CHAPTER 2  
Review of Literature

The search for the new sweeteners is gaining attention owing to some negative aspects like carcinogenicity, obesity, high calorie content, etc. displayed by the conventional sweetener, sucrose. Many synthetic sweeteners have been developed which have no food value, but preference still goes to natural sweetening compounds. An extensive search is going on to identify non-carbohydrate sweeteners which have no toxic effects and are also poor in calorie. A number of sweeteners like monellin, thaumatin, stevioside, etc. have been developed, but all these compounds are not yet produced in sufficient quantity for human consumption. Contrary, fructose as a natural occurring compound (honey and fruits), has been extensively tested in food applications. It is occupying an important place in modern world as a sweetener because of its nutraceutical and technical properties. Inulin, which is a polyfructan and second most abundant storage carbohydrate in nature, have recently received much interest as a renewable raw material for the production of fructose. To convert inulin into fructose, the most rational and economic option is its enzymatic hydrolysis. Microbial inulinases are an important class of enzymes that catalyzes the hydrolysis process for the production of fructose. Keeping in view the objectives of the present study, the literature has been reviewed under the following headings.

Production of inulinase,

Hydrolysis of inulin.

Production of Inulinase: Various microorganisms like moulds (Aspergillus aureus, A. awamori, A ficuum, A. fumigatus, A. niger, Penicillium rugulosum, P. trzebinskii, Fusarium oxysporum, etc.), yeasts
(Candida kefyr, C. pseudotropicalis, Kluyveromyces lactis, K. marxianus, K. fragilis, Pichia sp., etc.) and bacteria (Acetobacter sp., Achromobacter sp. Bacillus subtilis, Escherichia coli, Pseudomonas sp. Staphylococcus sp., etc.) have been reported as inulinase producers (Pandey et al., 1999, Singh and Gill, 2006). Raw materials used for the production of inulinase include a wide range of pure as well as natural substrates. Sugars like glucose, fructose, sucrose, lactose, maltose, fructan, inulin, etc. have been used as a substrate for the enzyme production. Various cheap plant materials like dahlia, Jerusalem artichoke, chicory, kuth roots, wheat bran, etc. have also been reported as substrate for the inulinase synthesis. Literature has been reviewed concentrating mainly on microbial sources, raw materials used and the process conditions for the fermentative production of inulinase.

Biotechnologically, plant inulinases are not popular in that their production is seasonal and not inducible. Furthermore, microbial inulinases, especially those recovered from fungi, are easy to isolate as they are secreted extracellularly and the fungi can be manipulated to secrete them in large amounts, either genetically or by altering carbon sources (Jing et al., 2003; Vandamme and Derycke, 1983; Nakamura et al., 1994). It has thus become evident that chemical hydrolysis of inulin is an unsustainable and undesirable approach as opposed to enzymatic methods.

Batch and continuous fermentation has been carried out for the production of inulinase from Kluyveromyces fragilis using sucrose (4%) as a carbon source at 50°C and pH 5.0. Inulinase production with sucrose was reported twice high as compared to inulin (Groot-wassink and Fleming, 1980).

Inulinase production by K. fragilis on various fermentable and non-fermentable carbon sources was examined in C-limited continuous culture. Fructose and sucrose supported higher inulinase yields, while other carbon sources like lactose, galactose, ethanol and lactate did not increased
inulinase activity beyond the basal levels. Fructose was identified as the primary physiological inducer and genetic evidence of inducible nature of inulinase in the wild type was also provided (Groot-wassink and Hewitt, 1983).

Inulinase has been produced from an isolate of *Aspergillus niger* at 28°C within 110 h of fermentation. The production was carried out in a medium containing corn steep liquor and maltose, in the absence of inulin (Derycke and Vandamme, 1984).

The simultaneous production of inulinase and lactase from *Kluyveromyces fragilis* has been studied and it is reported that synthesis of both the enzymes was suffered strongly from carbon catabolite repression in batch cultures grown on single and mixed inducers. The highest production of inulinase (25 IU/mg dry cell wt) and lactase (0.78 IU/mg dry cell wt) were obtained simultaneously in a continuous culture of an equal mixture of D-fructose and D-galactose (Hewitt and Groot-wassink, 1984). Inulin was reported best inducer for inulinase production from *Kluyveromyces marxianus* as compared to other carbon sources like glucose, fructose and sucrose. Overall, 212 IU/ml of inulinase has been produced with pure inulin (1%), which was four times higher as compared to the reducing sugars used (Parekh and Margaritis, 1985).

Inulinase production from *Clostridium acetobutylicum* ABKN8 grown in a basal medium containing inulin has been investigated by (Efstathiou et al. 1986). It was reported that inulinase production reached maximum at the end of exponential phase and most of the enzyme activity was detected in the supernatant. The inulinase was induced only by inulin and not by xylose, fructose or sucrose; however glucose was reported to repress the inulinase synthesis.

