Australian Journal of Basic and Applied Sciences, 5(10): 453-462, 2011
ISSN 1991-8178

Raw Garlic as a New Substrate for Inulinase Production in Comparison to Dry Garlic

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Abstract: Although several new substrates for the production of inulinase have been reported as being economically effective such as utilization of inulin-rich substrate rather than pure inulin, however, there is still need to develop the substrate to make the entire process much cheaper and more effective. Garlic has been suggested to have induction effect on inulinase production, but this induction effect is reduced in dried garlic. Therefore, raw garlic was adopted as a new substrate. Raw garlic improved inulinase activity from *Aspergillus niger* NRRL 3 up to 66 fold higher than pure inulin. Inulinase activity was 86 IU/L and 5652 IU/L for pure inulin and raw garlic respectively. Raw garlic improved inulinase activity up to 500 IU/L higher than dried garlic due to reduction of fructan level in the later. The study gives evidence that garlic constituents like calcium and phosphorus are responsible for induction of much higher inulinase activity as beneficial inulinase production was achieved using calcium and phosphorus in the form of $CaCl_2$ and K_2HPO_4 in concentrations of 0.1g/100ml medium and 0.25g/100mL medium respectively. Economically, raw garlic satisfies all the demands of industrial technologies because it is low cost, safety, healthy and save the time and energy required for drying. Furthermore, out of various nitrogen sources tested for production, urea gave the highest inulinase activity which is also cost effective.

Key words: *Aspergillus*, Inulinase, production, raw garlic, fructose.

INTRODUCTION

Plants like leek, onion and garlic is as old as the history of the human race and as extensive as civilization itself .References to these plants in the Bible and the Quran reflect their importance to ancient civilization both as flavorful foods and as healing herbs (Block, 2010). Onions, leeks and garlic are rich in fructan and are the most common sources of this polysaccharide in the Australian diet (Blakeney *et al.,* 1997). Not only these plants are rich in fructans but about 15% of flowering plant species have fructans as their main storage carbohydrate. Among these plants there are several economically important crops (e.g., wheat, barley, chicory, Jerusalem artichoke).

Fructans are fructose-based carbohydrates, but the precise type and degree of polymerization are species-specific and tissue-specific (Van den Ende *et al.,* 2001). Inulin is one of such fructans. Chemically, inulin is a linear fructan (degree of polymerization, DP, 2-60 or higher) consisting of fructose molecules linked by β (2-1) glycosidic bonds with, generally, a terminal glucose unit connected to the last fructose with a α (1-2) bond. Several inulin types occur in nature and they differ in the degree of polymerization and molecular weight, depending on the source, the harvest time, and processing conditions (Chiavaro *et al.,* 2007). Inulinases constitute an important class of enzymes for production of fructose and inulo-oligosaccharides, which are extensively used in pharmaceutical and food industry. Inulinases (EC 3.2.1.7) are enzymes that hydrolyze inulin and are classified in two types according to their mode of action on inulin. One, endo-inulinase produces oligosaccharides and the other, exo-inulinase liberates fructose and the production of them has been reported from various fungal, yeast and bacterial strains (Cazzeta *et al.,* 2005, Gill *et al.,* 2006, and Kushi *et al.,* 2000).

Since ancient times, garlic has been consumed as a seasoning or spice. In recent years, garlic has been found to contain a variety of physiologically active components, and therefore, garlic is widely used as a health food and a drug in most countries of the world. The garlic walks the fine line between food and medicine. Major emphasis in this study was placed upon established or developing uses rather than upon potential uses. In this respect this direction of research may be of interest in the innovative use of raw garlic as a substrate for inulinase production and consequently fructose production.

Inulin is reserve carbohydrate of garlic and many authors had assigned it an important nutritional value and few authors used inulin of dried garlic for Inulinase production (Sharma *et al.,* 2006). Despite this, there is no information about using raw garlic in inulinase production or about the effect of heated or stored garlic on inulinase activity. This is the first study on the effect of raw garlic fructan on inulinase production from

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Aspergillus niger NRRL 3 or may be any other sources of microbial inulinase. This study clearly demonstrates that not only raw garlic fructans contributes to the highest activity of inulinase but also the other nutrients already exist in garlic like, phosphorus and calcium. Our objective was to prove that raw garlic with its unique inulin and nutrients contents is the best new substrate for inulinase production from *Aspergillus niger* or may be any other microorganism. The industrial scale production of inulinase can be carried out using inexpensive substrates and nutrients.

