Alex. J. Fd. Sci. & Technol. *Vol. 7, No. 2, pp. 1-11, 2010*

Bioproduction of Fructose from Chicory (*Cichorium intybus*) Roots and Jerusalem Artichoke (*Helianthus tuberosus)*Tubers

Mona, I. Massoud[1], Amal, M. Abd El–Razek[2] & Hoda Mahrous[3]

[1] Sugar Crops Research Institute, Agric. Research Center, El–Sabahia, Alex. Egypt.

[2] Food Science and Technology Dept, Fac. of Agric., El–Shatby. , Alex. Univ., Egypt.

[3] Industrial Biotech. Dept, Genetic Eng. & Biotech.Res. Institute, Menufiya Univ., Egypt.

Received: 6 June 2010 ***Revised: 10 September 2010*** ***Accepted: 18 September 2010***

ABSTRACT

Fructose is a natural sugar widely used in beverage production and pharmaceutical industries . Therefore, the present study aimed to utilize the chicory roots (*Cichorium intybus*) and Jerusalem artichoke tubers (*Helianthus tuberosus*) extracts as inulin source for the production of fructose by inulinase (EC 3.2.1.80) hydrolysis . *Aspergillus* strain was isolated from sugarcane bagasse and identified as *A.niger* isolate MonEg (GQ890276) using PCR system. Under the optimized conditions, the aforementioned *A. niger* isolate produced maximum inulinase (54.70 U/ml) on a medium containing chicory roots powder as a carbon source after 72hr at 30°C. The specific activity of the enzyme was 21.06 U/mg toward inulin with a purification fold of 6.77 with ammonium sulphate precipitation at pH 5.5 and 50°C. The enzyme incubated with extracts of chicory roots (CR) and Jerusalem artichoke (JA) was prepared in 0.1M sodium acetate buffer (pH 5.5) at 50°C. The products of the enzymatic reaction were analyzed by dinitrosalicylic acid and HPLC. Data showed that the fructose yield ranged from 0.31 to 0.60 g/ g of inulin in the samples after 3 hr of reaction and nearly 58% and 39% of initial inulin had been hydrolysed under the same conditions in CR and JA samples, respectively. The fructose productivities were 0.57g /100ml/ hr and 0.31g /100ml/ hr for CR and JA samples, respectively. At a constant initial inulin concentration and changes in enzyme activity level, the fructose productivity increased to 0.48g /100ml/ hr in JA samples after 3 hr of incubation .So, chicory roots and Jerusalem artichoke are considered as natural inulin source that can be utilized for the production of fructose. The chicory powder is considered as a good source of carbon for inulinase production.

Key words: Aspergillus niger, inulinase , pharmaceutical industries, inulin, fructose.

INTRODUCTION

Fructose “a natural sugar” is one of the most widespread sugars in the world and is utilized by the food, drink and pharmaceutical industries. Fructose shows sweetening power 70% higher than sucrose. It is considered as a safe alternative sweetener to sucrose because it has beneficial effects in diabetic patients, it increases the iron absorption in children and has a higher sweetening capacity. It represents a suitable table sugar and as ingredient for some food products and as substrate for fermentative processes (Pawan, 1973).

Fructose can be produced from starch by enzymatic methods involving α–amylase, amyloglucosidase and glucose isomerase resulting in the production of products consisting of fructose (45%) ,oligosaccharides (8%) and glucose (50%) (Kochhar, *et al*., 1997, Zhang *et al*., 2004, Gill *et al*., 2006). Separation of fructose from high fructose corn syrup is costly and thus makes this method uneconomical. Fructose can also be obtained by acid hydrolysis of the inulin but it is easily degraded at low pH and the process gives rise to colouring of the inulin hydrolysate and by–product formation in the form of difructose anhydride, which has practically no sweetening properties (Vandamme & Derycke, 1983).

It is worthy to mention that, microbial fructanohydrolase plays an important role in the hydrolysis of inulin for its commercial exploitation. Enzymatic hydrolysis of inulin using inulinase is easier, more direct, cheaper and quicker (Zittan,1981, Pandey, *et al.,* 1999, Ettalibi & Baratti, 2001) and gives a yield of about 90–95% fructose (Gupta, *et al*., 1994, Vranesic *et al*., 2002). Inulinases are fructofuranosyl hydrolases produced by a wide array of organisms, bacteria, molds and yeasts and plants (Nakamura *et al*., 1996, Kato *et al*., 1999, Pessoni *et al.,* 1999, Shaheen *et al.,* 2008). Among them, *Aspergillus* strain is the most common and preferred choice

(Skowronek & Fiedurek,2004). The high–fructose syrup obtained from enzymatic hydrolysis of inulin can be utilized in the production of ethanol (Ohta *et al*.,1993, Nakamura ,*et al*., 1995).

Inulin is a linear polymer of D–fructose joined by β (2–1) linkages and terminated with a D–glucose molecule linked to fructose by β (1–2) bond, as in sucrose (Modler, 1994). Inulin serves as a storage polymer in many members of the compositae such as *Cichorium intybus* (chicory), and *Helianthus tuberosus* (Jerusalem artichoke) (Watherhouse & Chatterton, 1993). According to Barta (1995), these are the most suitable inulin sources for utilization at industrial scale, whose productivity is estimated at 2.5 and 4.5 ton/ha, respectively.

Although ,chicory is cultivated today for its roots which is utilized for the industrial production of inulin, chicory used in food ingredient (De Mastro, *et al.,* 2004), for non–food purposes, for which worldwide markets are growing rapidly and for forcing the growth of chicons, a winter vegetable used in Northern Europe. Jerusalem artichoke tubers have a good potential for production of fructose and as a feed stock for production of ethanol (Ongen–Baysal *et al.,* 1994). In chicory, inulin yield varies from 8 to 12.2 ton /ha comprising an average about 57% of total dry matter and 74% of the root. The inulin yield of Jerusalem artichoke varied between 4.0 to 6.7 ton /ha, with an average comprising 28% of total dry matter and 71% of the tuber (Meijer, *et al*., 1993).

