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## RESEARCH NOTE

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# OPTIMIZATION OF HFDS PRODUCTION FROM DATE SYRUP

*M. Jamshidi Mokhber, I. Alemzadeh\* and M. Vossoughi*

*Chemical and Petroleum Engineering Department, Sharif University of Technology  
P.O. Box 11365-6891, Tehran, Iran  
jamshidim2@yahoo.com - alemzadeh@sharif.edu – vosoughi@sharif.edu*

\*Corresponding Author

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**Abstract** Fructose is utilized as a common sweetener in many food industries. Today, a large amount of HFCS (high fructose corn syrup, an equilibrium mixture of glucose and fructose) is produced by glucose isomerase, immobilized enzymes from corn starch. There are a lot of date palms in Iran. Date is a favorable source of carbohydrates, and a suitable source for HFDS (high fructose date syrup) production. In this investigation, date syrup properties were first determined and the operational conditions for glucose isomerase application were optimized respectively. Ion exchange chromatography has been utilized for carbohydrate separation. Fructose obtained from chromatographic column reached 90 % fructose syrup and glucose obtained was converted to fructose (42 % conversion) in a fixed bed glucose isomerase enzyme reactor. A mixture of this equilibrium syrup with fructose syrup obtained from chromatographic column produced 55 % fructose syrup.

**Keywords** High Fructose Date Syrup (HFDS), Chromatographic Column, Enzyme Reactor, Glucose Isomerase, Ion Exchange Chromatography, Date Palm

**چکیده** فروکتوز به عنوان یک شیرین کننده متداول در بسیاری از صنایع غذایی مصرف می شود. امروزه مقدار زیادی از شربت غنی از فروکتوز که مخلوط تعادلی گلوکز و فروکتوز است که بوسیله آنزیم گلوکز ایزومراز نشاسته ذرت تولید می شود. کشور ایران دارای مقدار زیادی محصول خرما است که منبعی سرشار از کربوهیدراتهاست. از این رو منبع مناسبی برای تولید شربت غنی از فروکتوز می باشد. در این تحقیق، ابتدا خواص شربت خرما اندازه گیری و تعیین شده سپس شرایط عملیاتی برای کاربرد آنزیم گلوکز ایزومراز بهینه شده است. از کروماتوگرافی تبادل یونی جدا سازی کربوهیدراتها استفاده شده است. شربت فروکتوز بدست آمده از ستون کروماتوگرافی به میزان ۹۰ درصد غنی از فروکتوز می باشد و گلوکز جدا سازی شده توسط کروماتوگرافی، بوسیله آنزیم گلوکز ایزومراز در یک راکتور بستر ثابت آنزیمی به میزان ۴۲ درصد به فروکتوز تبدیل می شود. مخلوطی از شربت تعادلی گلوکز و فروکتوز بدست آمده از راکتور آنزیمی، با فروکتوز جدا سازی شده در ستون کروماتوگرافی شربتی با غلظت ۵۵ درصد فروکتوز تولید می کند.

## 1. INTRODUCTION

The fruit Date is produced largely in hot regions of South West Asia and North Africa, this fruit is full of carbohydrates as a rich source of calories. Date and its by products are used in food and pharmaceutical industries such as ethanol, citric acid, baker yeast and high fructose date syrup (HFDS) production [1].

The world production of date has increased to 5.4 million tones in 2002 and 65 percent of world

production is produced in Persian Gulf region. Iran which produces 18 percent of global date production, is a major date producing country [1,2]. The date crop in Iran during the farming year 1999-2000 was over one million tones but Iran exported only 100,000 tones during 1999-2000 and date consumption was only 100-200 thousand tones, therefore a large portion of date is unused in the country [3,4].

Sucrose is the main sweetener in the world, but after 1967 other sweetener such as high fructose

syrup replaced sucrose. Today, high fructose syrup is used as sweetener in many countries such as USA, Canada, European Community, Japan, South Korea and Argentina. The world production of high fructose syrup has increased from 3 million tones in 1980 to 8 million in 1995 and 12 million in 2004 [5,6]. The most common raw material used for the production of HFCS is corn starch. The production of HFCS from starch comprises of three major processes: liquefaction of starch by  $\alpha$ -amylase, saccharification of starch by the combined action of amyloglucosidase along with pullulanase and isomerization of glucose by glucose isomerase [7].