As far as we know, and according to the available literature, the present study could be considered as the first effort devoted to discover the potency of one of the natural (raw garlic), renewable biological source to produce inulinase and consequently fructose.

MATERIALS AND METHODS

Microorganisms and Media:

The strain used for screening of Inulinase production was obtained from NRRL (Northern Regional Research Laboratory). During the experiments, the culture was maintained at 4˚C on potato dextrose agar slants and sub-cultured every week. The composition of the basal culture medium used for screening the strains for inulinase was as follows (g/l): Jerusalem artichoke powder, 10.0, peptone, 3.0, K2HPO4, 1.0, MgSO4, 0.5, KCl, 0.5 (Sharma *et al.,* 2006) with some modifications. The pH was 5.5 before sterilization (autoclaving for 15 minutes at 121˚C and 1.5 atmospheric pressure).

Plants Used Instead of Inulin:

Different economically important crops were examined for their ability to induce inulinase production such as: garlic, onion, chicory, artichoke, wheat bran and Jerusalem artichoke. All of them were obtained from local market and dried in oven at 100˚C.

Growth Conditions:

The strain of *Aspergillus niger* NRRL 3, producing high inulinase activity in shaken cultures, was used and was incubated in 250 ml conical flasks, each containing 50 mL of the medium. The media were inoculated with two discs of fungal cultures from potato dextrose agar Petri dish and cultured at 30˚C for 7 days on a rotary shaker (160 rpm). Then at the end of the fermentation, the whole culture content was centrifuged in a cooling centrifuge at 4˚C at 400 rpm for 10 min. Inulinase activity was measured in the filtrate.

Enzyme Assay:

Inulinase activity was assayed by measuring the amount of reducing sugar released from inulin using Nelson's method(1944).Using 1% inulin, sodium acetate buffer(0.2 M, pH 5.6). One unit of Inulinase (IU) was defined as the amount of enzyme which librated 1µ mol of reducing sugar (fructose) per min under the assay conditions. The pure inulin used either for fermentation or enzyme assay was dahlia tubers inulin produced by Fluka company, Switzerland.

How to Select and Store Garlic?

Two different variety of garlic were used, Egyptian and Chinese garlic. Fresh garlic is recommended and selected by gently squeeze the garlic bulb between fingers to check that it feels firm and is not damp. Depending upon its age and variety, whole garlic bulbs will keep fresh for about a month if stored properly.

Fresh garlic cloves peeled and minced not pressed, to avoid the green pigments that formed as a result of enzymes reaction because when crushing is employed, juice rich of enzymes obtained. Store fresh garlic in either an uncovered or a loosely covered container in a cool, dark place away from exposure to heat and sunlight will help maintain its maximum freshens and will help avoiding reduction of fructan level.

RESULTS AND DISCUSSION

As more information has been gathered, it has become clear that garlic bulbs or cloves are susceptible to many microorganisms such as *Aspergillus*, *Penicillium*, *Botrytis* and *Fusarium* species particularly if the bulbs are damage but the most common organisms are *Aspergillus* and *Penicillium* (Dugan *et al.,* 2007).Therefore it was preferable to test some species of *Aspergillus* and *Penicillium* for their ability to produce inulinase as it is expected that these organisms which grow on a plant contain inulin probably capable to produce inulinase enzyme.

The results in Table (1) showed that all the tested fungi were capable of producing inulinase enzyme but with variable activities, the highest activity was recorded for *Penicillium melini* NRRL 848 and *Aspergillus niger* NRRL 3 and their activities were 522 and 245 U/L after incubation for 3 days respectively.

