yield of ethanol on sugar (g ethanol/g sugar).

6. Ethanol productivity

$$Q_P = \frac{E_L - E_0}{t_{fL} - t_{f0}} \quad (6)$$

7. Sucrose loses

$$Y_S(\%) = \frac{S_0 - S_L}{S_0} \times 100\% \quad (7)$$

Results and Discussions

Extraction Process

Tables II and III show the total sugars extracted, conductivity, and total dissolved solids (TDS) at various extraction times and temperatures, respectively. The TDS in the tables represent the minerals that are present in the date syrup (sugars are excluded). The total sugars increased with extraction temperature; however, the increase between 50°C and 60°C was less than half of increase between 38°C and 50°C. Also, the TDS and conductivity [due to the ash and acid content (Kaškonienė et al., 2010)] increased with temperature. Though TDS was almost constant after 50°C, the increase of conductivity at this point was related to the increase of sugar content (Terrab et al., 2003). Increasing extraction time increased the total sugars; however, the most significant increase of extracted sugars (32%) was obtained between 60 and 90 min. Obviously, TDS and conductivity increased with the extraction time.

Table II. Total sugars, TDS, and conductivity of date extract at 90 min

Extraction temperature (°C)	Total sugars (g/L)	TDS (mg/L)	Conductivity (mS)
24	88.6	1,650	3.17
38	132.6	2,190	3.79
50	204.0	2,900	4.91
60	247.8	2,870	5.35

Selective Fermentation by S. *Cerevisiae*

Figures 1(a) and 1(b) show the chromatograms of the date syrup before and after fermentation, respectively. Glucose that clearly appeared initially [Figure 1(a)] was converted to ethanol at the end of fermentation [Figure 1(b)]. As shown in the chromatograms, the fructose peak was almost unchanged. Small peaks of sucrose and glycerol are also observed in Figure 1(b).

Table III. Total sugars, TDS, and conductivity of date extract at 50°C

Extraction time (min)	Total sugars (g/L)	TDS (mg/L)	Conductivity (mS)
15	66.0	1,562	2.09
30	111.3	2,200	3.88
45	143.1	2,460	4.35
60	154.5	2,710	4.61
90	204.0	2,900	4.91
120	228.2	2,990	5.22

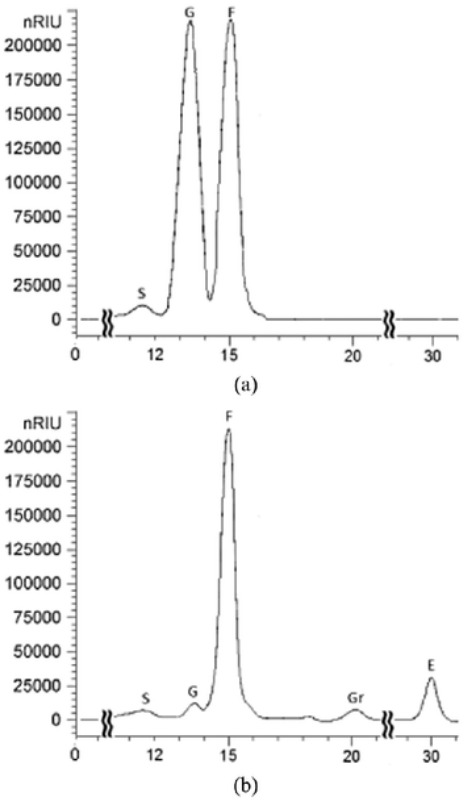

Fig. 1. Sample chromatograms of date syrup (a) before fermentation; (b) after fermentation (G = glucose; F = fructose; S = sucrose; E = ethanol, and Gr = glycerol).

