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Production Strategy of Inulinase by *Penicillium citrinum* AR-IN2 on Some Agricultural By-Products

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ABSTRACT

The factors controlling inulinase production by new source *Penicillium citrinum* AR-IN2 using some agricultural by-products as well as hydrolysis of some inulin containing substrates were the aim the of this study. The results revealed that 96 h was the best incubation period for enzyme production. Dahlia tuber at 3% in the fermentation media was the greatest inducer for enzyme production compared to the other carbon sources. As well as, corn steep liquor at 3%, pH 6 and 35°C were found to be the optimum for enzyme production. pH 5.5 and 55°C were the optimum reaction conditions for enzyme activity. *Penicillium citrinum* inulinase hydrolyzed Jerusalem artichoke containing inulin than pure inulin and the degree of hydrolysis reached 87.5 and 72.7%, respectively. Therefore, fructose may be produced with high sweetener from inulin containing materials.

Key words: Inulinase, *Penicillium citrinum*, fructose, dahlia tubers, Jerusalem artichoke

INTRODUCTION

Fructose and fructooligosaccharides are fast emerging as important ingredients in the food and pharmaceutical industry. Fructose is considered as safe alternative sweetener to sucrose because it has beneficial effects in diabetic patients, increases the iron absorption in children (Gill *et al*., 2006). Fructose has high sweetening capacity, so it can be used in the diet of obese persons (Roberfroid and Delzenne, 1998), it stimulates calcium absorption in postmenopausal women (Van den Heuvel *et al*., 2000) it stimulates growth of *Bifidobacteria* in large and small intestine (Durieux *et al*., 2001) and it prevents colon cancer (Rowland *et al*., 1998). Sucrose, on the other hand, is known to cause problems related to corpulence, cariogenecity, arthero-sclerosis and diabetes. Both fructose and fructooligosaccharides can be produced from inulin, a β-(2→1) polymer of fructose residues attached to a terminal glucose residue that ranges in its degree of polymerization from 2 to 60, or higher. Inulin is a natural storage polymer found widely in plants e,g., chicory roots, artichoke tubers, dandelion dahlia and Jerusalem artichoke tubers (*Helianthus tuberosus* L.) are an important potential source of inulin (Pandey *et al*., 1999; Stolzenburg, 2005). Fructose can be obtained by using acid hydrolysis of the inulin but fructose is easily degraded at low pH and the process gives rise to coloring of the inulin hydrolysate and

by-product formation in the form of difructose anhydrides (Barthomeuf *et al.*, 1991). Another method includes microbial sources which secrete high amount of inulinase and can cleave fructose from inulin, by a single step catalyzed reaction with yields up to 95% of fructose (Kaur *et al.*, 1992).

Microbial inulinases are an important class of industrial enzymes which hydrolyze inulin to produce fructose and fructo-oligosaccharides. Both fructose and fructo-oligosaccharides are fast emerging as important ingredients in the food and pharmaceutical industry (Singh and Lotey, 2010). Microbial inulinases are usually inducible and exo-acting enzymes. Inulinases are classified into endo- and exoinulinases, depending on their mode of action. Endoinulinases (2,1-β-D-fructan fructanohydrolase; EC 3.2.1.7) are specific for inulin and hydrolyse it by breaking bonds between fructose units that are located away from the ends of the polymer network, to produce oligosacch-arides. Exo-inulinases (β-D-fructohydrolase; EC 3.2.1 .80), split terminal fructose units from the non-reducing end of the inulin molecule to liberate fructose (Gill *et al.*, 2003; Sirisansaneeyakul *et al.*, 2007; Singh and Lotey, 2010). Hydrolysis causes inulin to liberate mostly fructose but also some glucose. Inulinases can be found in microorganisms as filamentous fungi, yeasts and bacteria. Among fungi, some well-known sources of these enzymes include *Aspergillus niger*, *A. ficuum*, *A. tamarii*, *Chrysosporium pannorum*, *Penicillium purpurogenum* and *Fusarium oxysporum* (Nakamura *et al.*, 1997, Sirisansaneeyakul *et al.*, 2007; Saber and El-Naggar, 2009). Among yeasts, the best-known producers are *Kluyveromyces marxianus*, *Debaryomyces cantarellii*, *Candida kefyr* and *Pichia polymorpha* (Kushi *et al.*, 2000; Pandey *et al.*, 1999). Inulinases have been found in several bacteria such as *Bacillus* sp. (Uzunova *et al.*, 2002) and *Pseudomonas* sp. (Kim *et al.*, 1997). Inulinases of filamentous fungi are used to optimize process of hydrolysis of the inulin related to food industries for the production of alcohol, acetone and butanol (Pandey *et al.*, 1999). Therefore, the factors controlling inulinase production by new source *Penicillium citrinum* AR-IN2 using some agricultural by-products as well as hydrolysis of some inulin containing substrates were the aim of this study.