The objective of the present study was to isolate and identify *Aspergillu*s strain from sugarcane bagasse as industrial waste and utilization of some agro–industrial sugar crops to produce highly active inulinase . Moreover, this research was carried out as an attempt to utilize inulin obtained from chicory roots and Jerusalem artichoke tubers to produce fructose.

MATERIALS AND METHODS

Materials

The chicory roots (CR) (*Cichorium intybus)* were harvested at maturity in experimental field of Sugar Crop Research Institute, Agricultural Research Center at Giza, Egypt. Jerusalem artichoke tubers (J.A) (*Helianthus tuberosus,* L.) cultivars were obtained from Sabahia Horticultural Research Station, Agricultural Research Center, Alexandria, Egypt. The CR and JA were washed with water, sliced, then dried and milled. The prepared samples with approximately 5.61% moisture content were stored in dry glass container for further use.

Sugarcane bagasse was collected from an experimental field of Sugar Crop Research Institute, Agric. Res. Center, El–Sabahia– Alexandria, Egypt.

Sugar beet molasses was obtained from Delta Beet Sugar Company, Kafr El–Sheikh Governorate, Egypt.

Pure inulin was purchased from Universal Fine Chemicals PVT–LTF and all the other chemicals were of the highest analytical grade.

Methods

Isolation and molecular identification of the microorganism

PCR and sequencing

The strain isolated from dried sugarcane bagasse was identified as *Aspergillus niger* according to the physiological and biochemical characteristics. The total genomic DNA of *Aspergillus niger* strain was isolated and purified according to the instructions of Qiagen's DNeasy Kit (Qiagen, USA). Amplification and sequencing of 18S rDNA and ITS from *A. niger* were performed using (MJ PTC 200), according to the methods described by Guillemant & Drouard (1992). Sequencing of the PCR products was performed by the service of (Macrogen, Korea).

Sequence analysis

The strain was identified by using NCBI–BLAST & Genbank data base (http://www.ncbi.nlm.nih. gov/BLAST). Multiple sequence analysis was done by Bioedit software.

Total fungal count

The isolate strain was preserved on potato dextrose agar (PDA) slants at 4°C, and was sub–cultured every month. The medium of PDA was prepared as given in Difco (1974). For inoculums preparation, the culture was inoculated on PDA slants at 30°C for 4–6 days and the spores were suspended in 8ml of sterile–distilled water. The pour plate method was used. Ten ml of sample were aseptically poured into sterile bottles and diluted serially in distilled water up to a 10 –8 dilution. One ml of a dilution was mixed with Sabouraud Dextrose Agar (SDA). The medium was allowed to solidify and then incubated at 37°C for 24 – 48 hr.

Preparation of chicory roots (CR)and Jerusalem artichoke tubers (JA) extracts

The CR and JA extracts were prepared according to the procedure described by Massoud, *et al.*

(2009) and were used as a source of inulin to study fructose production rate.

Culture media for enzyme production.

Two ml of a spores suspension (3.4 x 108 spores/ml) were inoculated in Erlenmeyers flasks containing 2.0% of carbon source (sucrose, bagasse, sugar beet molasses, inulin, CR and JA powders and extracts), enriched with 1.5% $(NH_4)_2SO_4$, 0.5% yeast extrat, 0.5% K_2HPO_4, 0.2% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4.7H_2O$, and final pH was adjusted to 5.5.The flasks were agitated in a rotatory incubator shaker (Innova 4230 ,Edison,NJ., USA), for 72 hr, at 200 rpm and temperature 30°C. After the cultivation, the mycelia were separated by centrifugation (8000xg, for 20 min at 4°C), filtration and the supernatant was used as a crude enzyme solution for enzyme assay and protein purification (Cruz, *et al.,*1998).

Assay of enzymes activities

Enzymes were assayed by measuring the concentration of reducing sugars released from inulin and sucrose.The reaction mixture containing 0.1 ml of diluted crude enzyme and 0.9 ml of 2% inulin or 2% sucrose (dissolved in 0.1 M acetate buffer, pH 5.5) was incubated at temperature 50°C. After incubating for 30 min, aliquots of 0.5 ml were withdrawn and the increase in reducing sugars was estimated by a 3,5–dinitrosalicylic acid (DNS) method (Miller,1959). Absorbance was measured at wavelength 575 nm. A higher absorbance indicated a high level of reducing sugars produced and consequently, a high enzyme activity. One inulinase unit is the amount of enzyme which forms 1 μmol fructose per min. One invertase unit is the amount of enzyme which hydrolyses 1 μmol sucrose per min under the same conditions. The ratio between these two activities is commonly expressed as inulin/sucrose (I/S) (Vandamme & Derycke ,1983). Protein content was measured according to the method described by Bradford (1976), using bovine serum albumin (BSA) as a standard.

Enzyme purification

The crude enzyme solution was first precipitated by ammonium sulfate (80% saturation) followed by overnight dialysis at 4°C against sodium acetate buffer (pH 5.5).Then, the resulting crude enzyme solution was freez–dried using lyophyilizer (Telstar, cryodos–50, Spain) and was stored at –18°C.

Properties of exoinulinase

The effect of pH on purified inulinase activity was determined by incubating the crude enzyme solution at different values of pH (from 3.0 to 7.0) using 0.1 M sodium acetate buffer at 50°C.

The optimum temperature was determined by measuring the enzymatic activity in acetate buffer, (pH 5.5) at the temperature ranges from 20°C to 70°C.

Determination of biomass

After incubation, the mycelia mass of *A. niger* was collected by centrifugation at 3500xg for 20 min at 4°C.The biomass was determined .