A bacterial strain of *Pseudomonas* sp. isolated from soil samples was investigated for the production of inulinase. The maximum production was observed in a medium containing inulin (1%), corn steep liquor
(1.5%), FeSO$_4$.7H$_2$O (0.003%), MgSO$_4$.7H$_2$O (0.05%) and (NH$_4$)$_2$HPO$_4$ (0.8%) with initial pH 7.0 at 45 °C for 60 h, under shaking conditions (Lee et al., 1987). Whereas, a mutant strain of K. fragilis having higher affinity for lactose has been used for the production of inulinase in a batch and continuous culture with lactose as a carbon source (Tsang and Groot-wassink, 1988).

Root extract of chicory, has been used for the production of mycelial and extracellular inulinase from Fusarium oxysporum. Inulin (3%) prepared from the extract has been found optimum for the production of inulinase. Maximum (86%) release of inulinase into the medium has been reported after a period of 12 days (Gupta et al., 1988). Pure inulin was used as an inducer for inulinase from Chrysosporium pannorum AHU 9700 isolated from soil. Sucrose, glucose or fructose did not induced inulinase synthesis. The highest inulinase activity of 115 IU/ml was obtained with inulin under the optimal conditions (Xiao et al., 1988).

Four strains of yeast namely Candida kefyr, C. pseudotropicalis var. lactosa, K. cicerisporus and K. fragilis were studied for the production of inulinase (Manzoni and Cavazzoni, 1988). High enzyme activity of 22.4 to 32.0 IU/ml with specific activity of 9.7-16.0 IU/mg of protein was detected in the culture supernatant when the yeasts were grown in a medium containing aqueous extract of fresh Jerusalem artichoke tubers as carbon source. The maximum inulinase activity was obtained at 28 °C from C. pseudotropicalis, and at 32 °C from K. cicerisporus as well as K. fragilis in 6 day old cultures.

Inulinase production from Fusarium oxysporum by using Cichorium intybus roots has been investigated by Gupta et al. (1989). Maximum extracellular inulinase was produced after 9 days of growth at 25 °C in a medium (pH 5.5) containing 3% fructan and 0.2% sodium nitrate. The level of enzyme decreased on the addition of either glucose, fructose, galactose or sucrose. Further, it has been reported that glycerol (10%)
gave better protection to inulinase against thermal denaturation at 50 °C as compared to ethylene glycol and sorbitol (Gupta et al., 1990).

A new strain of *Penicillium rugulosum* isolated from rotten dahlia tubers was found to produce a very active inulin hydrolyzing enzyme (Barthomeuf et al., 1991). The maximum enzyme production (54 IU/ml) was obtained after 40 h. The crude enzyme preparation was rapidly active on inulin suspensions and Jerusalem artichoke extracts.

*Arthrobacter* sp. isolated from chicory fields have been used for the production of inulinase in a fermenter with working capacity of 3 L. Fermentation was carried out at 30 °C in a medium containing inulin as a sole carbon source (pH 7.0). Inoculum (10%) of 12 h old culture was used for the fermentation, whereas, optimum aeration and agitation was reported to be 1.5 vvm and 105 rpm, respectively. Enzyme production of 0.4 IU/ml has been reported in 18 h incubation (Elyachioui et al., 1992).

An isolated *Streptomyces* sp. was investigated for the production of exoinulinase with inulin as carbon source and soybean meal as organic nitrogen source. The exoinulinase was reported to be a constitutive enzyme produced not only by inulin, but also by soluble starch or glucose (Ha and Kim, 1992).

Yeast strains like *Kluyveromyces fragilis*, *K. marxianus*, *Hansenula polymorpha*, *Pichia fermenta*, *P. polymorpha* and *Debaryomyces castellii* were studied and *Kluyveromyces fragilis* has been reported efficient for the production of inulinase. Sucrose and fructose were reported weak inducers, whereas inulin (1%) and peptone (1%) has been reported optimum for inulinase production. Inulinase activity of 6-7 IU/cm³ was produced in the medium (pH 6.4-6.8) at 25-27 °C after 36 h of incubation (Gupta et al., 1994).

Fontana et al. (1994) investigated the inulinase production by using chemically modified inulins. Caproyl and cholesteryl derivatives of native dahlia inulin were prepared from the respective chlorides. These inulin
derivatives were employed as carbon source and inducers using different strains of *K. marxianus*. Caproylated inulin was reported superior inducer as compared to the cholesterol derivative. It has been reported that inulinase induction or the secretion process is affected by the presence of ammonium phosphate.

Inulinase production in a lab scale fermenter has been reported from *A. niger* using kuth (*Saussurea lappa*) root powder (1%). Aeration of 1.5 vvm and agitation of 300 rpm has been reported optimal for the production of inulinase (290 IU/ml) at 30 °C after 72 h (Viswanathan and Kulkarni, 1995a). Further, full factorial design has been used for inulinase production with varying combinations of kuth roots, corn steep liquor and agitation rates at shake flask level. Kuth root powder (1%) and corn steep liquor (1%) was reported optimal for highest inulinase activity, and agitation contributed 18% in the inulinase production. Maximum inulinase activity (302 IU/ml) was reported at 30 °C after 72 h (Viswanathan and Kulkarni, 1995b). They also reported that inulinase is inducible and subjected to catabolic repression (Viswanathan and Kulkarni, 1995b).