The kinetic profiles of the selective fermentation by *S. cerevisiae* in medium FM4 is shown in Figure 2. The profiles of fructose yield and fraction, sucrose loss, and ethanol yield and productivity can be observed in Figure 3(a); whereas Figure 3(b) shows the cell mass yield and specific growth at 279 g initial sugar/L using medium FM4. It is clear that the strain has selectively converted glucose to ethanol and cell mass. During the early stages of fermentation, glucose was mainly used for cell growth. The exponential growth phase occurred before 24 h as shown by the profiles of the cell mass (Figure 2) and specific growth [Figure 3(b)]. The maximum cell mass yield was obtained at 8 h [Figure 3(b)]. The dry cell mass weight that increased from 1.2 to 5.45 g/L during the 60 h (Figure 2), led to a value for specific growth and cell mass yield of $0.025\,h^{-1}$ and 0.029 g/g, respectively, as listed in Table IV.

The production of ethanol started after 12 h of fermentation. The maximum ethanol yield and productivity were 75.6% and 1.3 g/(L · h) at 28 and 36 h, respectively, as shown in Figure 3(a). However, afterward the ethanol concentration slowly increased until the end of fermentation which corresponded to the ethanol yield and productivity of 75.4% and 0.95 g/(L · h), respectively, as presented in Table IV. The ethanol yield was calculated based on the consumption of total sugars.

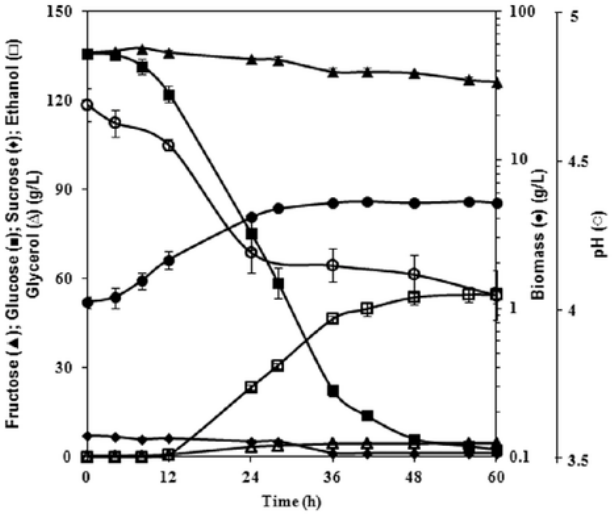

Fig. 2. Kinetic profiles of selective fermentation of date syrup (279 g/L) by *S. cerevisiae* using fermentation medium FM4. Medium FM4 was composed of 80% date extract as substrate and 20% inoculum medium LM4.

The glucose was completely converted after 60 h, while the fructose was minimally consumed (Figure 2). Thus, the fructose yield (i.e., amount of fructose at the end of fermentation to its initial amount) was 93% at the end of fermentation (Table IV). On the other hand, the fructose fraction increased from 49% to 97%. The pH dropped from 4.63 to 3.96 due to the production of acids (Stewart and Russell, 1987). About 5 g/L of glycerol was produced after 42 h of fermentation and remained constant until the end of the process; this confirms the dependence of glycerol production on glucose consumption. The decrease of sucrose during fermentation revealed the mutant's ability to hydrolyze the sucrose (84%) after 36 h due to the low glucose concentration.

Effect of Inoculum Media

Table IV shows the performance of *S. cerevisiae* in selective fermentation of date syrup using various fermentation media. The cell mass yield and specific growth rate of *S. cerevisiae* using medium FM1 were 0.017 g/g and $0.0155\,h^{-1}$, respectively. The specific growth rate with FM3 ($0.0155\,h^{-1}$) was similar to that of FM1. However, the cell mass yield, ethanol yield and productivity were greatly enhanced by 23.5%, 9.2%, and 10.3%, respectively. Although the fructose yield in medium FM3 was slightly lower than that of FM1, the fructose fraction (defined as the amount of fructose per total sugars) in the produced syrup was higher than that in medium FM1 (Table V) due to the hydrolyzed sucrose (44.6% improvement). This reveals the importance of medium composition in enhancing sucrose hydrolysis.