MATERIALS AND METHODS

Fungal organism and agricultural residues: *Penicillium citrinum* AR-IN2 was kindly provided from Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. The fungal strain was previously isolated from Jerusalem artichoke tubers and maintained on PDA with monthly transferring.

Tubers of Jerusalem artichoke were obtained from Al-Mansoura Horticulture Research Station, Agricultural Research Center, Egypt. Tubers were peeled and cut into slices, vacuum dried at 30°C and packed in laminated polyethylene packs. The other agricultural by-Products were cleaned, dried at 70°C and blended. Inulin was obtained from BDH chemicals LTd Pool England.

Fermentation technique: Spores of 72 h old culture were suspended in sterile saline solution (0.85%) containing 0.01% tween 80 to obtain 2.5×10^6 spores mL^{-1}. Fermentation was started using 5% spore suspension in 250 mL Erlenmeyer flask containing 50 mL liquid medium of Nakamura *et al.* (1978) which contains: inulin, 1%; corn steep liquor, 2%; $(NH_4)H_2PO_4$, 1.2%; KCl, 0.07%; $MgSO_4$, $7H_2O$, 0.05%; $FeSO_47H_2O$, 0.001% and pH 4.5 for 5 days at 30°C.

Optimization of fermentation conditions for inulinase production: The effects of incubation time (1 to 6 days), sugars and agro-wastes as carbon sources, various concentrations of dahlia tubers, nitrogen source, concentration of corn steep liquor, initial culture pH and finally, incubation

temperature were optimized for the production of inulinase. After incubation period, the mycelium was filtered through Whatman No. 1 filter paper and dried at 105°C until constant weight to determine the biomass. The culture filtrate was centrifuged at 3000 rpm for 20 min and used as a source of extracellular inulinase.

Enzyme assay: The reaction mixture (2 mL of 2% inulin, 0.5 mL of the crude enzyme and 2.0 mL of acetate buffer with pH 4.6) was incubated at 50°C for 20 min. The tubes were then kept in a boiling water bath for 10 min to inactivate enzyme (Viswanathan and Kulkarni, 1995). Reaction mixture was assayed for reducing sugars according to Smogyi (1952) using fructose as standard. One unit (U) of inulinase was considered as the amount of enzyme activity which liberates 1 μg fructose equivalent from substrate min^{-1} under the assay conditions.

Optimum pH and temperature for inulinase activity: To find out the optimum pH and temperature for inulinase activity, various buffers were used to cover the ranges of pH 3.0 to 8.0. The temperature range of 25-65°C was tested.

Hydrolysis of some inulin containing substrates: Twenty five unit of inulinase were added to 0.25 mL (4% w/v) of dalia tubers or Jerusalem artichoke and 0.75 mL of phosphate buffer (pH 5.5) and incubated at the optimum temperature of the enzyme (55 °C) for 15, 50, 60, 120, 180, 240 and 300 min. The degree of hydrolysis was expressed as the percent of reducing sugar against the total sugar×100. The total sugars in the hydrolyzed products were determined according to Anthrone method (Scott and Melvin, 1953).

Statistical analysis: Simple correlation coefficient (r) at $p \leq 0.05$ was performed to examine the relationships between individual properties using the statistical analysis software; CoStat v 6.4.