The inulinase production by a mixed culture of *A. niger* and *K. marxianus* in a medium containing Jerusalem artichoke powder was also investigated (Ongen-Baysal and Sukan, 1996). Inulinase produced by the mixed culture shown higher invertase activity from their respective monocultures.

Inulin was used as a carbon source for the production of inulinase from *Penicillium* sp. TN-88. Enzyme production was 9.9 IU/ml and after incubation of 4 days at 30 °C, the ratio of inulinase to invertase in the medium was 11.2 (Nakamura *et al.*, 1997).

Dahlia extract and pure inulin were reported best carbon sources as compared to monosaccharides, sucrose and sugar cane molasses for the production of inulinase from *Aspergillus niger*. Amongst various nitrogen
sources, best results have been reported with casein as compared to mineral nitrogen. Optimum culture conditions reported for the inulinase synthesis were pH 5.5, agitation 200 rpm at 28 °C and 60 h fermentation time (Cruz et al., 1998).

A response surface method was used to optimize the medium for the production of inulinase from *Kluyveromyces* sp. Y-85. Xylose and extract of Jerusalem artichoke tubers were reported best inducers, whereas reducing sugars such as fructose and glucose suppressed the enzyme production. Among nitrogen sources, all the organic sources especially pork were better than inorganic sources. Maximum inulinase activity (59.5 IU/l) at flask level was reported with Jerusalem artichoke extract (8%), urea (2%), beef extract (0.2%) and CSL (4%) at 30 °C after 24 h. Furthermore, scaling-up to 15 L and 1000 L tower type fermenter had produced 68.8 IU/ml inulinase (Wenling et al., 1998).

A comparative study on inulinase synthesis by *Staphylococcus* sp. RRL-1 and *Kluyveromyces marxianus* ATCC 52466 has been done in a medium containing inulin as a sole carbon source. Inulinase activity of 0.618 IU/ml was reported from a bacterial strain in a medium with optimal pH, temperature, agitation and inoculum size of 6.5, 37 °C, 150 rpm and 4%, respectively. Whereas, 0.47 IU/ml of inulinase activity was reported from yeast culture in a medium with optimum pH (6.0), temperature (30 °C), agitation (150 rpm) and inoculum size of 4% (Selvakumar and Pandey, 1999a). Further, wheat bran has been used in the solid state fermentation for the production of inulinase from *Staphylococcus* sp. RRL-1 and *Kluyveromyces marxianus* ATCC 52466. Under optimal conditions, inulinase production was maximum (107.64 U/gds) after 48 h with bacterial culture and 122.88 U/gds with the yeast culture after 72 h (Selvakumar and Pandey, 1999b).

A batch production of inulinase in a medium having pH 5.0 with aeration of 1.0 vvm and agitation of 120 rpm has been reported in a 15 L
fermenter at 30 °C using *Kluyveromyces marxianus* DSM 70106. Inulin (1%) was reported best carbon source in a medium containing yeast extract (0.2%), peptone (0.5%) and other salts. Glucose, fructose and sucrose reduced the inulinase production by 46%, 58% and 71%, respectively, whereas, corn steep liquor did not shown any effect on the production (Pessoa and Vitolo, 1999).

The production of extracellular inulinase from *K. marxianus* NRRL 2415 in a batch culture was studied and the enzyme was reported constitutive in nature. The highest inulinase production (10.6 IU/ml) was achieved when test organism was grown aerobically in a buffered medium (pH 7.0) containing 4% sucrose, 2% yeast extract and 0.6% sodium dihydrogen phosphate at 30 °C. After optimization, enzyme activity as well as productivity were increased by a factor of 4.6 each (Bazaraa and Al-Dagal, 1999).

*K. marxianus* var. *bulgaricus* has been used for the constitutive production of extracellular inulinase using inulin, sucrose, fructose and glucose as a carbon source. Highest inulinase activity was reported in a medium containing sucrose as the limiting substrate that was dependent on the dilution rate. Optimum pH, temperature, agitation and fermentation time at shake flask level were 3.0, 30 °C, 200 rpm and 48 h, respectively (Kushi *et al.*, 2000).

The production of inulinase from *K. marxianus* grown on agricultural wastes has been investigated (Shady *et al.*, 2000). Dahlia tubers, chicory roots, orange peel and beet pulp gave better production of inulinase as compared to other carbon sources. Among these, dahlia tubers were reported best carbon source and ammonium nitrate as best nitrogen source for inulinase synthesis from *K. marxianus* at 30 °C after 3 days of incubation.

The optimal culture conditions for endoinulinase production using chicory roots were studied in a shake flask culture (Park and Yun, 2001).
The higher enzyme production was achieved with Xanthomonas sp. (15 IU/ml) than the Pseudomonas sp. (3 IU/ml).