Production of fructose by hydrolysis of CR or JA tubers inulin extracts

A weight of 0.6mg of enzyme(562U) was incubated with extracts of CR and JA prepared in 0.1M sodium acetate buffer (pH 5.5) at 50°C. The products of enzymatic reaction were analyzed by HPLC (Agilent1100,USA) using a Thermo,APS–2Hypersil column (250 x 4.6mm) with a refraction index detector, RID 6–A (Shimadzu Co., Kyoto, Japan) as described by Kim, *et al.* (1997). Acetonitrile: water (80:20) served as the mobile phase. A sample volume of 20 μl was run at a flow rate of 2 ml/min for 15 min at 20ºC. Fructose and inulin were identified and quantified by comparing their retention times to known previously injected standards (inulin, fructose, glucose and sucrose).

Fructose yield (YF/S) and volumetric productivity of fructose (QF) were calculated according to the following equation, (Sirisansaneeyaku, *et al.,* 2007):–

- Fructose yield based on inulin ($Y_{F/S}$)= $[F_t]–[F_0]/[S_0]$; where $[F_t]$ and $[F_0]$ are concentrations of fructose at t and zero, time respectively, and $[S_0]$ is concentration of inulin at time zero
- Volumetric productivity of fructose (Q_F) $=[F_t]–[F_0]/t–t_0$, where t and t_0 time of incubation at t and zero, time respectively.

RESULTS AND DISCUSSION

3.1. Identification of microorganism

According to sequencing and sequence analysis results, the isolate was identified as *A .niger* isolate MonEg submitted into genbank database under accession No. of (GQ890276) (Figure 1).

```
Mon.    CCGCTTGTCGGCCGCCGGGGGGGCGCCTTTGCCCCCCGGGCCCGTGCCCGCCGGAGACCC 175
Asp.    CCGCTTGTCGGCCGCCGGGGGGGCGCCTTTGCCCCCCGGGCCCGTGCCCGCCGGAGACCC 175
0062    CCGCTTGTCGGCCGCCGGGGGGGCGCCTTTGCCCCCCGGGCCCGTGCCCGCCGGAGACCC 175
.LT3    CCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGACCC 300
        **************************** *******************************

Mon.    CAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAAC 235
Asp.    CAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAAC 235
0062    CAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAAC 235
.LT3    CAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAAC 360
        ************************************************************

Mon.    TTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACT 295
Asp.    TTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACT 295
0062    TTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACT 295
.LT3    TTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACT 420
        ************************************************************

Mon.    AATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGG 355
Asp.    AATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGG 355
0062    AATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGG 355
.LT3    AATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGG 480
        ************************************************************
```

Fig. 1: Multiple sequence alignment of 18 S rRNA gene of our isolate and other isolates from genbank data base

Inulinase Production by *A .niger* isolate MonEg (GQ890276)

Effect of carbon sources

The results indicate that inulinase production by *A. niger* isolate MonEg (GQ890276) was influenced greatly by different carbon sources in the medium as shown in Table (1) and Fig. (2a). The maximum inulinase production (54.70 U/ ml) was observed with media containing CR powder, followed by CR extract (42.58 U/ ml) as a source for carbon,whereas inulin (40.59 U/ ml) was tailed behind. However, the lowest inulinase (10.35 U/ ml) was that produced in the medium containing sugar beet molasses . Invertase activity was measured in the media, as a proportional correlation between invertase and inulinase. The medium which contained JA powder as a carbon source gave the highest invertase activity (13.17 U/ ml). Also, The ratio of inulinase activity to invertase activity (I/S) can be used as a criterion to characterize the enzyme. Maximum I/S ratio (4.77) was noticed in CR powder culture whereas the lowest I/S ratio (2.66) was observed in case of the culture containing sugar beet molasses as a source of carbon. This is an indication that chicory inulin is a potential inducer of inulinase. Similar results were previously recorded by Moriyama *et al.* (2002) and Kango (2008) .They reported that I/S ratio ranged between 0.02 and 7.9 for various microbial inulinases.

Table 1: Effect of different carbon sources on inulinase production

Carbon source	Inulinase activity (U/ml)	Invertase activity (U/ml)	I/S
Sucrose	35.28	9.02	3.91
Sugarbeet molasses	10.35	3.89	2.66
Sugarcane bagasse	30.03	6.55	4.58
Inulin	40.59	8.75	4.64
CRpowder	54.70	11.47	4.77
CRextract	42.58	9.21	4.62
JApowder	39.39	13.17	2.99
JAextract	33.89	10.72	3.16

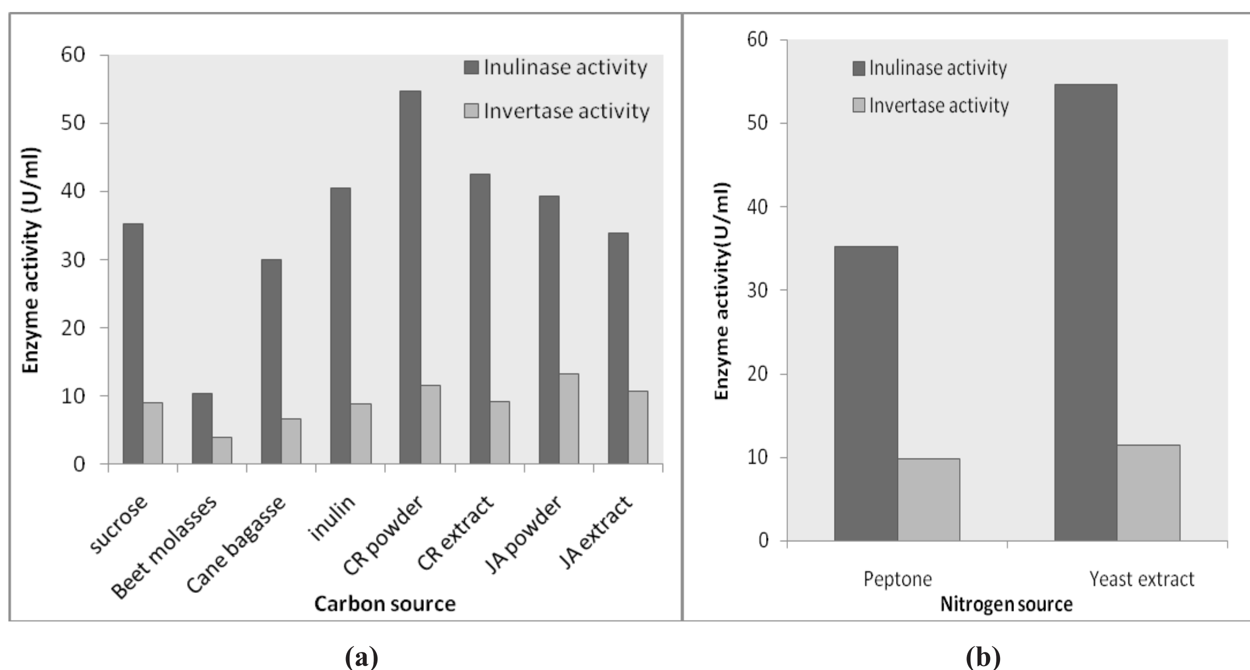
I/S= Inulinase activity/ Invertase activity