RESULTS AND DISCUSSION

Incubation period: Inulinase is an external glycoprotein produced by number of yeasts and filamentous fungi. However, fungi in general produce higher enzyme yields. Wherein, endogenous, *P. citrinum* AR-IN2 that previously isolated from Jerusalem artichoke tubers was chosen for studying its ability for inulinase production in the fermentation medium. Data plotted in Fig. 1, show inulinase production by *P. citrinum* as affected by time course. Inulinase synthesis was growth-associated and reached to the maximum activity (20.15 U mL^{-1}) after 4 days; however, it decreased down to 17.09 U mL^{-1} at the 5th day. The decline in enzyme activity after 5 days of fermentation may be due to the secretion of proteolytic enzymes which are known to cause the denaturation of inulinase (Gupta *et al.*, 1994). This may also be attributed to decrease in nutrient availability in the medium at the end of the cultivation process (Ongen-Baysal *et al.*, 1994). These data are in harmony with the finding of Parekh and Margaritis, (1986). Whereas, the time course reported for a maximum production of inulinase by *Penicillium* sp. under aeration conditions was 72 h (Derycke and Vandamme, 1984). As well as, a maximum inulinase yield (80 U mL^{-1}) was obtained after 60 h by shaking growth of *Aspergillus niger* (Poorna and Kulkarni, 1995).

Nutritional conditions of the fermentation medium: The carbon source has been estimated as a major cost factor in enzyme production. However, industrial application of inulinase would only be feasible if the carbon sources were available in large quantities at competitive price. A

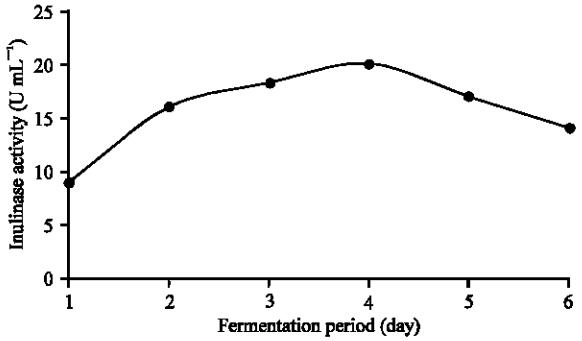

Fig. 1: Time course profile of inulinase production by *P. citrinum* AR-IN2

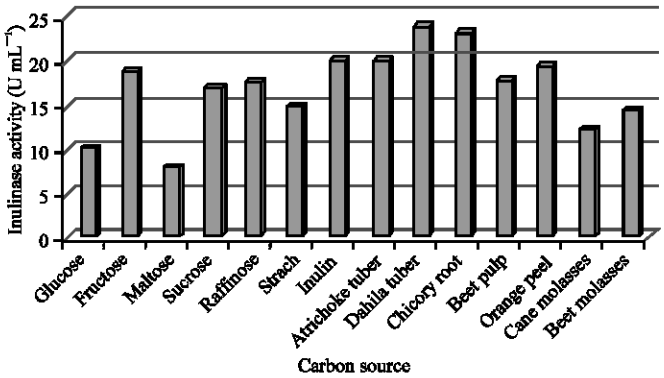

Fig. 2: Production of inulinase on various carbon sources by *P. citrinum* AR-IN2

reduction in the production cost can be achieved by the usage of inexpensive inulin-containing substrates such as chicory, dahlia, artichoke which are cheap, easily available, often abundant and alternative, for large-scale fermentation (Sharma *et al.*, 2006). The effect of carbon sources and agricultural by-products on inulinase production by *P. citrinum* has been studied and the data are shown in Fig. 2. Inulinase was produced over the different sources used but some of these sources reduced the enzyme biosynthesis while others induced it greatly. This means that, this enzyme is constitutive in nature and induced with its substrates. The inulin containing residues, i.e., dahlia tubers, chicory roots and artichoke tubers were found as potent inducers for inulinase production, in which the enzyme activities reached 23.9, 23.2 and 19.9 U mL^{-1}, respectively. Meanwhile, other carbon sources such as glucose, maltose and starch reduced the enzyme activity. These results are in harmony with the data obtained by Xiao *et al.* (1988) and Saber and El-Naggar (2009).

In order to determine the optimum concentration of the best carbon and energy source from the previous trial (dahlia tuber) for inulinase production, different concentrations of dahlia tuber (0.5-5%, w/v) were incorporated in the medium (Fig. 3). The results indicate that inulinase was produced at all concentrations of dahlia tuber used and a progressive increase in the production was observed with the increasing of the dahlia tuber concentration up to 3% and thereafter, a decline in enzyme production was observed. This means that the lower concentrations enhancing the enzyme productivity but higher concentrations repressed it. It has been well established that higher substrate concentration can lead to catabolic repression, consequently lowering the enzyme production (Jing *et al.*, 2003).