The addition of minerals [KH_2PO_4, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$] to either media LM1 or LM3 to form LM2 or

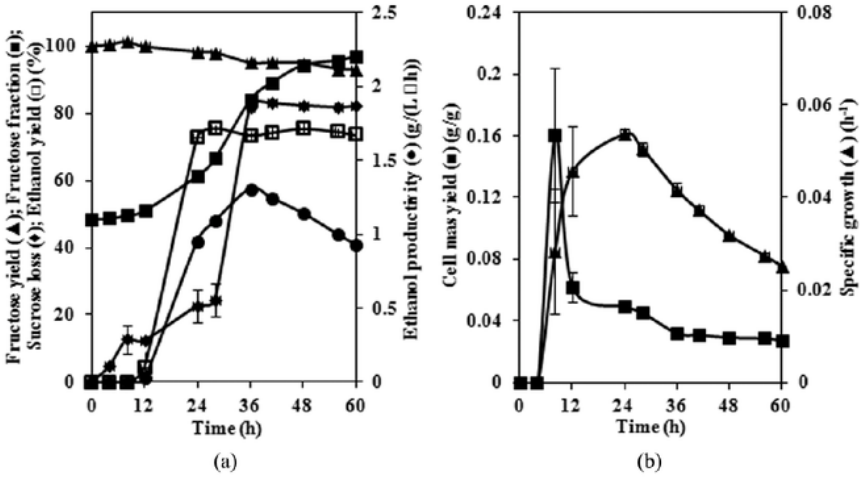

Fig. 3. Profiles of (a) fructose yield (▲) and fraction (■), sucrose losses (◆), and ethanol yield (□) and productivity (●); (b) cell mass yield (■), and specific growth of cell (▲) for 279 g/L initial date syrup using FM4.

Table IV. Performances of *S. cerevisiae* in selective fermentation of dates extract using various fermentation media (FM)

FM	Total initial sugar (g/L)	Initial cell mass (g/L)	Cell mass yield (g/g)	Specific growth rate, μ (h^{-1})	Ethanol yield (%)	Ethanol productivity [g/(L · h)]	Fructose yield (%)	Fructose fraction in sugars (%)	Sucrose hydrolyzed (%)	Glycerol (g/L)	Time* (h)
FM1	284	1.13	0.017	0.0155	69.5	0.68	94.3	92.9	18.0	5.0	76
FM2	281	1.13	0.019	0.0196	69.5	0.82	94.2	96.3	81.9	5.7	64
FM3	279	1.38	0.021	0.0155	75.9	0.75	91.3	94.2	62.6	5.0	76
FM4	279	1.20	0.029	0.0252	75.4	0.95	93.0	96.7	82.3	5.0	60
FM5	299	0.95	0.028	0.0254	78.2	0.91	94.3	95.1	75.2	6.2	70
FM6	365	1.25	0.020	0.0172	77.1	0.95	86.3	92.4	81.2	9.1	84
FM7	306	0.90	0.016	0.0183	68.7	0.74	97.4	95.6	76.3	8.2	76
FM8	358	0.95	0.015	0.0143	78.3	0.78	86.9	90.7	82.4	10.3	100
FM9	358	0.82	0.013	0.0140	64.7	0.58	94.4	92.9	21.2	9.1	100
FM10	358	0.85	0.016	0.0175	65.1	0.70	93.9	93.8	20.3	8.3	84
FM11	373	0.45	0.020	0.0128	57.2	0.29	100.0	85.5	83.8	9.2	166
FM12	367	0.75	0.014	0.0133	58.1	0.45	102.2	82.2	83.6	10.0	100
FM13	371	1.15	0.017	0.0151	64.6	0.70	99.8	91.1	82.8	10.0	88
FM14	370	0.95	0.017	0.0164	76.1	0.92	68.4	90.2	82.4	12.5	100
FM15	360	1.03	0.019	0.0189	69.1	0.82	93.6	94.8	55.9	7.4	80

*Time when the glucose was completely consumed or almost constant afterward.