_Bacillus polymyxa_ 29, _B. polymyxa_ 722 and _B. subtilis_ has been used for the biosynthesis of extracellular inulinase (Zherebtsov et al., 2002). Enzyme was reported to be constitutive in these strains, where starch and sucrose increased its synthesis. Maximum production of inulinase from these bacterial strains has been reported 76.1 IU/ml, 46.1 IU/ml and 41.1 IU/ml, respectively. The presence of reduced mineral nitrogen or organic nitrogen was reported necessary for enzyme production. The optimal pH, temperature and incubation time for the inulinase synthesis were 7.0, 33-35 ºC and 72 h, respectively.

The growing cells of thermophilic _Bacillus_ sp. 11 were immobilized on formaldehyde activated polysulphone membranes for the semicontinuous production of inulinase. The biocatalyst was reported to produce 1.5-2.0 times enzyme yield than those of free cells. It retained 60-90% of its original activity at the end of fifth cycle of repeated batch cultivation (Uzunova et al., 2002).

The production of both exo and endoinulinases by _Aspergillus ficuum_ JNSP5-06 has been investigated by Jing et al. (2003). Optimum fermentation conditions reported were inulin (2%), yeast extract (2%), (NH₄) H₂PO₄ (0.5%), NaCl (0.5%), MgSO₄, 7H₂SO₄ (0.05%), ZnSO₄, 7H₂O (0.01%) and initial pH 6.5. Inulinases were separated by native polyacrylamide gel electrophoresis (PAGE) and eight bands are reported.

An extracellular inulinase has been produced from _Streptomyces_ sp. GNDU 1 using inulin (1%) as an inducer. Glucose, fructose and sucrose repressed inulinase production. Yeast extract was reported best nitrogen source, whereas ammonium ions were inhibitory to the enzyme synthesis. Maximum inulinase (0.522 IU/ml) has been produced with optimum pH 7.5 at 46 ºC after 24 h of incubation (Gill et al., 2003).
A simplex method has been used to optimize production of *Aspergillus niger* strain 13/16 with respect to 5 components of the medium. Over 1.6-fold higher inulinase synthesis has been reported by this method as compared to classical one factor at a time method. Maximum enzyme activity achieved was 80 IU/ml and it was 2.5-folds higher in comparison with initial enzyme activity in the basal medium (Skowronek and Fiedurek, 2004).

Various physical and chemical parameters were optimized for the production of inulinase from an isolate of *A. niger*. Maximum productivity of inulinase (176 IU/ml) was reported in a medium containing inulin (5%, w/v), galactose, corn steep liquor and (NH$_4$)H$_2$PO$_4$ as nitrogen sources and adjusted to pH 6.5 at 28°C with a fermentation time of 72 h (Kumar *et al.*, 2004).

Effects of aeration, agitation and type of impeller (disk turbine, marine, pitched blade) were studied in a batch stirred reactor. Two factorial designs 22 were carried out. Agitation speed varied from 50 to 550 rpm (revolution per minute), aeration rate from 0.5 to 2.0 vvm (air volume/broth volume·minute). It has been shown that the enzyme production was strongly influenced by mixing conditions, while aeration rate was shown to be less significant. Additionally, the increase in the agitation speed is limited by the death rate, which increases drastically at high speeds, lowering the enzyme production (Bernardo et.al 2004).

A factorial design and response surface analysis were used for the production of inulinase from *Kluyveromyces marxianus* ATCC 16045 in a batch stirred reactor, using sucrose as a carbon source. Agitation and aeration were reported to be critical for the yeast, which affected viability of cells and enzyme production. Highest enzyme levels of 176 IU/ml were produced with aeration and agitation of 1 vvm and 450 rpm, respectively (Silva-Santistebean and Filho, 2005).
Inulinase production by yeast *Kluyveromyces* var. *bulgaricus* growing on yacon extract has been investigated. Maximum inulinase activity was reported with 40% (v/v) yacon extract. The optimum cultivation pH was 3.5, whereas a temperature range of 25-40 °C had shown no influence either on growth or on enzyme activity (Cazetta et al., 2005).

Three isolated strains of *Kluyveromyces* sp. A1, A2 and P7 isolated from agave sap and its fermented product have been used for the production of inulinase. With inulin (0.25%) as a carbon source, the enzyme production from these strains were 27.12 IU/mg, 23.12 IU/mg and 18.89 IU/mg, respectively which was 2.5 times more than the control strain *Kluyveromyces* sp. CDBB-L-278. All the reported strains had low susceptibility to catabolite repression (Cruz-Guerrero et al., 2006).

The production of inulinase from *Kluyveromyces marxianus* ATCC 16045 using sucrose and ammonium sulfate has been carried out. Intrinsic inulinase activity calculated was 879 IU/mg and material balances studies has shown no more than 0.1% either of carbon or nitrogen initially present in the medium were incorporated in the extracellular inulinase released under different cultivation conditions (Silva-Santisteban et al., 2006).