Figure 4 shows the effect of different nitrogen sources on inulinase production. Data obtained revealed that Corn Steep Liquor (CSL) and yeast extract were potent inducers for inulinase

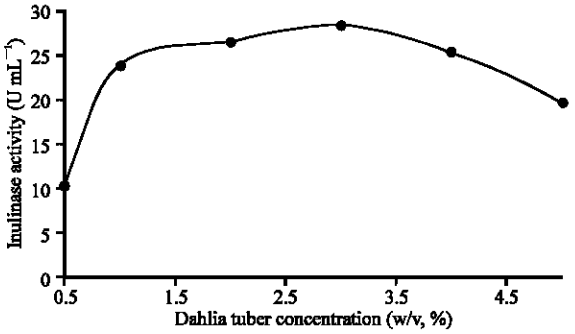

Fig. 3: Dahlia tuber concentration *vis P. citrinum* AR-IN2 inulinase production

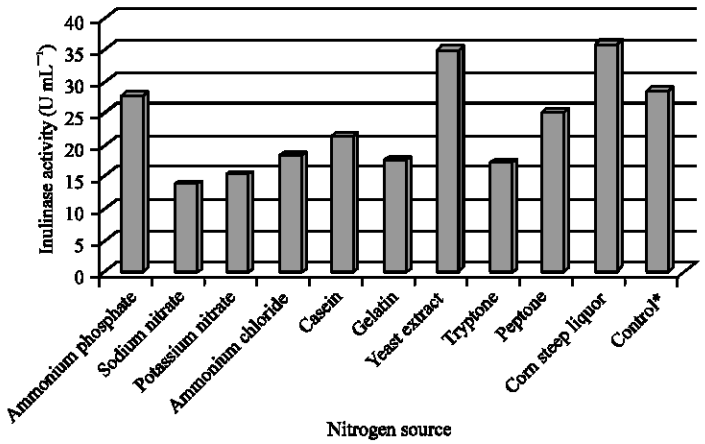

Fig. 4: Production of inulinase on various nitrogen sources by *P. citrinum* AR-IN2. *Control contains CSL+ Ammonium phosphate as nitrogen source

production by *P. citrinum*, in which the productivities were 35.6 and 34.8 U mL^{-1}, respectively. On the other hand, potassium and sodium nitrate were less inducer for enzyme biosynthesis. Similar data were reported by Nakamura *et al.* (1978) and Saber and El-Naggar (2009). Complex nitrogen sources were better than inorganic nitrogen sources. Meat extract has been reported as the best nitrogen source for the production of inulinase from *Chrysosporium pannorum* (Xiao *et al.*, 1988). Gupta *et al.* (1990) reported increased production of inulinase with sodium nitrate as a nitrogen source in the medium by *Fusarium oxysporum*, whereas it exerted an inhibitory effect on inulinase production in *Kluyveromyces fragilis*. CSL as organic nitrogen source was found to be the best for maximum inulinase production in *Aspergillus niger* AUP19 (Kumar *et al.*, 2005). Consequently, the corn steep liquor as the best nitrogen source for inulinase production was selected for further studies.

Production of inulinase by *P. citrinum* as affected by different concentrations of CSL is shown in Fig. 5. Results obtained showed that enzyme productivity increased gradually with the increase of CSL up to 3% (37.5 U mL^{-1}) and thereafter, a decline in this function was recorded. The higher concentration of CSL had inhibitory effect on inulinase synthesis. This could be due to the complex nature of this nitrogen source and some of its constituents at higher concentration might have a toxic effect on inulinase production. These results are in consistent with that obtained by Nakamura *et al.* (1978), Saber and El-Naggar (2009) and Dilipkumar *et al.* (2010) who reported that inulinase activity increased with increase in CSL and thereafter decreased with further