LM4, respectively, improved the fermentation efficiency, i.e., growth of the cell, ethanol productivity, and sucrose hydrolysis. Although the ethanol yields were similar, ethanol productivity was improved by 20.6% in medium FM2 compared to FM1 and by 26.7% in medium FM4 compared to FM3 (Table IV). Cell growth rate and the hydrolysis of sucrose were significantly enhanced. Although date syrups are very rich in minerals, vitamins, and nutrients

Table V. Fructose fraction and ethanol production for various fermentation media (FM)

	FM1	FM2	FM3	FM4	FM5	FM6	FM7	FM8	FM9	FM10	FM11	FM12	FM13	FM14	FM15
Fructose fraction in sugars (%)	92.9	96.3	94.2	96.7	95.1	92.4	95.6	90.7	92.9	93.8	85.5	82.2	91.1	90.2	94.8
Ethanol production (g/L)	51.7	52.5	57	57	63.7	79.8	56.2	78	58	58.8	48.1	45	61.6	92	65.6

(Sánchez-Zapata et al., 2011), the presence of minerals in inoculum medium was shown to play a positive role in enhancing the microbiological stability as well as improving the fermentation process (Somda et al., 2011). It has been reported that the presence of minerals in a medium will modify the cell membrane and protect the cell against the entry of extracellular ethanol without inhibiting intracellular ethanol excretion from the cell (Thomas and Rose, 1979). In the present study, the fermentation process was, on average, shortened by about 12 h (e.g., medium FM2 compared to FM1). The glycerol produced in medium FM2 was higher than that in FM1 due to the presence of minerals; however, similar amounts of glycerol were obtained in media FM3 and FM4, which could be related to the low osmoregulatory effect onto the yeast (Nevoigt and Stahl, 1997). Although the addition of minerals improved the fermentation, it is important to note that overdosing the culture with minerals reduces the fermentation efficiency (Sarris et al., 2014).

The overall performance of *S. cerevisiae* in media FM3 and FM4 was better compared to media FM1 and FM2, probably due to higher amounts of yeast extract (YE) or absence of malt extract or smaller amounts of peptone. It has been reported that in the glucose fermentation using *S. cerevisiae* ATCC 36858, higher YE led to an increase of ethanol productivity and cell mass yield (Atiyeh, 2003); however, increasing YE from 3 to 10 g/L resulted in a yield of cell mass that was three times higher and an ethanol productivity that was double that of increasing YE from 10 to 20 g/L. The other possibility is the role played by malt extract in improving fructose yield. These factors are investigated in the following sections using media FM5 and FM6 (both have the same composition of inoculum medium as FM4) as a basis for comparison.

Effect of Malt Extract

The effect of malt extract on the performance of *S. cerevisiae* can be observed by comparing medium FM7 and FM5. Although the total initial sugar was little higher in medium FM5 than FM4, the results are consistent as shown in Table IV. The presence of malt extract in medium LM7 decreased cell mass yield and the specific growth rate by 42.9% and 28.0%, respectively.

The medium composition has also been reported to influence the production of glycerol (Radler and Schütz, 1982). Glycerol produced in medium FM7 is 32% higher than that in FM5 without addition of malt extract as in medium LM4. The production of glycerol diverts the conversion of sugars to ethanol (Oura, 1977), which explains the decrease in ethanol yield and ethanol productivity. On the other hand, fructose yield increased by about 3%. The results showed that the addition of malt extract in medium LM5 had a minimum effect on sucrose hydrolysis.

Effect of Peptone

The effect of peptone on the performance of *S. cerevisiae* ATCC 36858 is seen in the fermentations performed in media FM6 and FM8. Compared to medium FM6, the fructose yield was not affected. The cell mass yield and specific growth rate dropped from 0.02 to 0.015 g/g and from 0.0173 to 0.0143 h^{-1}, respectively, thus resulting in slower fermentation (about 19% slower). This time elongation decreased ethanol productivity but had little effect on its yield. The availability of peptone to the yeast as a source of organic nitrogen and amino acids is essential to the cultivation of a microorganism (Kapich et al., 2004).