Inulin containing agricultural plant materials like rye, barley, banana, garlic, onion, wheat, chicory and dahlia were used for the production of inulinase using *Streptomyces* sp. Maximum inulinase activity (0.524 IU/ml) was reported using garlic as a carbon source in a medium having pH 7.0 and agitation 200 rpm at 37 °C after 24 h. Optimum pH and temperature for enzyme activity were 6.0 and 60 °C, respectively. Whereas, glycerol and mannitol were reported to show protective effect on enzyme activity (Sharma et al., 2006).

Conidia of *Aspergillus niger* 20 OSM were immobilized on pumice stones/polyurethane sponge and used in a repeated batch process for the production of extracellular inulinase. The maximal
inulinase production (22.1 IU/ml) has been reported in a medium adjusted to pH 6.0 and containing 0.5 g of carrier at 30 °C, with agitation speed of 200 rpm after 96 h. This procedure enabled six repeated-batches for enzyme production using the same carrier (Skowronek and Fiedurek, 2006).

A marine yeast *Pichia guilliermondii*, isolated from the surface of the marine alga has been used for the production of inulinase. As a medium sea water was used which contained inulin (4.0%) and yeast extract (0.5%). Optimized fermentation pH (8.0), temperature (28 °C) and shaking (170 rpm) were reported for the production of 60 IU/ml of inulinase after 48 h (Gong *et al.*, 2007).

A marine yeast strain *Cryptococcus aureus* G7a, isolated from sediment of China South Sea has been reported for the production of inulinase. The crude inulinase produced by this marine yeast showed the highest activity at pH 5.0 and 50 °C. The optimal medium for inulinase production was artificial seawater containing inulin 4.0% (w/v), K2HP04 0.3% (w/v), yeast extract 0.5% (w/v), KCl 0.5% (w/v), CaCl2 0.12% (w/v), NaCl 4.0% (w/v) and MgCl2·6H2O 0.6% (w/v), while the optimal cultivation conditions for inulinase production were pH 5.0, temperature of 28 °C and a shaking speed of 170 rpm. Under the optimal conditions, over 85.0 IU/ml of inulinase activity was produced within 42 h of fermentation at shake flask level (Sheng *et al.*, 2007).

A newly isolated strain of *Kluyveromyces marxianus* YS-1 was used for the production of extra cellular inulinase in a medium containing inulin, meat extract, CaCl2 and sodium dodecyl sulphate (SDS). Fermentation medium pH 6.5, cultivation temperature 30 °C and 5% (v/v) inoculum of 12 h-old culture were optimal for enzyme production (30.8 IU/ml) with a fermentation time of 72 h at shake flask level. Raw inulin (2%, w/v) extracted from dahlia tubers by processing at 15 kg/cm² for 10 min was optimum for bioreactor studies. Maximum enzyme production
(55.4 IU/ml) was obtained at an agitation rate of 200 rpm and aeration of 0.75 vvm in a stirred tank reactor with a fermentation time of 60 h (Singh et al., 2007).

The effects of sucrose concentration, pH, temperature and aeration rate on the production of biomass and inulinase (2,1-beta-D-fructan fructanohydrolase, EC 3.2.1.7) were investigated using fed-batch fermentation by *Kluyveromyces marxianus var. bulgaricus*. The best sucrose concentration for enzyme activity was 10 g L⁻¹, whereas activity was inhibited when the sucrose concentration was 20 g L⁻¹. Enzyme activity was strongly influenced by pH of the culture. pH 5.0 was the most suitable for both enzyme activity and cell growth, achieving 98.04 U mL⁻¹ and 14.05 g L⁻¹, respectively, after 72 hours of cultivation. The highest production of inulinase and biomass was observed at temperatures of 25°C and 30°C, whereas a decrease in activity was observed at 35°C and 40°C. The best aeration rate was 1.0 vvm. The increase in aeration rate or oxygen injection provided no increase in enzyme activity and biomass (Cazetta et al., 2008).

From the rotted Jerusalem artichoke tubers, 11 fungi were isolated on synthetic medium containing inulin as a sole carbon source. On the base of inulinase activity on inulin (I), one of them was selected and identified as *Aspergillus tamarii* AR-IN9. Incubation of *A. tamarii* AR-IN9 for 72 h, pretreatment of inulin-containing agro-wastes in autoclave at 20 lb/in², 3% corn steep liquor in the growth medium, pH 5.5 and 35°C were the best conditions for inulinase production. The overall production reached up to 71.97 U mL⁻¹. *Aspergillus tamarii* AR-IN9 showed invertase activity on sucrose (S), with high values of I/S ratio which indicating that the fungus is active in inulinase production. Inulinase activity reached its maximum at pH 5.2 and 45°C. The enzyme was still stable by 80% or more at the pH range from 4.4 to 7.2 for 24 h and by 75% at 50°C for 90 min. The metal ions; MgCl₂, CoCl₂ and MnCl₂ positively modulated inulinase activity.
The resultant inulinase showed high hydrolysis activity on Jerusalem artichoke (71.64%), dahlia tubers (67.55%) and chicory roots (55.11%). Therefore, various agro-wastes and inulin-containing materials could be economically hydrolyzed with *A. tamarii* AR-IN9 inulinase into fructose, which has many therapeutic and industrial aspects. Besides the beneficial environmental impact by the bioremediation of such agro-wastes (Saber et al., 2009).