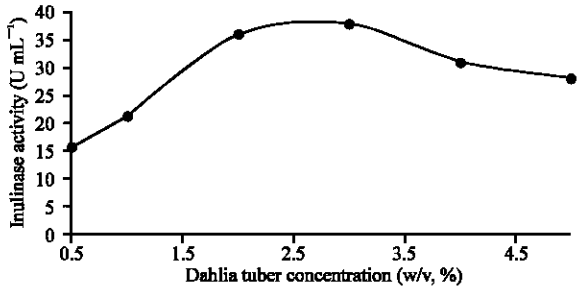

Fig. 5: Corn steep liquor concentration against *P. citrinum* AR-IN2 inulinase production

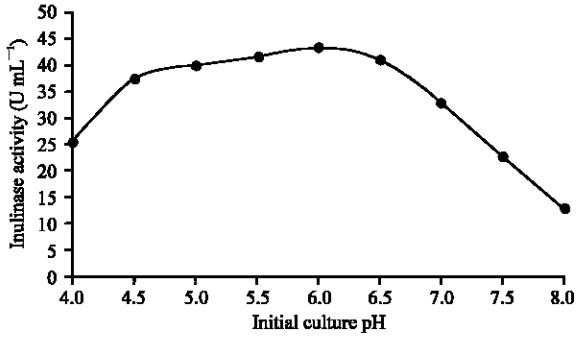

Fig. 6: Effect of initial culture pH on *P. citrinum* AR-IN2 inulinase production

increase in corn steep liquor. Inulinase also, appeared to be regulated by a double mechanism: increasing by the substrate and repression by the product (glucose and fructose), as the activity decreased with sucrose concentrations above 10 g L^{-1}, probably suppressed by reducing sugars (Cazetta *et al.*, 2010).

Physical conditions of the fermentation medium: As shown in Fig. 6 the initial pH of the fermentation medium had a pronounced effect on the enzyme production. Maximum inulinase activity was observed at pH 6. Above or below of this pH degree, the enzyme activity decreased. Shady *et al.* (2000) and Singh and Lotey (2010) found that pH 5.5 was the optimum pH for *Kluyveromyces marixanus* inulinase production, they added that the hydrogen ion concentration of the medium has a strong influence on the microbial growth. The results are also similar to those of Cazetta *et al.* (2010).

In addition, the maximum inulinase production (55.2 U mL^{-1}) was observed at 35°C as an optimum incubation temperature (Fig. 7). Above or below of this temperature, the enzyme activity decreased. The optimal temperature for inulinase production of *P. citrinum* is higher than *P. janczewski* which showed highest production at 28°C (Pessoni *et al.*, 1999). Shady *et al.* (2000) found that 30°C was the optimum temperature degree for *Kluyveromyces marxianus* inulinase production.

1. **Some properties of *P. citrinum* AR-IN2 inulinase:** The data presented in Fig. 8, show that *P. citrinum* AR-IN2 inulinase hydrolyzed inulin at wide pH level (3 to 8) but with different extent which reached its maximum at pH 5.5. Generally, the pH profile of inulinase tends to be acidic in nature. The enzyme was able to keep not less than 85% of its activity within a pH

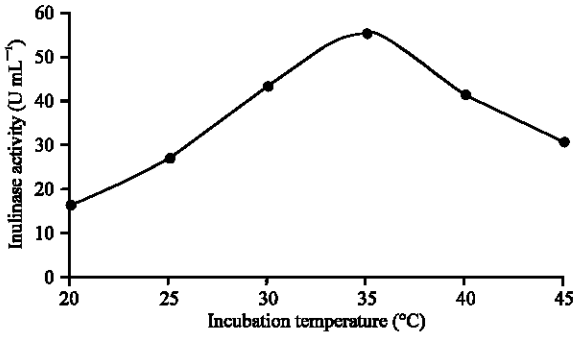

Fig. 7: Effect of incubation temperature on *P. citrinum* AR-IN2 inulinase production

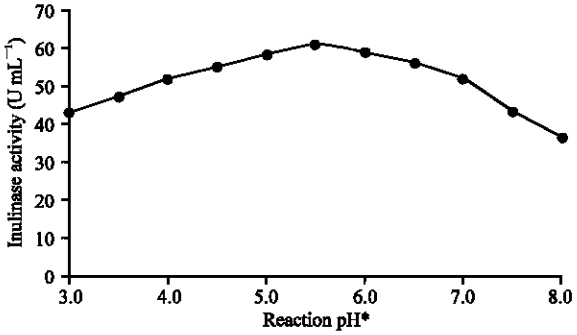

Fig. 8: Effect of the reaction pH on *P. citrinum* AR-IN2 inulinase activity. *The temperature of the reaction mixture is 50°C