The initial sugar concentration in medium FM6 (365 g/L) was higher than that in FM5 (299 g/L). There were slight differences in ethanol yield and productivity between the two media (FM5 and FM6) as shown in Table IV. On the other hand, at a higher initial sugar concentration (365 g/L), the cell mass yield and specific growth decreased by 28.6% and 32.3%, respectively. This was expected as higher initial sugar concentration caused higher osmotic stress on the cells (Jones et al., 1981). The fructose yield dropped due to the high substrate concentration; however, the fructose loss was still within the range reported previously for *S. cerevisiae* (Atiyeh and Duvnjak, 2001; Koren and Duvnjak, 1991).

Effect of Mineral Supplement

The addition of either Zn or Ni to medium LM6 enhanced the fructose yield from 86.3% to about 94.0%; however, cell growth and ethanol productivity decreased (Table IV). Compared to Zn-supplemented medium FM9, the Ni-supplemented medium FM10 showed higher cell mass yield (23.1%) and ethanol productivity (20.7%). Moreover, the fermentation time for Ni was 16% shorter.

The addition of any of the three minerals (Co, Mn, or Fe) to medium LM6 enhanced the fructose yield (close to 100%) compared to medium FM6. Moreover, the presence of Co in medium FM11 inhibited yeast growth, since very slow growth of the cell and much longer fermentation time were observed. The growth was slow during cultivation in inoculum medium as evidenced by the low initial cell mass concentration in medium FM11. For Mn-supplemented medium FM12, the cell mass and ethanol yield dropped by 30% and 24.6%, respectively. Values of fructose yield greater than 100% were obtained because the sucrose was hydrolyzed into glucose and fructose.

On the other hand, the addition of Fe to the medium provided better results compared to the addition of Co or Mn to medium LM6. The complete fermentation in medium FM13 was faster and quite close to the FM6. Very low consumption of fructose was observed in medium FM13. It has been reported that *S. cerevisiae* mutants were quite active in biosorption of Fe (Goyal et al., 2003), while the cultivation of the yeast in the media containing 2 g $FeSO_4$ per liter increased the total iron content of the yeast (Abbott et al., 2008). Furthermore, Fe has been found to be an essential nutrient for most major metabolic processes in the cell and the synthesis of organic and inorganic cofactors, and it further plays a positive role in the repair of DNA and the metabolism (Shakoury-Elizeh et al., 2010). These Fe characteristics could have played a positive role in reducing the consumption of fructose by the yeast. Cell mass yield and specific growth

dropped to 0.017 g/g and 0.0151 h^{-1}, respectively. Ethanol yield and productivity decreased by 16.2% and 26.3%, respectively. Since Fe plays an essential role in oxygen delivery in yeast cells (Shakoury-Elizeh et al., 2010), it might have suppressed acetaldehyde formation from pyruvate, causing inhibition of ethanol production. On the other hand, the presence of this mineral probably raised the osmolality (indicated by a slower process time) and lowered the ethanol production (Jones and Greenfield, 1984). It seems that the effect of Fe is similar to malt extract (see the section "Effect of Malt Extract"), but the enhancement of fructose yield was much more superior—15.6% as compared to that obtained with medium FM7 (only 3.3%). The results also imply that the presence of Fe is more beneficial for fructose production compared to ethanol production. Decreasing Fe concentration from 1 g/L in medium FM13 to 0.5 g/L in medium FM15 enhanced ethanol yield by 7% and cell mass yield by 11.8% but decreased the fructose yield from 99.8% to 93.6%. The fructose yields in either media FM13 or FM15 were higher than in FM6.

The addition of urea in medium FM14 decreased yeast growth (Table IV), probably due to the prevention of rapid acidification of the medium during fermentation that could negatively influence the growth of the mutant (Rodrigues et al., 2011). Little differences in ethanol yield and productivity were observed in media FM6 and FM14; however, the production of ethanol was much higher in FM14 (Table V), which can be attributed to higher consumption of fructose which was 31.6%. This was reflected in the higher production of glycerol due to the higher osmoregulatory response of the yeast (Nevoigt and Stahl, 1997). It was also reported (El-Refai et al., 1992) that the presence of urea enhanced the production of ethanol and the fermentation efficiency; therefore, addition of urea should be sought for systems that focus on the production of ethanol rather than fructose.