The successful development of fructose syrup production lies in the discovery of glucose-isomerizing enzyme. Isomerization of glucose to fructose using glucose isomerase is an equilibrium reaction; this reaction produces syrup containing 42 % fructose and 58 % glucose which is commonly used in sport drinks. Other typical types of HFCS are: HFCS 90 (most commonly used in baked goods) which is approximately 90 % fructose and 10 % glucose; HFCS 55 (most commonly used in soft drinks) which is approximately 55 % fructose and 45 % glucose. HFCS 55 is comparable to sucrose and HFCS 90 is sweeter than sucrose as fructose is sweeter than glucose [7]. These syrups are produced with ion exchange chromatography. A liquid chromatography method known as simulated moving bed (SMB) chromatography is the most economical method for an industrial-scale process. It needs an industrial separator technology that is being used in sugar industry to separate glucose and fructose [8,9].

Date is a source of fructose and glucose which could be separated by ion exchange chromatography column, then glucose could be converted to equilibrium mixture of fructose and glucose in isomerization column [10,11].

In this investigation, date syrup was analyzed, 90 % fructose syrup was produced by ion exchange chromatography column using date syrup and residual glucose was isomerized using immobilized glucose isomerase column.

## 2. MATERIAL AND METHODS

### 2.1. Materials Glucose Isomerase Sweetzyme T

from *Streptomyces murinus* was obtained from NOVO Nordisk, Ion exchange resin Amberlite CR1320Ca was obtained from Rohm and Hass and date syrup from Khorma-bon-jonoob.

**2.2. Methods** Iranian date syrup was analyzed and its properties such as Brix, density, calcium, protein and carbohydrates content were determined following the procedures [12]. EDTA method was used for calcium determination and Nelson-somogy and Cystein-carbazol methods were used for carbohydrates determination following the procedure used by also HPLC method for more accuracy [12]. Molisch test was used for carbohydrates determination for chromatography column effluent [12,13].

Enzyme assay: The enzyme reaction mixture contained 50 ml of Tris buffer (pH = 7.5), 9 g glucose (1M), 0.5 ml  $MgSO_4 \cdot 7H_2O$  (1M) as cofactor and 0.1 ml  $Na_2S_2O_5$  (1M) [14]. One unit of immobilized glucose isomerase activity (GI) was defined as micromoles of fructose produced per minute at 60°C and pH = 7.5. The reaction was stopped by separating enzyme and adding 1 ml of 0.5 N perchloric acid to 9 ml of reaction mixture, and produced fructose was determined with HPLC or Cystein-carbazol method [14].

The optimum temperature of GI ranges from 60 to 80°C and the optimum pH ranges generally between pH 7 to 9, but these ranges are a little different for different GI producing species [14]. Temperature ranges from 50 to 80°C was studied for determining optimum temperature for enzyme activity. Enzyme was added to reaction mixture and the amount of fructose produced was determined after 30 minutes. For enzyme stability, immobilized glucose isomerase was added to 50 ml Tris-HCl buffer at pH = 7.5 under temperature ranges 50-80°C for 2 hours, then enzyme was separated and added to reaction mixture at 60°C for 30 minutes and fructose was determined [14].

As optimum pH for enzyme activity, pH ranges from 6 to 8 was studied. Enzyme was added to reaction mixture and the amount of fructose produced at 60°C was determined after 30 minutes. For determining optimum pH for enzyme stability, enzyme was added to 50 ml phosphate buffer at pH ranges from 6 to 8 and it was maintained at 60°C for 2 hours, then enzyme was separated and added to reaction mixture and produced fructose was

determined after 30 minutes [14].

Isomerization was studied for 14 hours under optimum operational conditions. In this step two quantities of enzyme, 0.25 g and 0.5 g enzyme in 50 ml reaction mixture were examined.

Ion exchange chromatography: Glass column with the diameter of 3 cm and length of 40 cm was packed homogeneously with the ion exchange resin Amberlite CR1320Ca by slurry method [15], in this method slurry of resin in deionized water was poured into the column and the excess water was drained from the bottom of column. The packed height of glass chromatography column was generally 30 cm and bed volume was 200 ml.

To optimize feed concentration, three synthetic solution of glucose and fructose (50% w/w) with the concentration of 40, 80 and 90 g/l was injected to the column (10 ml or 5 percent of bed volume), then the column was rinsed with 5 ml/min deionized water for 75 minutes and the column effluent was collected in small sampling tubes from 5 to 60 minutes after rinsing the chromatography column. The eluted fractions were first determined by molisch test and then by HPLC method for more accuracy [13].

Date syrup with optimum feed concentration was injected to the column under the same conditions and the column effluent was collected and analyzed by molisch test and HPLC method. The operational condition was temperature 35°C.