Effect of nitrogen sources

As shown in Figure (2b), yeast extract in the culture was found to be suitable as an organic nitrogen source as compared to peptone. Many researchers found that yeast extract was the best nitrogen source for inulinase production (Ongen–Baysal *et al*., 1994 Viswanathan & Kulkarni, 1995, Kango, 2008), whereas Cruz *et al*. (1998) found that *A. niger*–245 produced maximum of inulinase on medium containing casein and dahlia extract.

Effect of incubation time

The results recorded in Figure (3) indicate that there was an increase in inulinase activity. It reached the maximum (54.70 U/ ml) after 72 hr of incubation and decreased thereafter. The invertase activity increased to 11.47 U/ ml at 72 hr of incubation. A decline in the inulinase activity after 72 hr of incubation could be attributed either to decrease in nutrient availability in the medium, or catabolic repression of the enzyme .The cell density increased to the maximum within 3 days and then declined gradually (Figure 3).



(a) **(b)**

Fig .2: Effect of different carbon (a) and nitrogen (b) sources on the production of inulinase

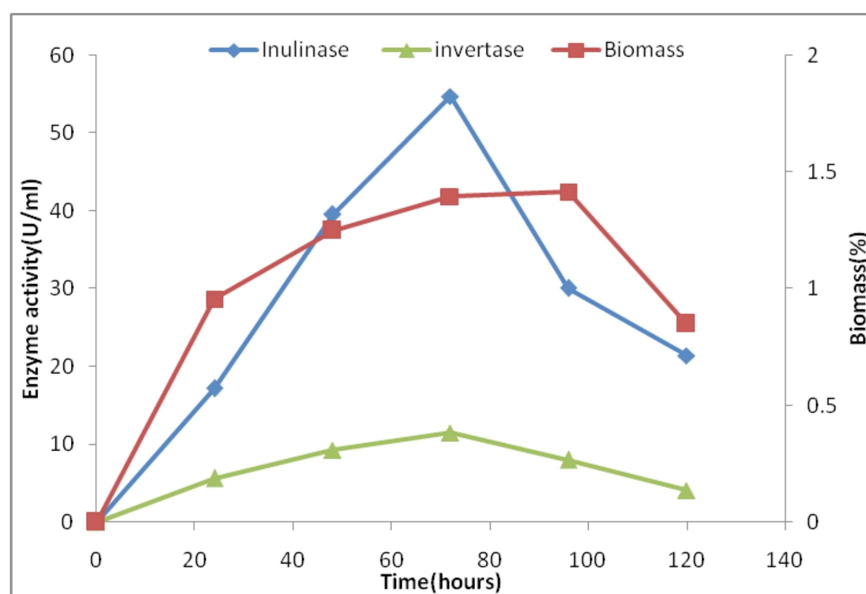


Fig .3: Enzymes activity and growth curve of *A.niger* isolate MonEg as a function of time

Cruz, *et al* (1998) reported that *A. niger*–245 culture gave the maximum inulinase activity (2U/ml) in 48–60 hr whereas Ohta *et al.* (1993) and Jing, *et al* (2003) reported that the maximum inulinase activity was achieved after 5 days. Viswanathan & Kulkarni (1995) obtained very high inulinase activity (290 U/ml) in 72 hr by growing *A. niger* van Tighem UV 11, a mutant, on kuth root powder in a fermenter.

Production of exo–inulinase

Data in Table (2) summarize the activity ,purification fold and yield recoveries of the enzyme precipitated with ammonium sulphate .As it can be observed, *A. niger* isolate MonEg (GQ890276) produced 54.70 U/ml of extracellular inulinase in CR powder culture after 3 days, and the culture filtrate had specific activities of 3.11U/mg towards inulin and 0.65 U/mg towards sucrose. After precipita-

Table 2: Purification of inulinase isolated from *A. niger* isolate MonEg (GQ 890276)

Steps	Volume (ml)	Enzyme activity (units)[a] toward:		Total protein (mg/ml)	Specific activity (U/mg)[b]		Recovery %	Purification factor (fold)
		Inulin	sucrose		Inulin	sucrose		
Culture supernatant	68	54.70	11.47	17.60	3.11	0.65	100	1
$(NH_4)_2$ SO_4 and Dialysis	57	31.29	6.54	1.49	21.06	4.40	47.95	6.77

$(NH_4)_2SO_4$ ammonium sulphate fraction, precipitated between 40% and 80%.

[a] One unit is defined as the amount of enzyme which forms 1 µmol fructose per min.at 50°C, pH 5.5.

[b] The specific activity is expressed in units of enzyme activity per milligram of protein .