The study on 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 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 (Mohammed et al., 2011).

The concentration of the carbon source had a repressive effect on the activity of inulinase. When the concentration was increased to 60 g/L, inulinase activity was only 50% compared with carbon source concentration of 20 g/L (Jiaoqi et al., 2012). Four different substrates banana peel, garlic peel, wheat bran and Rice bran were used. Among the four substrates the use of banana peel (12 g) at 40% moisture and reported organic nitrogen sources yeast extract resulted in maximum production of inulinase (Narayanan et al., 2013).

The effects of various carbon and nitrogen sources on inulinase activity were investigated, and the best ones were found to be Jerusalem artichoke
extract and peptone. Effects of medium composition and fermentation conditions were also determined for the production of extracellular inulinase. The addition of 1 % (by volume) tween 80 into fermentation medium enhanced the secretion of extracellular inulinase. In bench-scale fermentor, the age and amount of inoculum were also optimized and they were determined to be 40-hour-old culture and 5.0 % (by volume), respectively. The initial pH of the medium was adjusted to 6.5 and no further pH control was needed. Optimal aeration and agitation were 0.75 L/min and 150 rpm, respectively (Nguyen et al., 2013).

**Hydrolysis of Inulin for High Fructose Syrup Preparation:** Amongst various applications of inulinases, production of high fructose syrup is one of its major application. Raw materials used by various workers include commercially available pure inulin as well as cheap plant materials like chicory, Jerusalem artichoke, kuth roots, etc. Hydrolysis of inulin using both free as well as immobilized inulinase has been reported. Both batch and continuous systems have been used to hydrolyze inulin for the preparation of HFS.

The extract of Jerusalem artichoke tubers with 90% total sugars was hydrolyzed by inulinase to free sugars containing fructose (77%) and glucose (23%). Fructose in the hydrolysate was separated by Dowex ion-exchange chromatography with 61% recovery (Byun and Nahm, 1978).

The preparation of fructose by hydrolysis of inulin from chicory roots, belgian endive roots and dahlia tubers by inulinase followed by the recovery of fructose crystals has been reported (Kerkhoffs, 1981). Inulin solution (pH 5.0) obtained by freeze dried powder (35 g) of chicory roots was hydrolyzed at 50 ºC for 24 h and 24.5 g of fructose crystals were obtained from the hydrolyzed solution. Similarly, 16.8 g and 15.1 g of
crystalline fructose were obtained from 25 g belgian endive roots and 20 g dahlia tubers, respectively.

A complete hydrolysis of 15% carbohydrate in chicory and Jerusalem artichoke extracts was achieved in 10 h using free inulinase from *Pichia polymorpha*. A pH of 5 and temperature of 45 °C has been reported optimum for the hydrolysis (Guiraud and Galzy, 1981).

In a batch reactor at 45 °C, 90% conversion of artichoke tuber extract and 34 mg/ml of fructose yield has been reported in 20 h. Whereas, in a continuous system, 90% conversion of 7% inulin with the corresponding volumetric productivity of 102 mmol/l/h has been reported at 40 °C with a space time of 3.8 h (Kim and Byun, 1982). A continuous hydrolysis of chicory extract containing 15% sugars was carried out in a yeast cell reactor for 75 days. The system permitted complete bioconversion of inulin with sufficient cell growth. Reactor was operated for 75 days and the F/G ratio of the released product was 3 (Guiraud *et al.*, 1983).

The high fructose syrup has also been prepared by hydrolysis of inulin solution (pH 4.5-5.0) at 60-65 °C with an inulinase (1-5 IU) from *Aspergillus ficuum* containing both exoinulinase and endoinulinase with high degree of thermal stability (Zittan *et al.*, 1985).

Batch and continuous production of high fructose syrup from Jerusalem artichoke has been studied using *Kluyveromyces marxianus* cells entrapped in gelatin matrix. Overall 93% hydrolysis in 3 h with 42 g/l fructose yield has been reported in a batch system. Whereas, 90% conversion and 90 g/l/h volumetric productivity at a dilution rate 1.66 per hour has been reported in a continuous system. Bioreactor was run for 10 days with 2% loss of activity and no pellet disruption (Bajpai and Margaritis, 1985). Further, *Kluyveromyces marxianus* cells entrapped in sodium alginate beads were used by them for the continuous production of high fructose syrup from 15% Jerusalem artichoke inulin. Immobilized cell
concentration of 190 g/l beads and sodium alginate concentration of 1.4% has been reported optimum (Bajpai and Margaritis, 1986).