Batch isomerization of glucose: Fructose rich

product from chromatography column was used as 90 % fructose syrup and glucose rich product was used as isomerization feed. Glucose obtained from chromatography column was concentrated by a vacuum evaporator until the volume was reduced to one fifth of its initial volume, this syrup with concentration of 116 g/l glucose and 18 g/l fructose (86 % glucose and 14 % fructose) was a suitable feed for isomerization column. Enzyme was added to 50 ml of concentrated glucose syrup and maintained at 60°C and pH = 7.5, the production of fructose was measured at different intervals, for 14 hours.

Continuous isomerization: Glass column with a diameter of 3 cm was packed with glucose isomerase Sweetzyme T to the height level of 30 cm and concentrated glucose syrup obtained from vacuum evaporator was pumped through the column. Hot water (60°C) also was pumped through water jacket which surrounded the column for fixing temperature and the effluent was collected and determined by HPLC. Four different feed flow rates (4,6,8 and 10 ml/min) were pumped through the column, to optimize the feed flow rate.

### 3. RESULTS AND DISCUSSION

All the results obtained and presented in this investigation were the averages of two replicates. Table 1 shows the results of the date syrup analysis

TABLE 1. The Properties of Experimented Date Syrup.

Property	Value	Unit
Density	1.347	g/ml
Protein	0.5	%
Calcium	75	ppm
Fructose	37	%
Glucose	35	%
Sucrose	5	%
Brix	74	%

including density, Brix, calcium, protein and carbohydrates for 100 g date syrup [12,13].

### 3.1. Optimization of Operational Conditions

Figure 1 shows the effect of temperature on enzyme activity and stability. Enzyme activity increases with temperature to 70°C but enzyme stability decreases after 60°C. Enzyme activity was measured after 30 minutes, but for stability, the activity was measured after 2 hours, so the difference for reduction of stability comparing to activity is the length of time. The optimum temperature was selected 60°C, because enzyme column must be used for a long period of time. Figure 2 presents the optimum pH for enzyme activity and stability. The optimum pH for stability

was 7.5 and for activity was 8 as it is shown in Figure 2.

Figure 3 shows the effect of time on fructose production under experimental conditions, 12 hours is the optimum time in fructose production.

### 3.2. Glucose and Fructose Separation

Figure 4 shows the percent of glucose and fructose in the effluent of chromatography column for 40 g/l synthetic mixture of glucose and fructose. As it is shown in this figure, optimum glucose concentration was eluted from column after 15 minutes and optimum fructose after 35 minutes.

Glucose rich product defined as ion-exchange column effluent after 15 minutes and fructose rich product defined as column effluent after 35

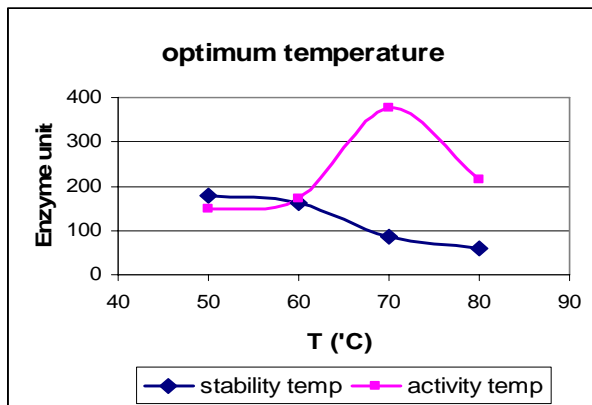


Figure 1. The effect of temperature on activity and stability of immobilized glucose isomerase, pH = 7.5.