Although *Penicillium melini* gave the highest inulinase activity but it produced dark brown pigments in the culture medium which causes problems during work on inulinase enzyme, therefore *Aspergillus niger* NRRL 3 was selected for inulinase production. Inulinase activity increased after incubation of *Aspergillus niger* NRRL 3 for 7 days in shaken culture. Therefore period of 7 days was chosen as a best incubation period for production. *Aspergillus niger* is one of the most important microorganisms used in biotechnology. It has been in use already for many decades to produce extra-cellular (food) enzymes (Roukas 2000). The safety of *Aspergillus* has already been substantiated on the basis of long-term dietary experience.

Table 1: Survey of some Aspergillus and Penicillium for the production of extra cellular inulinase.

Organism	Incubation Period	Final pH of *C.F	Protein Content (mg/ml)	Inulinase activity (IU/L)
Aspergillus niger NRRL 3	3 days	6.5	1.8	245
	7 days	6.5	2.2	272
Aspergillus awamori MEA NRRL 3112	3 days	7.5	0.78	0
	7 days	7.5	1.2	28
Aspergillus oryzae MEA NRRL 3487	3 days	7	0.92	11
	7 days	7.5	1.1	23
Penicillium funiculosum NRRL 13033	3 days	6.5	1	0
	7 days	7	0.9	12
Penicillium funiculosum NRRL 13041	3 days	6.5	0.75	179
	7 days	7.5	1.1	150
Penicillium pinophilum NRRL 1142	3 days	8	3.7	9
	7 days	7.5	3.8	22
Penicillium melini NRRL 848	3 days	7	2.7	522
	7 days	7.5	2.4	92

Aspergillus niger strains produce a series of secondary metabolites, but it is only ochratoxin A that can be regarded as a mycotoxin in the strict sense of the word. Only 3-10% of the strains examined for ochratoxin A production have tested positive under favorable conditions. New and unknown isolates should be checked for ochratoxin A production before they are developed as production organisms. It is concluded, with these restrictions, that *Aspergillus niger* is a safe production organism (Schuster *et al.,* 2002).

Aspergillus niger NRRL 3 of the present study is not considered as a new or unknown isolates moreover its culture medium is not one of that favorable condition for production of ochratoxin A therefore it is concluded that it can be considered as inulinase production organism, this result is in a good agreement with Gill and Singh (2006) who investigated that *Aspergillus* species are among the best known producers of inulinase (Singh and Gill, 2006).

Effect of Different Inulin Rich Substrates on Inulinase Activity:

A potent strain and a cheap carbon source not only raise the commercial status of fermentation process but may also affect the economy of any country, either directly or indirectly (Shankaranand and Lonsane, 1993). Therefore different cheap natural carbon sources like some economically important crops were examined for their ability to induce inulinase production such as garlic, onion, chicory, artichoke, wheat bran and Jerusalem artichoke by replacing carbon source in the medium with mentioned plants.

Table 2: Effect of different inulin rich substrates on inulinase activity *of Aspergillus niger.*

Carbon-source 1g/100ml	Final pH of *C.F	Protein Content (mg/ml)	Inulinase activity (IU/L)
Garlic	6.5	1.6	1632
Onion	6	1.8	848
Chicory	7.5	1.9	731
Artichoke	7.5	1.5	296
Wheat bran	6	1.9	284
Jerusalem artichoke	7	1.7	182

The extent of variability observed with respect to inulinases among different carbon sources is quite remarkable. The Results in Table (2) indicated that garlic was the most favorable inducers for inulinase production with maximum activity 1632 IU/L. The activity obtained was significantly higher than that earlier reported by Sharma *et al.,* (2006), who used dried garlic as a carbon source with maximum inulinase activity (524 IU/L) from *Streptomyces* sp. The enzyme activity of the present study was about 3.1-fold higher than activity obtained by the previous mentioned author. And also was higher than those obtained by other authors who, used pure inulin as a carbon source like *Fusarium oxysporum* (8 IU/L after 9 days) (Gupta *et al.,* 1990); *Panaeolus papillonaceous* (230 IU/L after 6 days) (Mukherjee & Sengupta, 1987) and *Aspergillus fumigatus* (400 IU/L after 5 days) (Sharma *et al.,* 1998) and comparable to *Penicillium* species (560 IU/L after 5 days) (Nakamura *et al.,* 1997).