Batch and continuous hydrolysis of Jerusalem artichoke inulin has been studied at 40 °C using inulinase from *Aspergillus ficuum* immobilized on chitin. In a batch system, extent of hydrolysis reported was 80% in 4 h and the ratio of fructose and glucose in the liberated product was 6.1. In a continuous system, maximum reducing sugar concentration of 88 g/l has been reported at a residence time of 4.4 h. The conversion at this residence time was 88%. Further, the immobilized inulinase was reported to be operated for two weeks with 4.8% loss in activity (Kim and Rhee, 1989).

A commercial inulinase preparation from *Aspergillus ficuum* (Novozym 230) was used in free form for the hydrolysis of 1% pure inulin. A high conversion of 90% with glucose and fructose as the sole products has been reported at 50 °C in 5 h (Carniti *et al*., 1991).

Whole cells of *Cladosporium cladosporioides* were used for batch production of fructose from Jerusalem artichoke extract. A complete hydrolysis was achieved at 60 °C in 150 min using 260 g/L of sugars. Thin layer chromatography of the enzymatic hydrolysate of inulin and Jerusalem artichoke extract had shown the exoaction mechanism of the enzyme (Ferreira *et al*., 1991).

Invertase and inulinase from *Cladosporium cladosporioides* have produced a glucose fructose mixture from which glucose was preferentially removed. The results have shown that 62-83% of the initial fructose was recovered from the fermentation of sucrose or an extract of Jerusalem artichoke. The fructose recovery was nearly 80% of the total fructose theoretically available. This yield was almost constant in 11 cycles using the same cells and fresh sterilized extract of Jerusalem artichoke (De-Andrade *et al*., 1992).

Partially purified inulinase from *Fusarium oxysporum* has been immobilized on DEAE-cellulose and used for the hydrolysis of inulin.
Hydrolysis of 2% inulin has been carried out at 37 °C at a flow rate of 10 cm$^3$/h. After three cycles, 90-95% conversion to fructose was reported (Gupta et al., 1992).

Hydrolysis of pure inulin and extracts of fresh and dried Jerusalem artichoke using partially purified inulinase from *K. marxianus* var. *bulgaricus* in soluble form. After 6 h, fructose yield was 33.4, 77.0 and 73.0% from pure inulin and extracts of fresh and dried Jerusalem artichoke, respectively with 10 IU of enzyme. Whereas, highest levels of fructose were 83.9 and 85.7% after 24 h, using 10 IU and 20 IU of free inulinase, respectively (Manzoni and Cavazzoni, 1992).

Inulinase from *A. niger* immobilized on Amino-cellulofine was used in a packed bed reactor for the continuous hydrolysis of inulin for the production of high fructose syrup. A complete hydrolysis of 5% pure inulin has been reported at a flow rate of 1.0ml/min at 40 °C over 45 days period of continuous operation. Volumetric productivity in the reactor was 410 g reducing sugars/l/h and the hydrolyzed product contained 97% fructose and 3% glucose. Half life of the bioreactor at 60 °C was reported to be 16 days (Nakamura et al., 1995).

Hydrolysis of inulin extracted and purified from kuth root powder and standard inulin was carried out with purified inulinase from *A. niger* Van Tieghem UV 11. Fructose yields were 70% and 68%, respectively under same conditions of operation (Viswanathan and Kulkarni, 1995d). Inulinase produced from mixed culture of *A. niger* and *K. marxianus* was used for hydrolysis of Jerusalem artichoke extract having total sugar of 16% (w/v) and pH 5.0 at 50 °C. A conversion of 90% was achieved with 5% (v/v) of crude cell free enzyme preparation after 4 h (Ongen-Baysal and Sukan, 1996).

A process of producing fructose syrup from agave plants was developed using a mixture of exo and endoinulinase (2880 IU) from *A. niger* (Partida et al., 1998). A pulp of milled agave plant heads was
liquified and concentrated to produce a polyfructose concentrate. The polyfructose extract thus obtained was treated with activated charcoal followed by cationic and anionic resins to produce a demineralized, partially hydrolyzed polyfructose extract. The extract having pH 4.5 was mixed with enzyme and incubated at 50 °C for 6 h. The hydrolyzed extract was then concentrated using conventional techniques in a vacuum evaporator and filtered through a membrane having a 0.45 μm nominal pore size, to produce a fructose concentrate of 77.5 °Brix.

Aqueous extracts of dahlia, chicory and artichoke has been prepared by mashing and agitating the tubercles in distilled water for 20 min followed by sieving and centrifuging the supernatant. Hydrolysis of 5% inulin from these extracts (pH 5.0) has been carried out at 50 °C using 14 units of inulinase from *Aspergillus niger*. Enzyme was reported to show higher activity on inulin from chicory than on Jerusalem artichoke and dahlia inulin. A complete hydrolysis within 3 h of incubation has been reported for all the three plants materials and fructose content obtained was 90% (Cruz *et al.*, 1998).