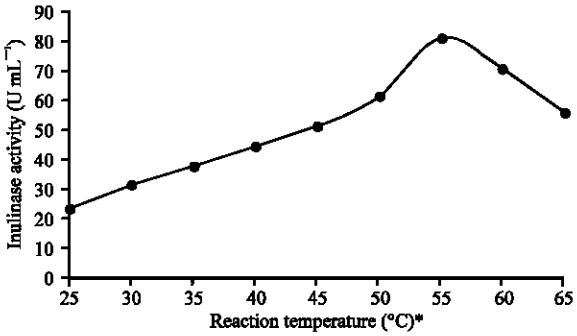

Fig. 9: Effect of reaction temperature on *P. citrinum* AR-IN2 inulinase activity. *The pH of the reaction mixture is 5.5

range of 4 to 7 which means that the produced inulinase can hydrolyze inulin at a broad pH levels. So, this enzyme may be used in a wide range of industrial processes depending on the degree of pH. Out of this optimum pH, the degree of hydrolysis decreased. The optimum pH of 5.5 is in consistent with the general range of many microbial sources reported such as *Penicillium janczewskii* (4.8-5.0) (Pessoni *et al.*, 1999). Drent and Gottschal (1991) found that inulinase activity was optimum at neutral pH.

The effect of temperature on inulinase activity (Fig. 9) shows that inulinase of *P. citrinum* reached to the maximum activity at 55°C. Above or below of this temperature degree, the enzyme

activity decreased. This means that the enzyme is thermolabiale one and can be efficiently used in the industrial processes depending on high temperature. These results are similar to those obtained by Beluche *et al.* (1980). However, as compared to inulinases from *F. oxysporum* and *P. janczewskii* which have temperature optima of 35 and 45°C, respectively (Kaur *et al.*, 1992; Pessoni *et al.*, 1999), the optimum temperature of *P. citrinum* AR-IN2 inulinase in the present study is significantly higher.

Application of *P. citrinum* AR-IN2 inulinase: In an effort to evaluate the suitability of the obtained inulinase to be used for saccharifying of inulin-containing substrates, an application trial was performed, it could be easily observed from the data in Table 1 that *P. citrinum* AR-IN2 inulinase hydrolyzed dahlia tubers and Jerusalem artichoke containing inulin when both substrates were treated with fungal inulinase. The degree of hydrolysis of two substrates differed and increased with prolonged time of hydrolysis and reached its maximum (61.8 and 74.4%, respectively) at the end of hydrolysis. Regardless the substrate, there was strong correlation coefficient between the time of reaction and degree of hydrolysis (0.942 and 0.965 $p \leq 0.01$) that means the efficient of the obtained inulinase in hydrolysis of different inulinase containing substrates. However, this is the first report on production of inulinase by *P. citrinum* AR-IN2.

During the hydrolysis of inulin containing substrates, high fructose is produced, this is due to the hydrolysis of inulin to fructose in a single step from the non-reducing end of fructosidic chains (Shady *et al.*, 2000). The results also show that Jerusalem artichoke more hydrolysable than the other one. The difference between the degrees of hydrolysis of both substrates may be due to the difference in affinity between two substrates and enzyme, this is also, may be due to the degree of polymerization and the presence of polyfructans and other ingredients in the tested substrates which induce the enzyme activity. The results are similar to those obtained by Shady *et al.* (2000) and Saber and El-Naggar (2009).

The use of agro-industrial residues as substrate for inulinase bio-production is a good choice to reduce production costs, since enzyme activity will be improved and the downstream step of the process will be viable technically and economically. In addition, the screening of microorganisms that are able to overproduce inulinase using these substrates is fundamental to guarantee successful compatibility with medium constituents (Makino *et al.*, 2009). In conclusion, the bioremediation of such agricultural by-products by *P. citrinum* AR-IN2 inulinase introduces feasible benefit trends in various economic and environmental aspects, wherein fructose is produced during the fermentation process.