It is interesting to note that although plants considered in this study all contain fructan but there is distinct variable differences in inulinase activity. The possible differences in fructan synthesis in these species, probable number and concentration of polymers present in addition to their relative molecular weights, all these reasons may be responsible for the variable activities.

The tested plants contain fructan of inulin type but inulins themselves are differ in that, inulins with a terminal glucose are known as alpha-D-glucopyranosyl-[beta-D-fructofuranosyl] (n-1)-D-fructofuranosides, while inulins without glucose are beta-D-fructopyranosyl-[D fructofuranosyl] (n-1)-D-fructofuranosides. The structure of purified garlic fructan determined by ^{1}H NMR and ^{13}C NMR spectroscopy adopted from Puthanapura *et al.,* (2011) revealed that garlic fructan have (2 → 1) β-D-fructofuranosyl bonds linked to a terminal glucose at the non-reducing end and β-D-fructofuranosyl branching on its backbone. Baumgartner *et al.,* (2000) reported that fructan isolated from garlic characterized by being with high molecular weight. This structure obtained by both previous authors may explain why the activity of inulinase is higher with garlic as a substrate and varied among the other examined plants. Moreover, the fructan of chicory have a simple linear structure and are prone to degradation (Weyens *et al.,* 2004).

Garlic and onion have the same type of fructan however they differ in that garlic has larger polymer reach to degree of polymerization 50 while onion reach to degree of polymerization 5 (Darbyshir, 1981). The activity obtained from garlic was 1.9-fold higher than that obtained from onion and this result may not have been due to degree of polymerization only but may have been due to differences in some nutrients of both as shown in Table (3).

Table 3: Some nutritional value of garlic and onion.

Nutritional value per 100 g (3.5 oz) garlic and onion		
Nutrient	Garlic	onion
Manganese	0.47 mg	0.129 mg
Vitamin C	8.85 mg	7.4 mg
calcium	51.31 mg	23 mg
phosphorus	43.38 mg	29 mg
Vitamin B1(thiamin)	0.06 mg	0.04 mg
protein	1.80 g	1.1 g

This table adopted from the first chapter of *World's Healthiest Foods* book from www.whfood.org and website of onion wikipedia with some modification.

The differences between phosphorus and calcium content in both garlic and onion is obvious as shown in Table (3), while the other content is nearly the same, therefore the authors suggested that concentration of phosphorus and calcium may affect inulinase activity and this suggestion will prove later in other section of this study. Garlic is superior on onion in enhancing inulinase activity even if the peels only were used, this result proved by Ayyachamy *et al.* (2007) who reported that the inulinase production level of *Xanthomonas campestris* was 6.7-fold higher in garlic and 5.8-fold in onion, under optimized solid state fermentation conditions using garlic and onion peels compared with the submerged culture.

Effect of Different Carbon on Inulinase Activity:

To investigate the effects of other carbon sources for comparison with garlic, *Aspergillus niger* NRRL 3 was incubated in medium contain 10 different carbon sources separately and they were, sugarcane molasses, sugar beet molasses, sucrose, inulin, lactose, glucose, soluble starch, fructose, galactose, and arabinose. The results of inulinase enzyme activity of *Aspergillus niger* NRRL3 are shown in Table (4).

Table 4: Effect of different sugars on inulinase activity of *Aspergillus niger*.

Carbon-source 1g/100ml	Final pH of *C.F	Protein Content (mg/ml)	Inulinase activity (IU/L)
Sugarcane molasses	7.5	1.8	236
Sugar beet molasses	7	3	215
Sucrose	7	0.9	179
Extracted inulin	7	1.2	107
Pure Inulin	7	0.7	86
Lactose	5	1	65
Glucose	7	1	48
Soluble Starch	7	0.9	28
Fructose	7	1.1	17
Galactose	5.5	2.1	16
Arabinose	6.5	1	11

Among the different carbon sources tested, sugarcane molasses and sugar beet molasses were found to support inulinase synthesis, whereas glucose, lactose, galactose, arabinose and soluble starch showed a repressive effect on inulinase. Skikander (2006) reported that raw materials like beet or cane molasses are abundantly available as substrates for microbial fermentations. They contain various levels of available sugars

and, in addition, a range of certain metal salts, this result may explain why different types of molasses gave higher inulinase activity than the other pure sugars.