The powdered tubers of Jerusalem artichoke were extracted in boiling water and used as a substrate for the preparation of high fructose syrup. A complete hydrolysis of the extract containing 4.5% fructans in a bed column reactor has been reported at 50 °C with a dilution rate of 1.7/h (flow rate 30 ml/h). Percent hydrolysis was 75% and corresponding volumetric productivity was 234.9 g reducing sugars/l/h. The hydrolytic product was a mixture of 85% fructose and 15% glucose. The half life of the developed bioreactor was reported to be 32 days (Wenling *et al.*, 1999).

The continuous production of high fructose syrup from inulin using amino-cellulofine immobilized recombinant inulinase has also been investigated in a packed bed reactor (Kim *et al.*, 2000).

The production of high fructose glucose syrup from dried tubers of girasole by immobilized inulinase has been reported. The reactor
containing immobilized inulinase was operated using 20% inulin liquor having pH 4.0-5.0 at 57-63 °C with flow rate of 1500-2250 ml/h (Zexiang, 2003). A method of producing fructose from girasole extract using gene engineered inulinase from *A. niger* has also been reported recently (Zhang *et al.*, 2004).

An exoinulinase from *Kluyveromyces marxianus* and expressed in *Pichia pastoris* has been studied for the hydrolysis of inulin. The optimum pH and temperature were 4.5 and 55 °C, respectively. Glucose showed competitive inhibition on enzyme activity for hydrolysis of inulin which had a degree of polymerization (DP) not less than 10, but no inhibition effect on enzyme activity for hydrolysis of oligosaccharides (DP≤ 9). It was also reported that inulinase had the synthetic activity of inulin (Zhang *et al.*, 2005).

Inulinase extracted from *Aspergillus fumigatus* and immobilized on chitin, QAE-Sephadex and ConA linked amino activated silica beads was used for continuous hydrolysis of inulin (2.5%) at 60 °C. Half lives of the enzyme immobilized on these supports have been reported 35, 22 and 45 days, respectively and the corresponding volumetric productivities reported are 2.7, 2.3 and 3.4 g/l/h (Gill *et al.*, 2006).

A commercial inulinase preparation was immobilized on Amberlite IRC-50 and used for the hydrolysis of inulin. Activation energies of free and immobilized inulinase were 30.0 and 26.6 kJ/mol, respectively. Immobilized inulinase was reported stable for one hour at 60 °C. Repeated biotransformation for 24 h has been reported without any decay in activity at 40 °C, whereas 20% loss was reported for trials at 50 °C (Catana *et al.*, 2007).

Inulinase (2,1-β-D fructan fructanohydrolase, EC 3.2.1.7) targets on the β-2, 1 linkage of inulin, which is a polyfructan consisting of linear fructose units and hydrolyzes it into fructose. This is a single step method,
which can produce as high as 95% of fructose. D-fructose is amongst those sweeteners, which are important primarily because of their functional properties rather than sweetness. The demand of fructose is ever increasing due to its beneficial role for diabetics, increased iron absorption in children, increased ethanol removal from blood of highly intoxicated persons, apart from being used as a low calorie sweetener, because it is twice sweeter than sucrose and organoleptically desirable (Vandamme et.al., 1983). Its low water activity makes it’s a preferred choice for preservation.

Being a sweetener with low cost, its scope extends from food and beverage industries to pharmaceuticals. In pharmaceutical, its uses include formulation of tablets or solutions for injections and infusions (Hanover and White, 1993). Currently, the commercial production of high fructose syrup relies on the multienzymatic hydrolysis of starch (Huisman and Gray, 2002). By this method, not more than 45% (approx.) yield of fructose can be obtained. To increase the fructose content over 90%, ion-exchange techniques have been developed, but they add to the cost of production (Fleming and Groot-wassink, 1979; Toumi and Engell, 2004). Lower complexity, higher fructose yield and concomitant cost reduction are easily foreseen for the production process based on inulin hydrolysis. Recently, it is coming up as a more promising technology that includes single step hydrolysis of inulin which can produce as high as 95% fructose content.

Therefore, inulinase can be an enzyme of choice for the production of high fructose syrup. Second important application of inulinase is in the production of ethanol. The process generally involves simultaneous saccharification and fermentation using inulinase or an inulinase producing microorganism in combination with other microorganisms (Kim and Rhee, 1990) and (Nakamura et.al., 1996). Apart from this, inulinases are also used for the production of inulo-oligosaccharides (Yun et.al., 1997).
gluconic acid, sorbitol (Kim DM, and Kim HS, 1992), pullulan (Shin et.al., 1989) and acetone-butanol (Oiwa et.al., 1987). Inulinase production is effected by medium composition and type of the organism used for fermentation. For commercial production of an enzyme, the factors like high yielding microorganism, a cheap raw material and its scaling-up feasibility are considered. High inulinase yielding microorganisms are Aspergillus, Kluyveromyces and Penicillium strains (Cruz et.al., 1995) and (Ongen-Baysal et.al., 1994). Various plant materials like dahlia, chicory and Jerusalem artichoke are reported as cheap raw materials for inulinase production.