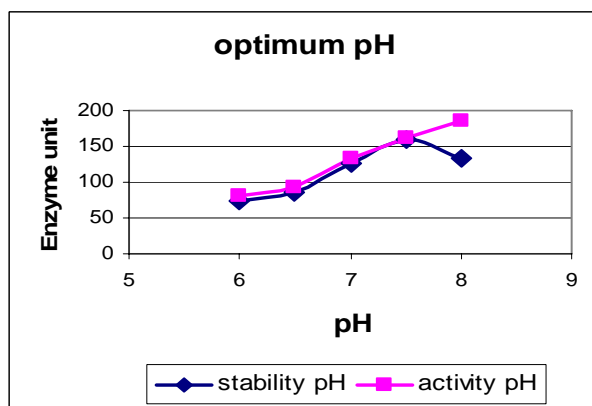
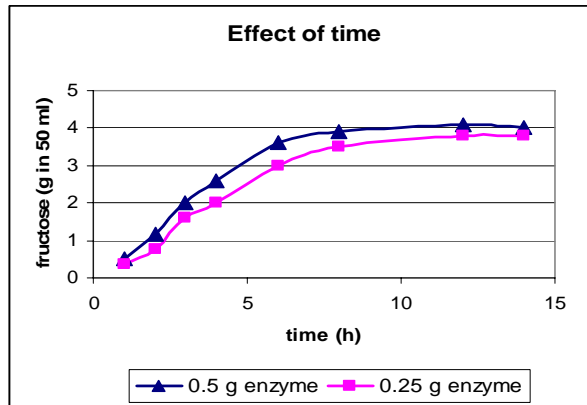
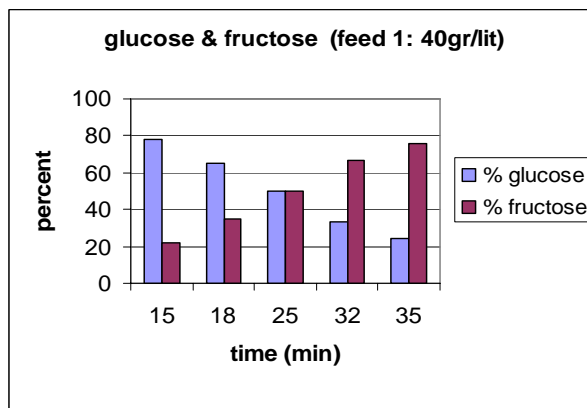


Figure 2. The effect of pH on activity and stability of immobilized glucose isomerase at 60°C.



**Figure 3.** The effect of time on glucose isomerization using glucose isomerase at 60°C, pH = 7.5 and initial glucose concentration was 9 g in 50 ml.



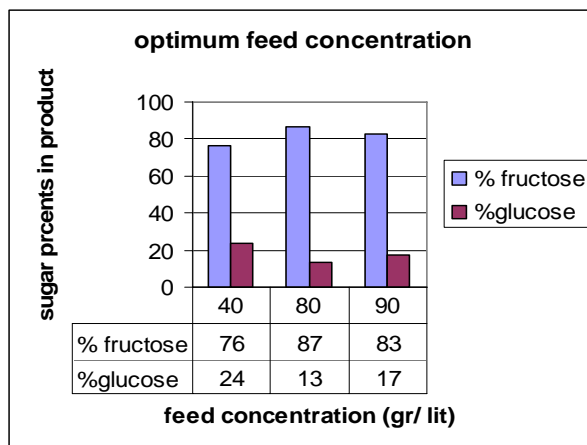
**Figure 4.** Percents of carbohydrates in the chromatography column effluent for 40g/l synthetic mixture of glucose and fructose at 35°C.

minutes. Figure 5 presents the percents of glucose and fructose in fructose rich product for three synthetic solution of 40, 80 and 90 g/l. Fructose obtained from chromatography column after 35 minutes was 76 percent for feed 1 (40 g/l) and 87 percent for feed 2 (80 g/l) and 83 percent for feed 3 (90 g/l), so the optimum feed concentration for these solutions was 80 g/l.

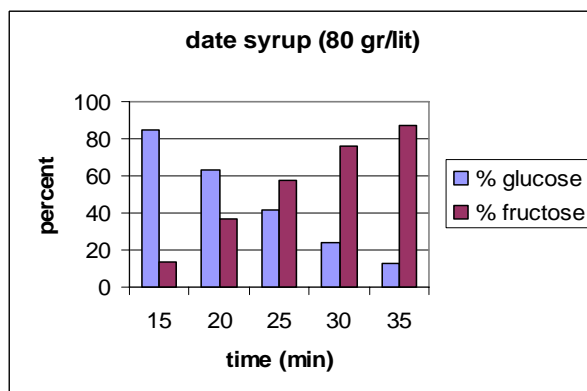
Date syrup with the concentration of 80 g/l was injected to the column and the effluent was collected and the concentration of sugars determined by HPLC method. Sucrose eluted the column at first, then glucose and fructose respectively.

Figure 6 shows the percentages of carbohydrates in the effluent of chromatography column with time. Fructose was 87 percent in column effluent after 35 minutes and glucose was 85 percent after 15 minutes. These figure present changes in the percentages of glucose and fructose in the eluted fractions.