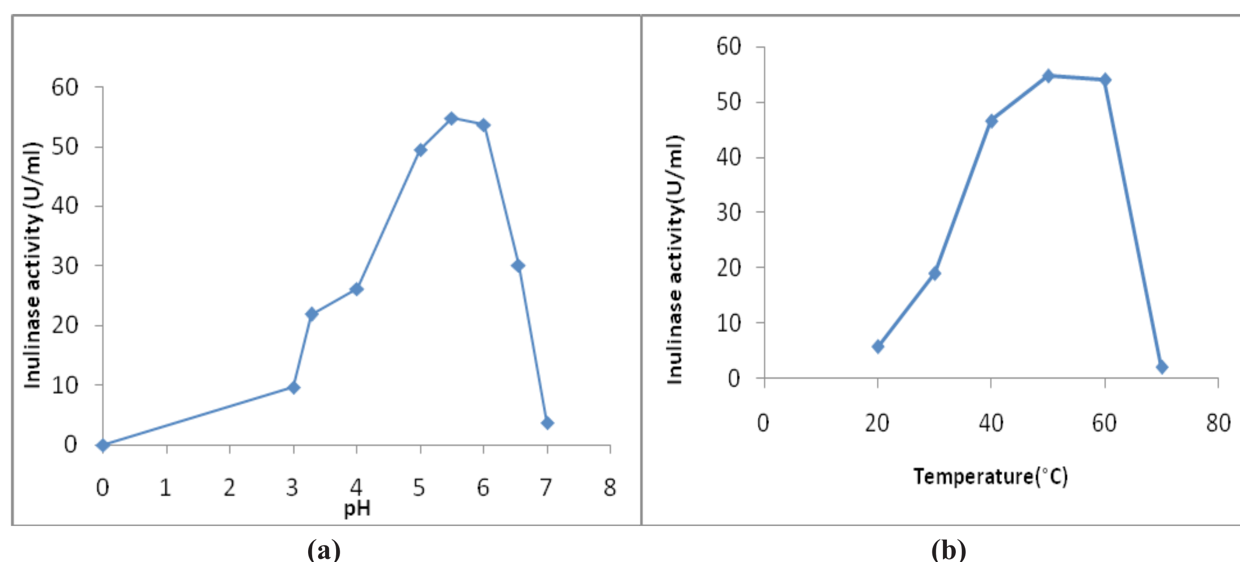
tion with ammonium sulphate (80% saturation), the exo– inulinase solution showed 31.29 U/ml of inulinase activity with a recovery of 47.95%. and the specific activity increased to 21.06 U/mg towards inulin with a purification fold of 6.77. These results are to with that of Nakamura *et al.* (1995) who recommended that *A.niger* 817 produced 67 U/ml of extracellular inulinase in a 5–day–old, and the culture filtrate had specific activities of 2.9 U/mg towards inulin and 0.35 U/mg towards sucrose and the purification steps elevated the specific activities to 71 U/mg towards inulin and 7.6 U/mg towards sucrose (I/S ratio, 9.4).

Effect of pH and temperature on inulinase activity

The results recorded in Fig. (4a, b) show that the optimal pH and temperature for the crude inulinase were 5.5 and 50°C,respectively .The enzyme was stable up to 60°C, but it was inactivated at70°C. Sharma,*et al*. (2006) reported that the optimum temperature and pH for inulinase activity of *Streptomyces* sp. were 60°C and 6.0, respectively. The enzyme of *A. fumigates* showed maximum activity at pH 6.0 and was stable over a pH range of 4.0–7.0, whereas the optimum temperature for the enzyme activity was 60°C (Gill *et al.,* 2006). Kushi *et al*. (2000) reported that the optimum temperature ranged from 55°C to 60°C for the free inulinase from *K. marxianus var. bulgaricus*. The optimum pH for inulinase secreted by *K.marxianus* was 4.4 (Singh *et al.*, 2007).

Fructose production and inulin hydrolysis

Fructose and inulin contents were determined in the studied CR and JA samples using DNS method. The results recorded that the inulin content of CR and JA samples were 41.07% DM and 29.50% DM respectively. On the other hand, the



(a) (b)

Fig .4: Effect of pH (a) and temperature (b) on activities of inulinase from *A.niger* isolate MonEg

fructose content ranged between 2.44and 3.59% for CR and JA samples, respectively. The variation in carbohydrate content may be attributed to the difference in chemical composition of crop type (Gupta & Kaur, 2000).Data presented in Table (3) show the products of enzymatic inulin hydrolysis as determined by HPLC. The fructose content increased gradually up to 1.88 g/100ml and 1.28 g/100 ml for CR and JA extracts, respectively after 3 hr of the reaction. Therefore, the rate of hydrolysis of the inulin reached 58.89 % in CR extract and 38.98% in JA extract after 3hr under the same conditions . As it can be seen from Table (3), the fructose yield based on inulin ($Y_{F/S}$) was 0.60g/ g of inulin and the fructose productivity (Q_F) was 0.57g /100ml/ hr for CR extracts, whereas the $Y_{F/S}$ was 0.31g/ g of inulin and Q_F was 0.31g /100ml/ hr for JA extracts. No oligosaccharides were traced from the HPLC data during the reaction. The crude enzymes hydrolysed a large amount of the initial inulin of CR extract as compared to that of JA extracts. Nakamura, *et al.* (1995) found that 5.0% solution of pure dahlia inulin was completely hydrolyzed by inulinase isolated from *Aspergillus niger* mutant 817 and resulted the volumetric productivity of 410 g reducing sugars/l/ hr. Also, Sirisansaneeyaku, *et al.* (2007) reported that the inulinases produced by *Aspergillus niger* TISTR 3570 and *Candida guilliermondii* TISTR 5844 were appropriate for hydrolysing inulin to fructose.

