ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Production of Dextranase from Agro-industrial Wastes by *Aspergillus awamori* F-234 under Solid State Fermentation.

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ABSTRACT

Dextranase (α-1, 6-D-glucan, 6-glucanohydrolase; E.C.3.2.1.11) is an enzyme which hydrolizes the α-1,6 glucosidic linkages in dextran, found in plants, mammalian tissues, and microorganisms. Five fungal strains (*A. niger* F-93, *A. fumigatus* F-993, *P. funculosum* NRC289, *T. koningii* F-25 and *A. awamori*F-234) were screened for their capacity for dextranase enzyme production using four agro-mechanical wastes (sugar beet pulp, olive mill solid waste, jojoba mill solid waste and sugar cane bagasse) under solid state fermentation. Among all tested fungi *Aspergillus awamori* F-234 was selected as the best dextranase producer. The optimization of dextranase production was investigated under various influencing factors, including incubation time; temperature; pH, also additional nitrogen sources were optimized .The highest dextranase acquired after 3 days incubation time. The optimum pH and temperature for dextranase production were at pH 6 and 35°C when ammonium nitrate was supplemented as a nitrogen source. Owing to addition of dextranase ethanol yield was increased during sugar cane molasses fermentation by ***Saccharomyces*** *cerevisiae*.

Keywords: Dextranase, *Aspergillus awamori,* agro-industrial wastes, SSF.

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INTRODUCTION

Dextrans are undesirable compounds in sugar production that integrated by contaminant microorganisms from sucrose, expanding the viscosity of the flow and lessening industrial recovery, achieving noteworthy losses [1]. The utilization of the dextranase enzyme is the most proficient strategy for dextrans hydrolyzing. Dextranase (α-1, 6-D-glucan, 6-glucanohydrolase; E.C.3.2.1.11) is an enzyme that hydrolizes the α-1,6glucosidic linkages in dextran, found in plants, mammalian tissues, and microorganisms. Dextranase enzyme has various applications in medicine and sugar industry in view of its hydrolyzing limit of dextran also; utilizing dextranase can take care of numerous issues in sugar processing because of microbial dextran deposit [2]. Since dextranase can hydrolyze or hinder glucans synthesising, it can be utilized as a part of the treatment of dental plaque [3]. Likewise it is utilized for preparing low molecular weight dextran and cytotoxic dextran conjugate, In addition dextranase appears as an enhancer of antibiotic activity in endocarditis [4, 5]. Dextranase has picked up consideration as a result of coordinated isomaltooligosaccharides synthesising of which have been appeared to display pre biotic impacts [2] .What's more one of the diverse methods for obtaining isomaltooligosaccharides is the assimilation of dextran polymer by dextranase enzyme [6] Dextranase has been represented from various microorganisms, as *Chaetomium erraticum* [7], *Penicillium aculeatum, Hypocrea lixii* [8,9] and *Bacillus licheniformis* [10].The utilization of dextranases in the sugar industry was recommended by Tilbury over 30 years back. When the enzyme had been studied for the preparations of medicinal dextrans used as substitutes for blood plasma. Moreover, recently in toothpaste formulations to hydrolyze the dextrans present in tooth glue details to hydrolyze the dextrans present in the dental surfaces [11]. Utilization of dextranase has an economic worth, with the use of the enzyme it could be expected a large improvement of the sugar production cost [12, 13]. The aim of the present work was to screen dextranase production by five fungal species using different wastes, and to develop the optimization of dextranase production conditions by *Aspergillus awamori* F-234.

MATERIALS AND METHODS

Microorganisms and Media

Aspergillus awamori F-234*, A. niger* F-93, *A. fumigatus* F-993, *P. funculosum* NRC289 and *Trichoderma koningii* F-25 were obtained from Microbial Chemistry Department collection, National Research Centre, Dokki, Giza, Egypt. The fungal cultures were maintained on PDA and kept at 4°C. Subculture was made at 2 weeks intervals.

Inoculum preparation

A spore suspension of $2x10^5$spores/mL from routine subcultures was used to inoculate 250 ml capacity Erlenmeyer flasks containing 5 g of crushed substrate moistened with water to 70% v/w. Cultures were grown for 5days at 30°C statically .Two flasks for each strain were taken daily for dextranase determination (Protein and specific activity). One hundred ml of 0.05M citrate buffer was added to each flask. Then flasks were shacked on a rotary shaker (150 rpm) for one hour at 30°C. , filtered on Whatman No1 filter paper then the filtrate was clarified by centrifugation at 6000 g for 20 min. The supernatant was assayed for protein and enzyme activity.

Measurement of dextranase activity

Dextranase activity was analyzed with the 3, 5 dinitrosalicylic acid (DNS) method by assaying the reducing sugars released during a 25 min reaction (1% (w/v) dextran, 0.05 M acetate buffer, pH 5.0 at 50°C). The absorbance was read at 550 nm using a spectrophotometer. The amount of reducing sugar was calculated from the standard curve based on the equivalent glucose. One enzyme activity unit (U) was defined as the amount of enzyme that liberates one micromole of glucose per min reaction under assay conditions (14). The results of the analysis are the mean values of duplicate separate experiments.