**3.3. Isomerization of Glucose Obtained from Date Syrup Chromatography** Glucose rich product obtained from date syrup ion-exchange chromatography was isomerized to fructose in the presence of glucose isomerase. This syrup was first concentrated by a vacuum



**Figure 5.** Percents of carbohydrates in fructose rich product, column effluent after 35 minutes for three synthetic mixtures of glucose and fructose at 35°C.



**Figure 6.** Percents of carbohydrates in the chromatography column effluent for initial date syrup of 80g/l at 35°C.

evaporator, such as 50 ml of this glucose syrup contained 86 % glucose and 14 % fructose. Figure 7 presents glucose consumption and fructose production with time on a batch scale; the reaction was in equilibrium after 12 hours.

Concentrated glucose rich product from date syrup chromatographically was pumped through the enzyme column with different flow rates. Figure 8 shows glucose consumption and fructose production in isomerization column with different feed flow rate. As it is shown in this figure, the optimum flow rate was 4 ml/min. Fructose production and glucose consumption was optimum at this flow rate and the equilibrium syrup of glucose and fructose was

produced. At lower flow rates, increasing in the settling time of equilibrium syrup of glucose and fructose in enzyme column led to some by-products production such as psicose [16].

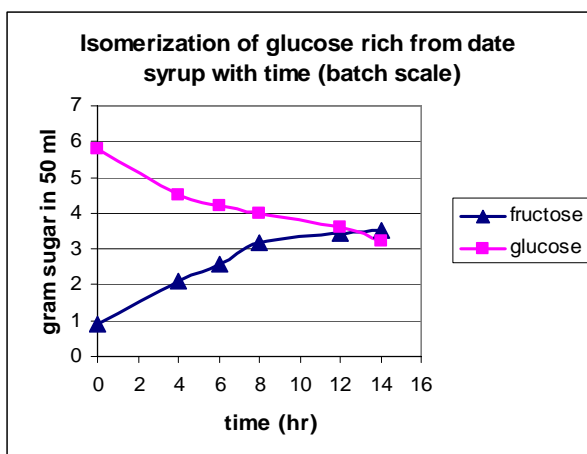
#### 4. CONCLUSION

Date syrup as a valuable source of carbohydrates was analyzed. Enrichment of date syrup sugars using immobilized glucose isomerase and ion exchange separation chromatography column was studied.

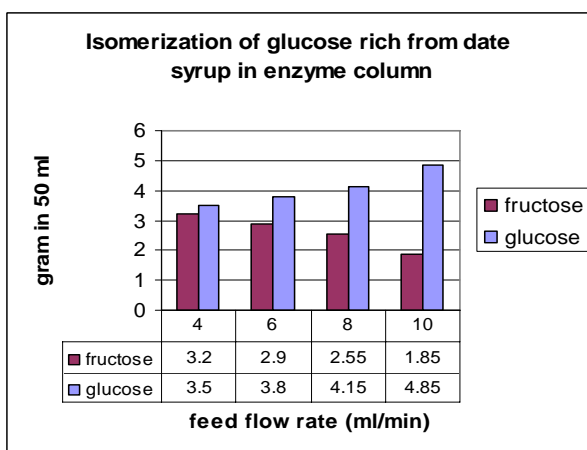
Optimum operational conditions for enzyme activity and stability of glucose isomerase (NOVO NORDISK) were determined: temperature 60°C and pH 7.5.

Date syrup is a mixture of carbohydrates: sucrose, glucose and fructose. In ion-exchange chromatography column, sucrose eluted the column at first, then glucose and fructose respectively. Glucose and fructose form complexes with calcium ion of resin with different stabilities, so fructose eluted the column at last because fructose-calcium

complex was more stable than glucose-calcium complex [16]. Each test lasted for 75 minutes in order to wash the column properly for next injection. The height to diameter ratio of isomerization column was 10 and the operational condition temperature were 60°C and pH = 7.5. Feed was pumped through the column with different flow rates (H/D = 10) and optimum flow rate was 4 ml/min. At higher flow rates, produced fructose was low and at lower flow rates undesirable by-products such as psicose was produced [16].



**Figure 7.** Glucose consumption and fructose production in 50 ml of concentrated glucose syrup obtained from date syrup in the presence of GI in batch scale (glucose syrup contained 116 g/l glucose and 18 g/l fructose) T = 60°C and pH = 7.5.



**Figure 8.** Glucose consumption and fructose production in 50 ml of enzyme column effluent for concentrated glucose syrup obtained from date syrup isomerization (glucose syrup contained 116 g/l glucose and 18 g/l fructose) T = 60°C and pH = 7.5.

## 5. ACKNOWLEDGMENTS

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