Effect of change inulinase activity on fructose production

The effect of change of inulinase activity level from 562U to 810U on fructose production from both CR and JA extracts was studied under the same preparation conditions. The results indicate that the change in inulinase activity did not affect the fructose production of CR extract (Table 4). On the other hand, the fructose content of JA extract increased by increasing the enzyme activity to 810U, it reached to 1.81g/ 100ml and the rate

Table 3. The production of fructose from hydrolysis of inulin chicory roots and Jerusalem artichoke tubers extracts using crude inulinase

Sample	Time(min)	Inulin (g/100ml)	Fructose (g/100ml)	Inulin hydrolysate(%)[a]	$Y_{F/S}$[b]	Q_F[c] (g/100ml/hr)
CR extract	0	2.87	0.17	–	–	–
	30	2.07	0.89	27.87	0.25	1.44
	60	1.73	1.27	39.72	0.38	1.10
	90	1.22	1.84	57.49	0.58	1.11
	120	1.18	1.85	58.89	0.59	0.84
	150	1.21	1.86	57.84	0.59	0.67
	180	1.18	1.88	58.89	0.60	0.57
JA extract	0	2.95	0.36	–	–	–
	30	2.34	0.68	20.68	0.11	0.64
	60	2.29	0.73	22.37	0.13	0.37
	90	2.04	1.01	30.85	0.22	0.43
	120	1.99	1.06	32.54	0.24	0.35
	150	1.95	1.11	33.90	0.26	0.30
	180	1.80	1.28	38.98	0.31	0.31

CR extract chicory roots extract(2.87%inulin).

JA extract: Jerusalem artichoke tubers extract (2.95%inulin).

a Inulin hydrolysate (%)=$[S_0]–[S_t]$ / $[S_0]$x 100; where $[S_0]$ and $[S_t]$ are concentrations of inulin at time zero and t, respectively.

b $Y_{F/S}$ = fructose yield based on inulin =$[F_t]–[F_0]$ / $[S_0]$; where $[F_t]$ and $[F_0]$ are concentrations of fructose at time t and zero, respectively.S0 was always measured by the DNS method prior to hydrolysis.

c Q_F = volumetric productivity of fructose= $[F_t]–[F_0]$ / $t – t_0$, where t and t_0 time of incubation at time t and zero.

Table 4. Effect of change in inulinase activity on production of fructose from hydrolysis of inulin of Jerusalem artichoke (JA) and chicory root(CR) extract*

Inulinase activity (U)	Fructose (g/100ml)		Inulin hydrolysate (%)		$Y_{F/S}$		Q_F	
	CR	JA	CR	JA	CR	JA	CR	JA
562	1.88	1.28	58.89	38.98	0.60	0.31	0.57	0.31
810	1.87	1.81	58.54	55.05	0.59	0.49	0.57	0.48

*After 3hr of the reaction

CR extract chicory roots extract(2.87%inulin).

JA extract: Jerusalem artichoke tubers extract (2.95%inulin).

[a] Inulin hydrolysate (%)=$[S_0]$–$[S_t]$ / $[S_0]$ × 100; where $[S_0]$ and $[S_t]$ are concentrations of inulin at time zero and t, respectively.

[b] $Y_{F/S}$ = fructose yield based on inulin =$[F_t]$–$[F_0]$ / $[S_0]$; where $[F_t]$ and $[F_0]$ are concentrations of fructose at time t and zero, respectively.S0 was always measured by the DNS method prior to hydrolysis.

[c]Q_F = volumetric productivity of fructose= $[F_t]$–$[F_0]$ / t – t_0, where t and t_0 time of incubation at time t and zero.

of hydrolysis of the inulin increased from 38.98% to 55.05% after 3hr of the reaction. Also, it can be seen from the data in Table (4) that the fructose yield based on inulin ($Y_{F/S}$) increased to 0.49g/g of inulin and the fructose productivity (Q_F) was 0.48g /100ml/ hr in JA samples. The obtained data showed the same trend as those achieved by other authors who reported that exoinulinase can successively release fructose from the inulin (Kumiko *et al.,* 1999, Kushi*,et al*., 2000, Gong, *et al.*, 2008 & Sheng, *et al.,* 2008).

According these data, it can be concluded that ,100g of CR crop give 26.82g fructose calculated on dry weight basis when using 562U of enzyme activity under the experimental conditions used in the present study. Only, 18.10g of fructose produced from 100g of JA crop as calculated on dry weight basis when 810U of enzyme activity was used.

In conclusion, chicory roots and Jerusalem artichoke are considered as a natural source of inulin that can be utilized for the production of fructose and thereby a significant impact of their use in industrial processes can be invested. The addition of chicory powder as a carbon source was significantly higher than pure inulin for inulinase production.

ACKNOWLEDGEMENTS

The authors are thankful to Prof.Dr Ahmed Rafik El–Mahdy, Prof. of Food Science and Technology, Faculty of Agric., El–Shatby., Alex. University for his support and valuable help.

REFERENCES

Barta, J. **1995**. Inulin containing plants in food processing. Third International Fructan Conference, Logan, Utah, USA, p. 31.

Bradford, M.M. **1976**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein—dye binding. Analytical Biochemistry,**72**: 248–254.

Cruz, V. A., Belote, J. G., Belline, M. Z. & Cruz, R. **1998**. Production and action pattern of inulinase from *Aspergillus niger*–245: Hydrolysis of inulin from several sources. Revista de Microbiologia, **29**: 301–306.

De Mastro G,Manolio, G & Marzi,V. **2004.** Jerusalem artichoke (*Hellanthus Tuberosus* L.) and chicory (*Cichorium intybus* L.): Potential crops for inulin production in the Mediterranean area. Acta Horticulturae (ISHS), **629**: 365–374.

Difco **1974**. Manmal of dehyhrated culture media and reagents of microbiological and clinical laboratory procedures ,Ninth Edition. Reprinted. Difco Laboratores. Detriol,Michigan,USA.

Ettalibi, M. & Baratti, J. C. **2001**. Sucrose hydrolysis by thermostable immobilized inulinases from *Aspergillus ficuum*. Enzyme Microbial and Technology, **28**: 596–60

Gill, P.K., Manhas, R.K.& Singh, P. **2006**. Purification and properties of a heat–stable exoinulinase isoform from *Aspergillus fumigatus*. Bioresource Technology, **97**: 894–902.