Sucrose slightly favored inulinase production compared with pure inulin which suppressed enzyme. This result in good agreement with Ayyachamy *et al.,* (2007) who reported that culture of Xanthomonas grown on sucrose showed considerable inulinase production. Comparatively, lower inulinase production was observed when inulin was used as a carbon source. The enzyme activity of the present study obtained by using extracted inulin as a carbon source was about 1.2 -fold higher than activity obtained by using pure inulin as a carbon source. Maximum inulinase production of 0.552 IU/ ml from inulin by Streptomyces sp. (Gill *et al.,* 2003) and 2.92 IU/ ml by *Aspergillus niger* 245 (Cruz *et al.,* 1998) have been reported.

Effect of Combination of Different Sugars With Garlic on Inulinase Activity of Aspergillus Niger NRRL 3:

Use of various carbon sources, singly and in combination, suggested that inulinase production by *Aspergillus niger* NRRL 3 is probably inducible and subject to catabolite repression Table (5).

Table 5: Effect of combination of different sugars with garlic on inulinase activity of *Aspergillus niger*.

C-source	Final pH of *C.F	Protein Content (mg/ml)	Inulinase activity (U/L)
Garlic + chicory	6.0	1.7	615
Garlic + sugarcane molasses	6.5	1.6	477
Garlic + inulin	6.0	1.4	408
Garlic + glucose	6.0	2.0	346
Garlic + wheat bran	6.0	1.8	333
Garlic + fructose	6.0	1.6	272
Garlic + sugar beet molasses	6.5	2.0	255
Garlic + sucrose	6.0	1.8	173

Garlic and chicory combination had higher inulinase yields than other combined carbon sources. However, all combinations of garlic with other carbon sources inhibited inulinase activity.

Comparison Between Effect of Raw and Dry Garlic on Inulinase Activity:

In an attempt to investigate the effect of heat of drying on garlic fructan content and consequently on inulinase activity, garlic was utilized in different forms as follows: oven dried garlic (under 50˚C and 100˚C) and raw garlic. All different forms of garlic were added separately in the fermentation medium as sole carbon source then sterilized and inoculated as mentioned before in material and methods section. Results in Table (6) has revealed that raw garlic induces much higher inulinase production than both dry garlic forms.

Table 6: Effect of heat of drying on garlic and inulinase.

Form of garlic	Color of garlic	Inulinase activity (U/L)
Oven dried garlic 100˚C	Dark Brown	3148
Oven dried garlic 50˚C	Light Brown	3171
Raw garlic	white	3611

Also it was of interest to note the change of garlic color due to different heat treatments, garlic changed into brown color after drying at 50˚C and became darker after drying at 100˚C,while raw garlic still keeping its color. It was interesting to observe that increasing temperature of drying from 50˚C to 100˚C can reduce inulinase activity from 3171 IU/L to 3148 IU/L. Bőhm *et al.,* (2005) stated that dry heating of inulin from Jerusalem artichoke at high temperature for 30 minutes induced complete degradation of the fructan chains and the concomitant formation of low-molecular degradation products, most likely di-D-fructose dianhydrides.

All the above results together suggested that heat of drying may be responsible for degradation or reduction of fructan level in garlic and hence reduces inulinase production. This suggestion is in agreement with Mahmoud (2006) who reported that different heat temperatures had irreversible change in the chemical structure of microbial fructan produced from Bacillus subtilis NRC33a such as degradation to a lower molecular weight structure and in agreement with Flores (2005) who reported that fructan changes in garlic stored at different temperatures. The authors of the present study tested this assumption by evaluating the total carbohydrates and free fructose content of fructans extracted from both raw garlic and dried garlic after and before hydrolysis. The initial concentration of inulin prior to any hydrolysis was measured with the phenol-sulfuric acid method (Dobois *et al.,* 1956) using dahlia tubers inulin produced by Fluka company, Switzerland. as standard. Inulin extraction yield (crude inulin) from fresh garlic was 41% while from dry garlic it was 31%. The amount of fructose for both fresh and dry garlic was determined in 0.01 g of extracted inulin after dialysis and hydrolysis to be 4278 µ g fructose for both fresh and dry garlic. The librated sugars from enzymatic hydrolysis of standard inulin, fresh garlic extract and dry garlic extract was analyzed using paper chromatography and illustrated in Fig. (1).