Table 1: Hydrolysis of dahlia tuber and jerusalem artichoke by *P. citrinum* inulinase

	Degree of hydrolysis (%)	
Time of reaction (min)	Dahlia tubers	Jerusalem artichoke
15	12.240	15.215
30	25.075	27.965
60	36.380	40.375
120	44.115	48.110
180	51.425	58.820
240	57.630	66.810
300	61.795	74.375
Correlation coefficient (r) at $p \leq 0.01$	0.942**	0.965**

**The correlation between time of reaction and degree of hydrolysis is very significant.

REFERENCES

Barthomeuf, C., F. Regerat and H. Pourrat, 1991. Production of inulinase by a new mold of *Penicillium rugulosum*. J. Ferment. Bioeng., 72: 491-494.

Beluche, I., J.P. Guiraud and P. Galzy, 1980. Inulinase activity of *Debaromyces cantarellii*. Folia Microbiol., 25: 32-39.

Cazetta, M.L., R. Monti and J. Contiero, 2010. Effects of culture conditions on the production of inulinase by *Kluyveromyces marxianus*. Braz. Arch. Biol. Technol., 53: 701-707.

Derycke, D.G. and E.J. Vandamme, 1984. Production and properties of *Aspergillus niger* inulinase. J. Chem. Technol. Biotechnol., 34: 45-51.

Dilipkumar, M., M. Rajasimman and N. Rajamohan, 2010. Optimization of inulinase production using copra waste by *Kluyveromyces marxianus* var. *marxianus*. Chem. Ind. Chem. Eng. Q., 16: 319-327.

Drent, W.J. and J.C. Gottschal, 1991. Fermentation of inulin by a new strain of *Clostridium thermoautotrophicum* isolated from dahlia tubers. FEMS Microbiol. Lett., 78: 285-291.

Durieux, A., C. Fougnies, H. Jacobs and J.P. Simon, 2001. Metabolism of chicory fructooligosaccharides by bifidobacteria. Biotechnol. Lett., 23: 1523-1527.

Gill, P.K., A.D. Sharma, R.K. Harchand and P. Singh, 2003. Effect of media supplements and culture conditions on inulinase production by an actinomycete strain. Bioresour. Technol., 87: 359-362.

Gill, P.K., R.K. Manhas and P. Singh, 2006. Purification and properties of a heat-stable exo-inulinase isoform from *Aspergillus fumigatus*. Bioresour. Technol., 97: 894-902.

Gupta, A.K., A. Gill, N. Kaur and R. Singh, 1994. High thermal stability of inulinases from *Aspergilus* species. Biotechnol. Lett., 16: 733-734.

Gupta, A.K., P. Rathore, N. Kaur and R. Sing, 1990. Production, thermal stability and immobilization of inulinase from *Fusarium oxysporum*. J. Chem. Biotechnol., 47: 245-257.

Jing, W., J. Zhengyu, J. Bo and A. Augustine, 2003. Production and separation of exo and endoinulinase from *Aspergillus ficuum*. Process Biochem., 39: 5-11.

Kaur, N., M. Kaur, A.K. Gupta and R. Singh, 1992. Properties of β-fructosidases (invertases and inulinases) of *Fusarium oxysporum* grown on an aqueous extract of *Cichorium intybus* roots. J. Chem. Technol. Biotechnol., 53: 279-284.

Kim, D.H., Y.J. Choi, S.K. Song and J.W. Yun, 1997. Production of inulo-oligosaccharides using endo-inulinase from a *Pseudomonas* sp. Biotechnol. Lett., 9: 369-372.

Kumar, G.P., A. Kunamneni, T. Prabhakar and P. Ellaiah, 2005. Optimization of process parameters for the production of inulinase from a newly isolated *Aspergillus niger* AUP19. World J. Microbiol. Biotechnol., 21: 1359-1361.

Kushi, R.T., R. Monti and J. Contiero, 2000. Production, purification and characterization of an extracellular inulinase from *Kluyveromyces marxianus* var. *bulgaricus*. J. Ind. Microbiol. Biotechnol., 25: 63-69.

Makino, Y., H. Treichel, M.A. Mazutti, F. Maugeri and M.I. Rodrigues, 2009. Inulinase bio-production using agroindustrial residues: Screening of microorganisms and process parameters optimization. J. Chem. Technol. Biotechnol., 84: 1056-1062.