Optimization of culture conditions for dextranase production.

The culture conditions (cultivation time, temperature, initial pH and nitrogen source) were investigated. Optimum temperature and pH were determined by changing separately the conditions of activity

assays for pH from 3.5 to 7.5 (buffer solutions of 0.05 M citrate phosphate and phosphate buffer pH from 3.5 to 7.5 was used), incubation temperatures from 20 to 40 °C were studied under optimum pH value.

Soluble Protein determination

The amount of total protein was determined according to the method of15 Lowry *et al. (*15) using bovine serum albumin as a standard protein.

Enzyme precipitation

Different concentrations of ammonium sulphate were used to precipitate the enzyme. Each ammonium sulphate concentration was added slowly to a 15 ml centrifuge tube containing 5 ml of the supernatant culture solution (pH 5.0). The tubes were agitated for 15 s in a vortex at room temperature and then refrigerated overnight. The protein precipitate was collected by centrifugation under cooling (6000 rpm, for 45 min at 4 °C) and then dissolved in 0.05 phosphate buffer (pH 5.0) up to the initial volume (5ml)

Investigating the optimum pH and temperature for enzyme activity

For detecting optimum pH of crude enzyme activity, the activities were detected at different buffer solutions of 0.05M as mentioned above (pH from 3.5-7.5). For optimum temperature of crude enzyme activity, it was carried out at the optimum pH value (6), under different temperatures from 35 to 70°C**..**

Determination of thermostability of dextranase

Different aliquots of dextranase were incubated in 0.05M phosphate buffer (pH 6) at temperatures from 35 to 75°C for 90 min and the remaining activity was determined.

(All experiments were performed in duplicates)

RESULTS AND DISCUSSION

Fungal strain

By extraordinary features, as thermostability and pH stability besides, shabby agro -industrial wastes would be a better option than the already available expensive dextran for enzyme production. Where most of the production expense of industrial enzymes are accounted by of the cost of the growth medium. Data presented in **Table (1)** exhibited that *A. awamori F-234* was the potent strain utilized for dextranase production and sugar beet pulp was the most suitable substrate used for enzyme production. Where dextranase activity was 1057U/g. Comparative perceptions had been demonstrated by other specialists with distinctive fungi as *Chaetomium erraticum* [7], *Paecilomyces lilacinus, Penicillium aculeatum* [8, 10]

Table 1: Production of dextranase by fungal strains cultivated on some agro-industrial wastes under SSF system.

Fungal strain	Dextranase activity U/g			
	olive mill solid waste	sugar beet pulp	jojoba mill solid waste	sugar cane bagasse
A. niger F-93	722	984	825	612
A. fumigatus F-993	794	1012	960	678
A. awamori F-234	834	1057	914	589
P. funculosum NRC289	814	826	685	680
T. koningii F-25	768	634	816	712

Time course

The time course of dextranase production by *Aspergillus awamori* F-234 is shown in **Figure (1)**. Maximum dextranase activity came to following 3 days. It was 1197 U/g, likewise, the heights protein (33mg) with high specific activity (52 U/mg) were after 3 days. The consequences of this study exhibited inclinations

preferences in time course for dextranase production. In comparison, other previous studies demonstrated longer incubation period for enzyme production. Where incubation period for dextranase production was 10 and 7 days by *L. dextranicus* and *P. aculeatum* [8].Also, reported that maximum dextranase activity by *Paecilomyces lilacinus* was reached after 7 days; thereafter the enzyme activity declined [2].

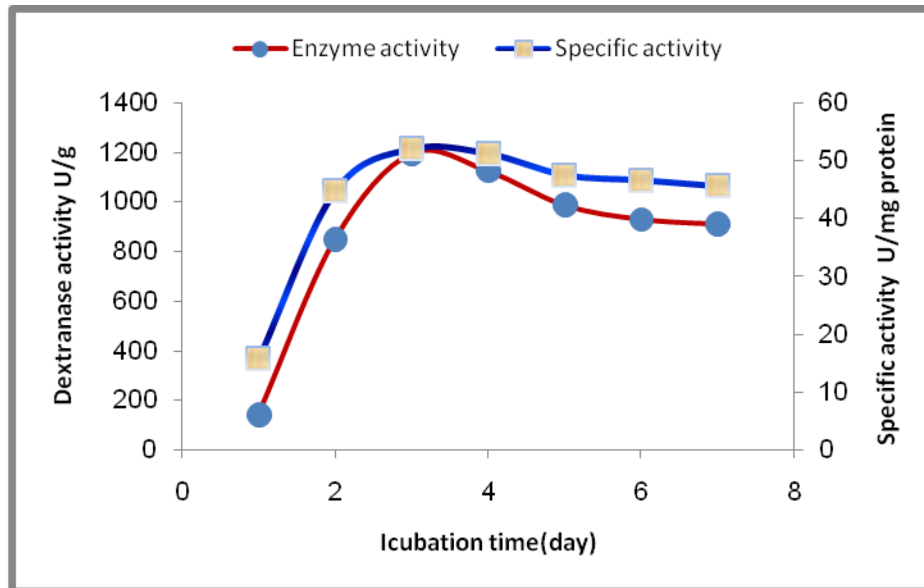

Figure 1: Effect of the incubation period on dextranase production by *Aspergillus awamori* F-234