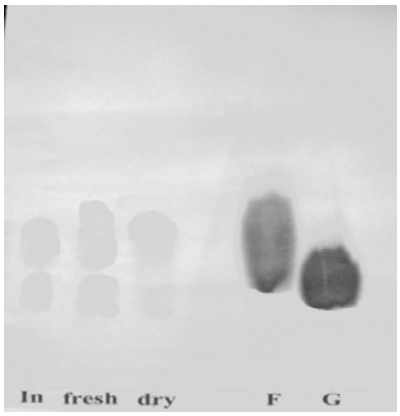

Fig. 1: Chromatographic analysis of enzymatic hydrolysis products of standard inulin, fresh garlic and dry garlic extracts.

The superior induction effect of raw garlic over dry garlic on inulinase production encouraged the authors to examine the influence of using raw garlic in the fermentation medium without sterilization in order to avoid heat effect and pressure effect of autoclaving. Unfortunately the un-sterilized garlic inhibited the growth of *Aspergillus niger* NRRL 3. Garlic could not be fermented by *Aspergillus* since garlic exhibits antimicrobial activity (Antonio *et al.,* 1998). No process of fermentation employing garlic as a predominant source has been reported. As mentioned before autoclaved raw garlic gave much higher activity, this distinct highest activity undoubtedly linked to the level of fructan. This may be due to that dry heating changes inulin to a different kind of fructan in contrast to wet heating (cooking) which has different effect since inulin is hot water soluble.

According to Puthanapura et al., 2011 and Ferris et al., (2008) heating and processing techniques reduces levels of fructan in garlic and onion. It can be concluded that high production of inulinase require certain length of fructan with certain degree of polymerization in addition to certain branching in fructan backbone. Raw garlic satisfies all the demands. Garlic has had an amazing array of inulinase production because fructan in garlic have shown to play a significant role in Inulinase activity.

It is important to mention that during the present study, Chinese garlic was used because it gave higher inulinase activity than Egyptian one in preliminary test, the results were 2514 and 3148 IU/L for Egyptian and Chinese garlic respectively. This is may be due to the higher levels of inulin-type fructans in Chinese garlic than common garlic as Judprasong *et al.,* (2011) reported that Chinese garlic contain 29.2 ± 5.62 g fructan/100 g fresh weight while common garlic contain, 22.4 ± 2.86 g fructan/100 g fresh weight.

Effect of Different Concentration of Garlic on Inulinase Activity:

The effect of substrate concentration on inulinase was studied, using different concentrations of raw garlic as the substrate ranging from 0.5 g % to 3.0 g %.

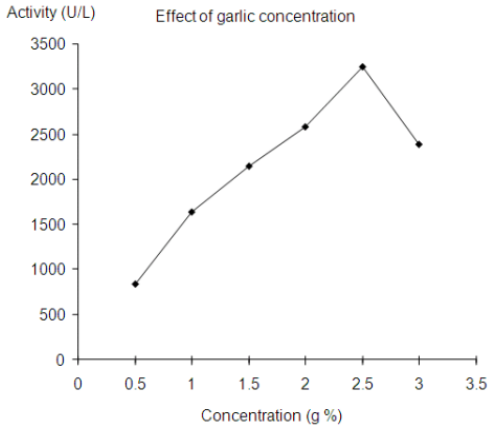

Fig. 2: Effect of different concentration of raw Garlic on inulinase activity.

Effect of Nitrogen Sources on Production of Inulinase:

Application of appropriate nitrogen source is very important for optimal production of enzymes. Therefore the effect of nitrogen sources was tested by replacing peptone in the medium with other compounds, maintaining equi-molar amount of nitrogen at 0.44 g/liter keeping the rest of medium composition the same. The cultures were grown for7 days, harvested and processed for enzyme assays. Inorganic nitrogen sources including NH_4Cl, $(NH_4)_2 SO_4$, NH_4NO_3 and organic nitrogen sources namely beef extract, yeast extract, casein, bakers yeast, soybean and urea were examined. Nitrogen constituent has a profound effect on production of inulinase activity in culture medium as shown in Tables (7& 8).