Gong, F., Zhang, T,Chi, .Z.M,Sheng, J., Li, J. & Wang, X.H. **2008.** Purification and characterization of extracellular inulinase from a marine yeast *Pichia guilliermondii* and inulin hydrolysis by the purified inulinase. Biotechnolgy and Bioprocess Bioengineering, **13**: 511–517.

Guillemant, P.M. & Drouard, L. **1992**. Isolation of plant DNA: a fast, inexpensive and reliable method. Plant Moleculer Biology Reporter, **10:** 60–65.

Gupta, A.K., Singh, D.P., Kaur, N.& Singh, R. **1994.** Production, purification and immobilization of inulinase from *Kluyveromyces fragilis*. Journal of Chemical Technology and Biotechnology, **59**: 377–385.

Gupta, A.K.& Kaur, N. **2000**. Fructan metabolism in Jerusalem artichoke and chicory .In Carbohydrate Reserves in Plants, A.K. Gupta & N. Kaur (Eds), Elsevier Science B.V., 223–248.

Jing, W., Zhengyu, J., Bo, J.& Augustine, A**. 2003**. Production and separation of exo and endoinulinase from *Aspergillus ficuu*m. Process Biochemistry, **39**: 5–11

Kango, N. **2008**. Production of inulinase using tap roots of dandelion (Taraxacum officinale) by *Aspergillus niger*. Jounal of Food Engineering, **85**: 473–478.

Kato, K., Araki, T., Kitamura, T., Morita, N., Moori, M. & Suzuki, Y. **1999**. Purification and properties of a thermostable inulinase (b–D–fructan fructohydrolase) from *Bacillus stearothermophilus* KP1289. Starch, **51**: 253–258.

Kim DH, Choi Y.J, Song S.K. & Yun JW. **1997**. Production of inulooligo– saccharides using endo–inulinase from Pseudomonas sp. Biotechnology Letters, **19**: 369 –71

Kochhar, A., Kaur, N.& Gupta, A. K. **1997**. Inulinase from *Aspergillus versicolor* a potent enzyme for producing fructose from inulin. Journal of Scientific and Industrial Research, **56**: 721–726.

Kumiko K, Toshihiro A, &Tae K. **1999**. Purification and properties of a thermostable inulinase (b–D–fructan fructohydrolase) from *Bacillus stearothermophilus* KP1289. Starch, **51**:253–261.

Kushi, R.T Monti, R. & Contiero, J. **2000.** Production, purification and characterization of an extracellular inulinase came from *Kluyveromyces marxianus* var. *bulgaricus*. Journal of Industrial Microbiology and Biotechnology, **25**: 63–69.

Massoud, M.I., Amin, W. A. & Elgindy, A. A. **2009**. Chemical and technological studies on chicory (*Cichorium Intybus L*) and its applications in some functional foods. Journal of the Advances in Agricultural Researches,**14**:739–755.

Meijer W.J.M., Mathijssen E.W.J.M. & Borm G.E. L. 1**993**. Inulin: Aversatile fibre ingredient. In: Inulin and Inulin–Containing Crops ,A. Fuchs. (Ed.),Elsevier Science Publishers B.V. p. 29

Miller, G. L. **1959**. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Analytical Chemistry, **31**: 426–428.

Modler, H. W. **1994**. Bifidogenic factors sources, metabolism and applications . International Dairy Journal, **4**: 383–407.

Moriyama, S., Akimoto, H., Suetsugu, N., Kawasaki, S., Nakamura, T. & Ohta, K. **2002**. Purification and properties of an extracellular exoinulinase from *Penicillium* sp. strain TN–88 and sequence analysis of the encoding gene. Bioscience Biotechnology and Biochemistry, **66**: 1887 –1896.

Nakamura, T., Ogata, Y., Shitara ,A., Nakamura ,A. & Ohta, K. **1995**. Continuous production of fructose syrups from inulin by immobilised inulinase from *Aspergillus niger* mutant 817. Journal of Fermentation and Bioengineering, **80**: 164–8.

Nakamura, T. Ogata, Y., Hamada, S. & Ohta, K. **1996**. Ethanol production from Jerusalem artichoke tubers by *Aspergillus niger* and *Saccharomyces cerevisiae*. Journal of Fermentation and Bioengineering, **81**: 564–566.

Ohta, K., Hamada, S& Nakamura, T. **1993**. Production of high concentrations of ethanol from inulin by simultaneous saccharification using *Aspergillus niger* and *Saccharomyces cerevisiae*. Applied Environmental Microbiology, **59**: 729–733.

Ongen–Baysal, G., Sukan, S.S. & Vassilev, N. **1994**. Production and properties of inulinase from *Aspergillus niger*. Biotechnology Letters, **16** : 275–280.

Pandey, A., Soccol, C.R., Selvakumar, P., Soccol, V.T., Krieger, N.& Fontana, J.D. **1999.** Re-

cent developments in microbial inulinases: Its production, properties and industrial applications. Applied Biochemistry and Biotechnology, **81**: 35–52.

Pawan, G.L.C. **1973**. Fructose. In:Molecular Structure and Function of Food Carbohydrates., Birch, G.G., Green, L.F. (Eds.), Appl. Sci. Publishers, London, pp. 65–80.

Pessoni, R.A.B., Figueiredo–Ribeiro, R.C.I.& Braga, M.R. **1999**. Extracelluar inulinase from *Penicillium janczewskii,* a fungus isolated from the rhizosphere of *Vernonia herbacea*. Journal Applied Microbiology, **87**: 141–147.

Singh, R.S ., Sooch, B S. & Puri, M. **2007**. Optimization of medium and process parameters for the production of inulinase from a newly isolated *Kluyveromyces marxianus* YS–1.Bioresource Technology, **98**: 2518–2525.

Shaheen, I., Bhatti, H.N.& Ashraf, T. **2008**. Production, purification and thermalcharacterisation of invertase from a newly isolated Fusarium sp under solidstate fermentation. International Journal of Food Science and Technology, **43**: 1152–1158.