As depicted in Table V, fructose fractions in syrups obtained using selective fermentation of date extract were between 82.2% and 96.7%, which was much higher than the commonly marketed 55% HFCS. On the other hand, the bioethanol concentrations were in the range 45–92 g/L.

Effect of ClN Ratio in Inoculum Medium

The effect of carbon to nitrogen ratio in fermentation process is an important factor in a biological process, and it has been widely studied in many fermentation processes (Brzonkalik et al., 2012; Kalil et al., 2008). Microorganisms utilize carbon about 25 times faster than nitrogen during anaerobic digestion (Kalil et al., 2008); on the other hand, in some cases, the influence of nitrogen source is more significant than the carbon source (Brzonkalik et al., 2012), and alcoholic fermentation is favored in media that have a low initial C/N ratio (Sarris et al., 2013). Here, the effect of C/N ratio applied in inoculum medium on fermentation process was studied. Table VI presents C/N ratio for various inoculum LM. Higher C/N ratio in inoculum medium led to higher fructose yield as observed for LM1 and LM2 (compared to LM3 and LM4) and LM7 (compared to LM5). On the other hand, lower C/N ratio led to the higher fermentation performances, i.e., increase in cell growth and ethanol productivity. The presence of urea that lowered C/N ratio also resulted in higher ethanol production.

Kinetic Model for Fructose Fraction, Ethanol, and Fructose Production

Mathematical and kinetic models have been applied as useful tools for further process development such as pilot plant or industrial implementation (Gorsek and Zajsek, 2010). In this study, the modified Gompertz kinetic model equation [Equation (8)] that was usually used for prediction of ethanol production will be used, for the first time, to predict fructose production. Monod kinetic model and others have been used to describe the profiles of yeast growth and substrates (Farias et al., 2014); on the other hand, Gompertz model provides the prediction of lag time, the specific production rate, and the maximum product concentration (Mu et al., 2006; Zajsek and Gorsek, 2010). Since the presence of iron (FM13) and urea (FM14) led to very high fructose yield and ethanol production, the modified Gompertz equation will be applied to both as examples [Figures 4(a) and 4(b)]. The same model was expanded [Equation (9)] to enable predictions of the fraction of fructose in sugars. The modified Gompertz equation is defined as follows:

$$\gamma_i = \gamma_{i,\mathrm{m}} \exp\left[-\exp\left[\frac{r_{i,\mathrm{m}} \cdot \exp(1)}{\gamma_{i,\mathrm{m}}}\left(t_{\mathrm{L,i}} - t\right) + 1\right]\right] \quad (8)$$

where i denotes ethanol (Eth) or fructose (Frcts). For ethanol: γ_{Eth} represents the ethanol concentration (g/L), $\gamma_{\mathrm{Eth,m}}$ is the potential maximum ethanol concentration (g/L), $r_{\mathrm{Eth,m}}$ is the maximum ethanol production rate [g/(L · h)], and $t_{\mathrm{L,Eth}}$ is the lag phase or the time to exponential ethanol production (h). For fructose: γ_{Frcts} represents the fructose concentration (g/L), $\gamma_{\mathrm{Frcts,m}}$ is the initial fructose concentration (g/L), $r_{\mathrm{Frcts,m}}$ is the maximum fructose loss rate [g/(L · h)], and $t_{\mathrm{L,Frcts}}$ is the expected maximum time for the fructose to be zero (h).