Nakamura, T., A. Shitara, S. Matsuda, T. Matsuo, M. Suiko and K. Ohta, 1997. Production, purification and properties of an endoinulinase of *Penicillium* sp. TN-88 that liberates inulotriose. J. Ferment. Bioeng., 84: 313-318.

Nakamura, T., S. Hoashi and S. Nakatsu, 1978. Culture conditions for inulase production by *Aspergillus*. Nippon Kogeikagaku Kaishi, 52: 105-110.

Ongen-Baysal, G., S. Sukan and N. Vassilev, 1994. Production and properties of inulinase from *Aspergillus niger*. Biotechnol. Lett., 16: 275-280.

Pandey, A., C.R. Soccol, P. Selvakumar, V.T. Soccol, N. Krieger and J.D. Fontana, 1999. Recent developments in microbial inulinases: Its production, properties and industrial applications. Applied Biochem. Biotechnol., 81: 35-52.

Parekh, S. and A. Margaritis, 1986. Production of inulinase (β-fructan fructanohydrolase) by *Kluyveromyces marxianus*. Agric. Biol. Chem., 50: 1085-1087.

Pessoni, R.A.B., R.C.L. Figueiredo-Ribeiro and M.R. Braga, 1999. Extracellular inulinases from *Penicillium janczewskii*, a fungus isolated from the rhizosphere of *Veronia herbacea* (*Asteraceae*). J. Applied Microbiol., 87: 141-147.

Poorna, V. and P.R. Kulkarni, 1995. A study of inulinase production in *Aspergillus niger* using fractional factorial design. Bioresour. Technol., 54: 315-320.

Roberfroid, M.B. and N.M. Delzenne, 1998. Dietary fructans. Ann. Rev. Nutr., 18: 117-143.

Rowland, I.R., C.J. Rumney, J.T. Coutts and L.C. Lievense, 1998. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis, 19: 281-285.

Saber, W.I.A. and N.E. El-Naggar, 2009. Optimization of fermentation conditions for the biosynthesis of inulinase by the new source; *Aspergillus tamarii* and hydrolysis of some inulin containing agro-wastes. Biotechnology, 8: 425-433.

Scott Jr., T.A. and E.H. Melvin, 1953. Determination of dextran with anthrone. Anal. Chem., 25: 1656-1661.

Shady, T.S.M., F.I.A. Hauka and M.M.A. El-Sawah, 2000. Inulin hydrolysis by *Kluyveromyces marxianus* inulase produced on some agricultural wastes. Egypt. J. Microbiol., 35: 139-154.

Sharma, A.D., S. Kainth and P.K. Gill, 2006. Inulinase production using garlic (*Allium sativum*) powder as a potential substrate in *Streptomyces* sp. J. Food Eng., 77: 486-491.

Singh, R.S. and S. Lotey, 2010. Enhanced exoinulinase production from *Kluyveromyces marxianus* YS-1 using response surface methodology. Braz. Arch. Biol. Technol., 53: 1005-1013.

Sirisansaneeyakul, S., N. Worawuthiyanan, W. Vanichsriratana, P. Srinophakun and Y. Chisti, 2007. Production of fructose from inulin using mixed inulinases from *Aspergillus niger* and *Candida guilliermondii*. World J. Microbiol. Biotechnol., 23: 543-552.

Smogyi, M., 1952. Notes on sugar determination. J. Biol. Chem., 195: 19-23.

Stolzenburg, K., 2005. Jerusalem artichokes-raw material for inulin and fructose production. Zuckerindustrie, 130: 193-200.

Uzunova, K., A. Vassileva, V. Ivanova, D. Spasova and A. Tonkova, 2002. Thermostable exo-inulinase production by semicontinuous cultivation of membrane-immobilized *Bacillus* sp. 11 cells. Process Biochem., 37: 863-868.

Van den Heuvel, E.G.H.M., M.H.C. Schoterman and T. Muijs, 2000. Transgalactooligo-saccharides stimulate calcium absorption in postmenopausal women. J. Nutr., 130: 2938-2942.

Viswanathan, P. and P.R. Kulkarni, 1995. Enhancement of inulinase production by *Aspergillus niger* van Teighem. J. Applied Microbiol., 78: 384-386.

Xiao, R., M. Tanida and S. Takao, 1988. Inulinase from *Chrysosporium pannorum*. J. Ferment. Technol., 65: 553-558.