Table 7: Effect of organic Nitrogen source on Inulinase activity.

Nitrogen-source	Final pH of *C.F	Protein Content (mg/ml)	Inulinase activity (U/L)
Urea	8.0	2.8	4093
Beef extract	6.9	2.5	3504
Casein	6.7	2.9	3378
Peptone(control)	6.8	2.1	3244
Soy bean	7.0	2.6	2182
Bakers yeast	7.0	3.0	1286
Yeast extract	4.3	1.8	1217

Yeast extract was the poor nitrogen sources of inulinase synthesis. Beef extract, casein and peptone were the best sources. Urea significantly improved the inulinase activity, compared to other nitrogen; urea supported the maximum production of inulinase. It was obvious that organic nitrogen sources have stimulating effect. Ammonium nitrate, ammonium sulphate and ammonium chloride found to be inhibitory for inulinase synthesis compared with urea, presumably because of the release of ammonium ions (Singh *et al.,* 2006).

Table 8: Effect of inorganic Nitrogen source on Inulinase activity.

Nitrogen-source	Final pH of *C.F	Protein Content (mg/ml)	Inulinase activity (U/L)
Ammonium nitrate	6.8	2	2290
Ammonium sulphate	6.8	1.7	1952
Ammonium chloride	6.6	2	1731

Effect of Different Concentrations of Urea on Inulinase Activity:

Fig. (3) shows the effect of different concentrations of urea incorporated in basal medium raining from 0.05 g % to 0.4 g % and the production of enzyme was enhanced (4093 IU/L) with addition of 0.1% urea. Higher concentrations of urea induce denaturizing of cells by increasing in cell pore size there by reducing enzyme (Ahsen *et al.,* 2003).

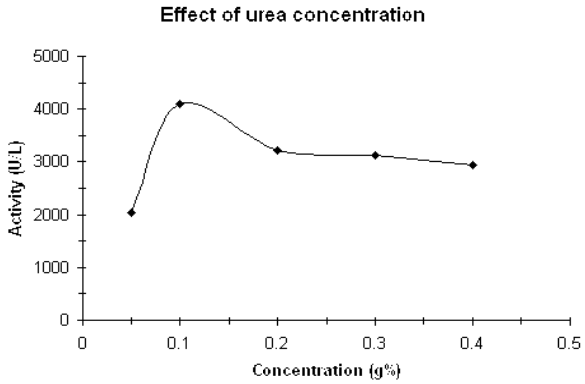

Fig. 3: effect of different concentrations of urea on inulinase activity.

Effect of Different Concentrations of Phosphate on Inulinase Activity:

In attempt to prove that some garlic constituent is responsible for substantial enhancement in inulinase production, different concentrations of phosphorus in form of K2HPO4 were added to the fermentation medium of *Aspergillus niger* NRRL 3.

It has been found that a definite relationship exists between the phosphorus content of the medium and their inulinase activity. The effect of inorganic phosphate in this study indicated that inulinase production is controlled by phosphate, phosphate concentrations higher than optimal level (0.25%) suppressed inulinase production. Thus an improvement in inulinase production of 1.13 fold over control and 1.4 fold over production at lowest phosphate concentration was achieved. The results represented in Fig. (4). Highly active preparations of inulinase can be obtained from a species of *Aspergillus* which is grown on a medium containing phosphate,

culture experiments have revealed that phosphates definitely stimulate the formation and enrich the inulinase content of the preparations (Nuggehalli and Montnahalli, 1936).

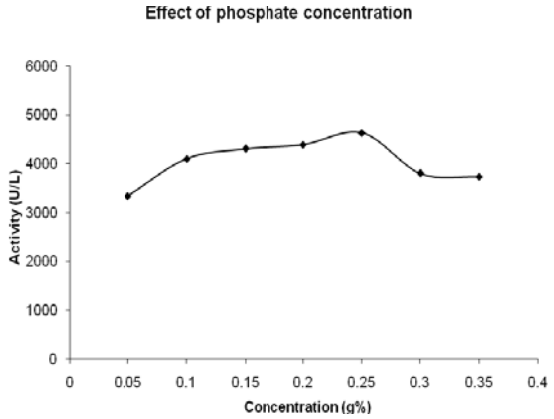

Fig. 4: Effect of different concentrations of phosphate on inulinase activity.