The modified Gompertz equation was further expanded to allow for the prediction of the fructose fraction in sugar as follows:

$$\eta_{\mathrm{Frcts}} = \eta_{\mathrm{Frcts,0}} + \left(0.5\eta_{\mathrm{Frcts,m}}\right) \exp\left[-\exp\left[\frac{r_{\mathrm{Frcts,m}} \cdot \exp(1)}{\left(0.5\eta_{\mathrm{Frcts,m}}\right)}\left(t_{\mathrm{L}} - t\right) + 1\right]\right] \quad (9)$$

where η_{Frcts} is the fructose fraction in sugar (%), $\eta_{\mathrm{Frcts,0}}$ is the initial fructose fraction in sugar (%) which is 47.45%, $\eta_{\mathrm{Frcts,m}}$ is the potential maximum fructose fraction in sugar (%), $r_{\mathrm{Frcts,m}}$ is the maximum fructose fraction in sugar (%), and t_{L} is the time to exponential fructose fraction (h).

The comparison between experimental and model predictions of fructose and ethanol production [Equation (8)] and fructose fraction in sugar [Equation (9)] for FM13 and FM14 are shown in Figures 4(a) and 4(b), respectively.

Table VI. Carbon to nitrogen ratio in various liquid media (LM)

	LM1	LM2	LM3	LM4	LM5	LM6	LM7	LM8	LM9	LM10	LM11	LM12	LM13	LM14	LM15
C/N	11.0	8.1	4.6	4.0	4.0	4.0	4.9	3.8	4.0	4.0	4.0	4.0	4.0	3.0	4.0

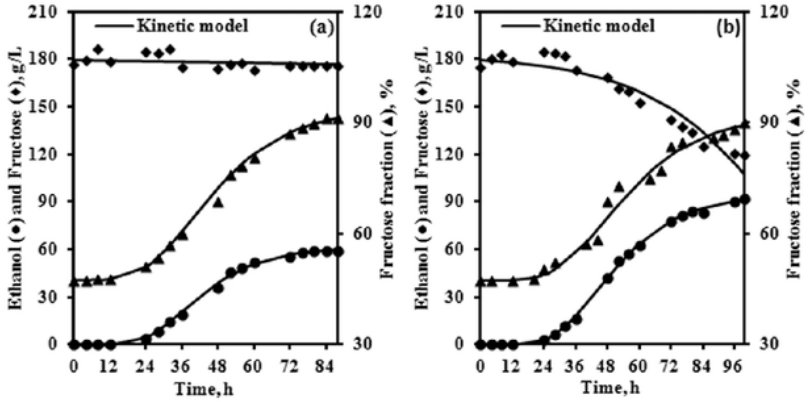

Fig. 4. Kinetic models predictions (lines) versus experimental data (symbols) for fructose fraction [Equation (9)], fructose, and ethanol production [Equation (8)] for (a) FM13 and (b) FM14.

Table VII. Estimated parameters of the kinetic models for ethanol and fructose production [Equation (1)] and fructose fraction [Equation (2)]

	Estimated parameters											
	Ethanol production				Fructose concentration				Fructose fraction			
	$\gamma_{Eth,m}$ (g/L)	$r_{Eth,m}$ [g/(L·h)]	$t_{L,Eth}$ (h)	R^2	$\gamma_{Frcts,m}$ (g/L)	$r_{Frcts,m}$ [g/(L·h)]	$t_{L,Frcts}$ (h)	R^2	$\eta_{Frcts,m}$ (g/L)	$r_{Frcts,m}$ [g/(L·h)]	t_L (h)	R^2
FM13	61.5	1.61	23.2	0.99	195.4	−0.138	1805	0.89	46.7	1.00	23.0	0.99
FM14	95.0	2.05	27.6	0.99	182.0	−2.32	147	0.92	45.1	0.85	28.2	0.98

Excellent fit was obtained as shown in Figure 4 and the coefficients of regression (R^2) in Table VII. As shown in Table VII, the maximum ethanol production rate ($\gamma_{Eth,m}$) for FM14 was higher than that for FM13, thus leading to higher potential maximum ethanol concentration ($r_{Eth,m}$). However, the rate of maximum fructose loss ($r_{Frcts,m}$) for FM 13 was much lower than it is for FM14; the negative signal on $r_{Frcts,m}$ indicates the decrease in fructose during fermentation. Furthermore, the predictions of the expanded Gompertz equation fitted very well the experimental data of fructose fraction in sugar (Figure 4). This expanded equation [Equation (9)] is quite beneficial in predicting the fermentation time required to have a desired fructose fraction in sugar.