Sharma, A. D., Kainth, S. & Gill, P. K. **2006**. Inulinase production using garlic (*Allium sativum*) powder as a potential substrate in *Streptomyces sp.* Journal of Food Engineering, **77**: 486–491.

Sheng, J. ,Chi, Z.M, Gong, F.& Li, J. **2008** .Purification and characterization of extracellular inulinase from a marine yeast *Cryptococcus aureus* G7a and inulin hydrolysis by the purified inulinase. Applied Biochemistry and Biotechnology, **144**:111–121.

Sirisansaneeyaku, S.,Worawuthiyanan, N., Vanichsriratana, W., Srinophakun P. & Chisti, Y. **2007**. Production of fructose from inulin using mixed inulinases from *Aspergillus niger* and *Candida guilliermondii* .World Journal Microbiology and Biotechnology, **23**:543–552.

Skowronek, M. & Fiedurek, J. **2004**. Optimization of inulinase production by *Aspergillus niger* using simplex and classical method. Food Technology and Biotechnology, **42**: 141–146.

Vandamme, E.J. & Derycke, D.G. **1983.** Microbial inulinases: fermentation process, properties, and applications. Advances in Applied Microbiology, **29**: 139–176.

Vranesic, D., Kurtanjek, Z., Santos, A.M.P.& Maugeri, F. **2002**. Optimisation of inulinase production by *Kluyveromyces bulgaricus*. Food Technology and Biotechnology, **40:** 67–73.

Viswanathan, P. & Kulkarni, P. R. **1995**. *Saussurea lappa* (kuth) as a new source of inulin for fermentative production of inulinase in a laboratory stirred fermenter. Bioresource Technology, **52**: 181–184.

Watherhouse, M. L. & Chatterton, N. J. **1993**. Glossary of fructan terms. In: Science and Technology of Fructans .M. Suzuki & N. J. Chatteron (Eds.), Boca Raton, FL: CRC Press, pp. 2–7.

Zhang, L., Zhao, C., Zhu, D., Ohta, Y. &Wang, Y. **2004**. Purification and characterization of inulinase from *Aspergillus n*iger AF10 expressed in *Pichia pastoris*. Protein Expression and Purification, **35**: 272–275

Zittan, L. **1981.** Enzymatic hydrolysis of inulin –an alternative way to fructose production. Starch, **33**: 373–377.

الإنتاج الحيوي لمحلي الفركتوز من الأنيولين المستخلص من جذور نبات الشيكوريا ودرنات خرشوفة القدس (الطرطوفة)

منى ابراهيم مسعود(١)، أمل محمد عبد الرازق(٢)،هدى محروس(٣)

(١) معهد بحوث المحاصيل السكرية– مركز البحوث الزراعية– الصبحية– إسكندرية– مصر

(٢) قسم علوم وتكنولوجيا الأغذية –كلية الزراعة (الشاطبى)–جامعة الأسكندرية –مصر

(٣) قسم التكنولوجيا الحيوية –معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية –جامعة المنوفية–مصر

يستخدم سكر الفركتوز على نطاق واسع في تحلية المشروبات، كما يدخل في بعض الصناعات الدوائية. و تهدف هذه الدراسة إلى الاستفادة من مستخلصات جذور نبات الشيكوريا ودرنات خرشوفة القدس (الطرطوفة) كمصدر طبيعي للأنيولين في إنتاج الفركتوز بالتحلل الأنزيمي. وقد تم عزل سلالة من فطر الأسبرجلس من مصاصة قصب السكر، وباستخدام PCR وGenbank data base و تم التعرف عليه وسمى *A. niger* isolate MonEg (GQ890276). وتحت الظروف المثلى للنمو ، أمكن الحصول على أعلى إنتاجية لإنزيم الأنيوليناز (٥٤,٧٠ وحدة/ مل) خارج الخلايا ، وذلك بتنميه الفطر لمدة ٧٢ ساعة على بيئة غذائية تحتوي على جذور الشيكوريا المجففة كمصدر كربوني وعلى درجة حرارة ٣٠°م. وبعد الترسيب بكبريتات الأمونيوم المشبعة كان النشاط النوعي للإنزيم ٢١,٠٦ وحدة/ مجم بروتين، وعدد مرات التنقية للإنزيم ٦,٧٧ عند pH ٥,٥، ودرجة حرارة ٥٠°م. و بتحضين الأنزيم مع مستخلص جذور نبات الشيكوريا ودرنات الطرطوفة المستخلص بالمحلول المنظم من خلات الصوديوم بتركيز يصل إلى ٠,١ مول، وباستخدام الظروف المثلى من حيث pH ودرجة الحرارة، وتتبع نواتج التحلل الإنزيمي باستخدام طريقة DNS وHPLC. أوضحت النتائج، إنه بعد ثلاث ساعات من التفاعل ،تراوحت إنتاجية الفركتوز بين ٠,٣١–٠,٦٠ جرام/ جرام أنيولين مع حدوث تحلل للأنيولين بنسبة تقارب ٣٩,٥٩ ٪ تحت نفس الظروف في كل من مستخلص جذور نبات الشيكوريا ودرنات الطرطوفة على الترتيب. وكذلك كان معدل إنتاج الفركتوز هو ٠,٥٧ و٠,٣١ جرام/ ١٠٠ مل/ ساعة في عينات الشيكوريا والطرطوفة على الترتيب. كما أوضحت النتائج زيادة معدل إنتاج الفركتوز إلى ٠,٤٨ جرام/ ١٠٠مل/ ساعة بتغير نشاط الإنزيم وثبات نسبة الأنيولين في مستخلص درنات الطرطوفة بعد ثلاث ساعات من التفاعل. لذا، فإنه يمكن الإستفادة من تلك النباتات كمصدر طبيعي للأنيولين لإنتاج الفركتوز، بالإضافة إلى أن جذور الشيكوريا تعتبر مصدرا كربونيا جيدا لإنتاج إنزيم الأنيوليناز.