Effect of Different Concentrations of Calcium Chloride on Inulinase Activity:

A study of the effect of adding calcium in the form of calcium chloride on the yield of inulinase in a fermentation by *Aspergillus niger* NRRL3 was undertaken (Fig. 5). The concentration of calcium chloride in the medium ranged from 0.025 g% to 0.175%.

When a 0.1 % of calcium chloride was added to the fermentation medium, a maximum activity of inulinase (5652 IU/L) was observed. Thus an improvement in inulinase production of 1.22 fold over production without adding calcium chloride and 1.1 fold over production at lower calcium chloride concentration was achieved. It can be concluded that calcium, markedly stimulated inulinase production.

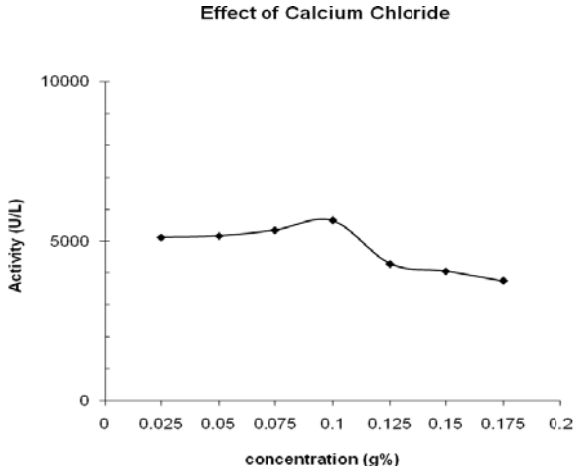

Fig. 5: effect of different concentrations of calcium chloride on inulinase activity.

Effect of Different Concentrations of Magnesium Sulphate on Inulinase Activity:

The addition of MgSO4·7H2O did not stimulate inulinase production Fig. (6). An inhibition of the production of enzymes at a higher concentration of this compound was observed .This result in agreement with Skowronek *et al.*, (2004).

Conclusion:

The results obtained from this study lead to the suggestion that a further scaling up is possible especially at using fresh raw garlic while adjusting problem of sterilization and remove characteristic garlic enzymes that inhibit growth but keeping fructan level. The results of calcium and phosphorus experiments prove the assumption suggested by the authors that higher calcium and phosphorus content of garlic is preferable than the lower calcium and phosphorus content in onion in enhancing inulinase activity which is also prove that not only

fructan content of garlic is responsible for inulinase induction. Raw garlic satisfies all the demands of industrial technologies, low cost, safety, healthy and saving time and energy required for drying.

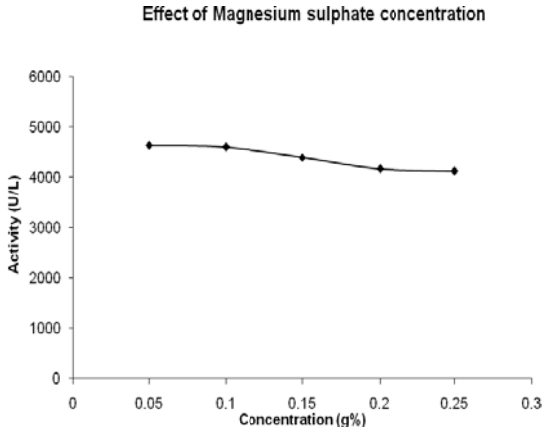

Fig. 6: effect of different concentrations of magnesium sulphate on inulinase activity.

ACKNOWLEDGMENT

The authors would like to express their acknowledgment to the National Research Center, Cairo-Egypt for funding this work. Also, the authors would like to express their gratitude and deep appreciations to Dr. Abu-Bakr Gomaa, Professor of Agricultural Microbiology, NRC, Cairo, Egypt for his kind help and reviewing the manuscript.

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