Conclusions

Date extract has been used as a substrate for the production of fructose solutions with concentrations higher than 82%. The reformulation of the inoculum medium had significant effects on the fructose purity and yield in the selective fermentation process by *S. cerevisiae*. Malt extract decreased ethanol yield and inhibited cell growth with little increase in fructose yield, whereas the presence of peptone improved cell growth and ethanol productivity. The addition of some minerals improved the performance of *S. cerevisiae*, i.e., enhanced cell mass yield and ethanol productivity as well as sucrose hydrolysis. The addition of Fe significantly minimized fructose losses. The modified Gompertz equation was successfully used to predict the ethanol and fructose production. Gompertz equation was expanded to enable excellent predictions of the profiles of fructose fraction. Such a model and its expansion are quite useful and will pave the way for further process development or industrial applications.

Nomenclature

E_0 ethanol concentration at the beginning of fermentation, (g/L)

E_L	ethanol concentration at the end of fermentation, (g/L)
F_0	fructose concentration at the beginning of fermentation, (g/L)
F_L	fructose concentration at the end of fermentation, (g/L)
F_T	fructose fraction at certain fermentation time, (g/L)
Q_P	ethanol productivity, (g/(L·.h))
$r_{Eth,m}$	maximum ethanol production rate, (g/(L·.h))
$r_{Frcts,m}$	maximum fructose fraction in sugar, (%)
$r_{Frcts,m}$	maximum fructose loss rate, (g/(L.·h))
S_0	sucrose concentration at the beginning of fermentation, (g/L)
S_0	total sugar concentration at the beginning of fermentation, (g/L)
S_L	sucrose concentration at the end of fermentation, (g/L)
S_L	total sugar concentration at the end of fermentation, (g/L)
S_T	total sugar concentration at the certain fermentation time, (g/L)
t_{f0}	fermentation time at the beginning of process, (g/L)
t_{fL}	fermentation time at the end of process, (g/L)
$t_{L,Frcts}$	expected maximum time for the fructose to be zero, (h)
$t_{L,Eth}$	lag phase or time to exponential ethanol production, (h)
t_L	time to exponential fructose fraction, (h)
X_0	biomass concentration at the beginning of fermentation, (g/L)
X_0	cell mass concentration at the beginning of fermentation, (g/L)
X_L	biomass concentration at the end of fermentation, (g/L)
X_L	cell mass concentration at the end of fermentation, (g/L)
$Y_{E/S}$	ethanol yield of its theoretical value, (%)
Y_F	fructose yield, (%)
Y_S	sucrose lose, (%)
$Y_{X/S}$	biomass yield, (g/g)

Greek Letters

γ_{Frcts}	fructose concentration (g/L)
$\gamma_{Frcts,m}$	initial fructose concentration (g/L)
η	fructose fraction in sugar (%)
η_{Frcts}	fructose fraction in sugar (%)
$\eta_{Frcts,0}$	initial fructose fraction in sugar (%)
$\eta_{Frcts,m}$	potential maximum fructose fraction in sugar (%)
μ	specific growth rate of cell mass (h^{-1})

Greek Letters

γ_{Eth}	ethanol concentration, g/L
$\gamma_{Eth,m}$	potential maximum ethanol concentration, g/L
γ_{Frcts}	fructose concentration, g/L
$\gamma_{Frcts,m}$	initial fructose concentration, g/L
η_{Frcts}	fructose fraction in sugar, %
$\eta_{Frcts,0}$	initial fructose fraction in sugar, %
$\eta_{Frcts,m}$	potential maximum fructose fraction in sugar, %
η	fructose fraction in sugar, %
μ	specific growth rate of cell mass, h^{-1}

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2. Kinetic Modeling and Enhanced Production of Fructose and Ethanol From Date Fruit Extract

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