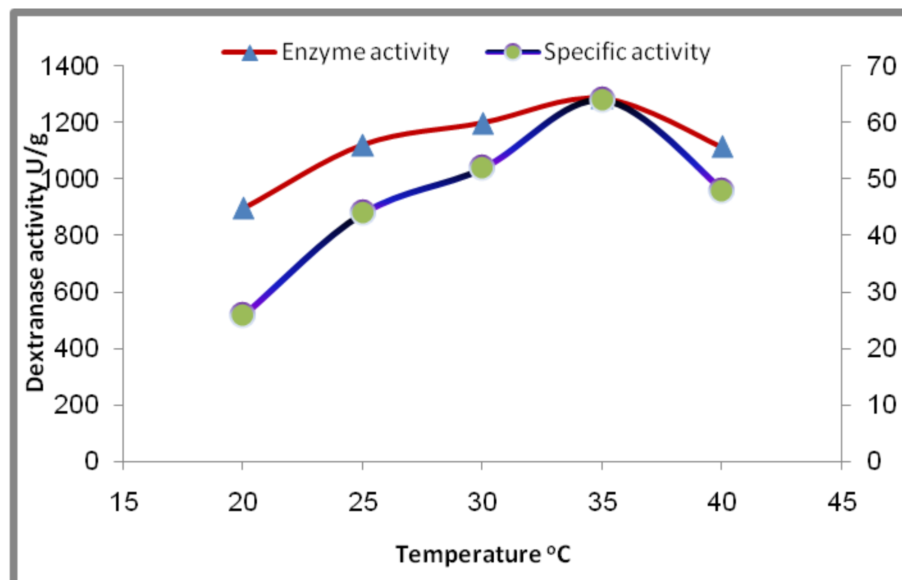

Figure 2: Effect of the incubation temperature on dextranase production by *Aspergillus awamori* F-234.

Effects of temperature

To set up the impact of growth temperature, dextranase activity and total protein content were determined at various growth temperatures running from 20 to 40°C while different circumstances were stable. The results showed that 35°C appears to support dextranase activity **(Figure 2)**. Similar observations were reported for dextranase production by *streptococcus sobrinus* [16]. While 30°C was the optimal incubation temperature for dextranase production by *Paecilomyces lilacinus* and *Penicillium aculeatum* [2] .It could be concluded that certain temperatures which are not suitable for the microorganism could influence the microbial metabolism and cause low enzyme production, in this manner, Experiments are gone to at 35°C in next study.

Effect of pH

The change of Initial medium pH prompts the change of cell membrane nature and/or cell wall that affected fungal growth and dextranase production too. Figure (3) demonstrates dextranase activities obtained by *Aspergillus awamori* F-234 under various initial pH values. It could be concluded that at pH value higher than 4.0, the growth of the fungus is stimulated and dextranase production is favored at pH range 5.5-6.5 values while the maximum specific activity of dextranase production was observed at initial pH 6.0. This result is in accordance with [17] who demonstrated an optimum pH 6-0 for dextranase production by *Streptococcus sobrinus and Paecilomyces lilacinus*. What's more, the acquired results are in current with reported pH 5.6-6.0 for optimum dextranase production by *Penicillium ilacinum* [13]

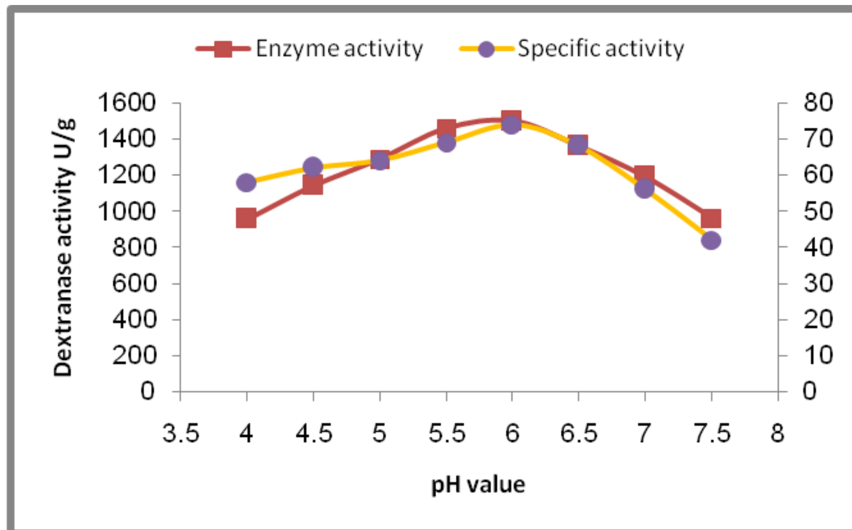

Figure 3: Effect of initial pH on dextranase production by *Aspergillus awamori* F-234.

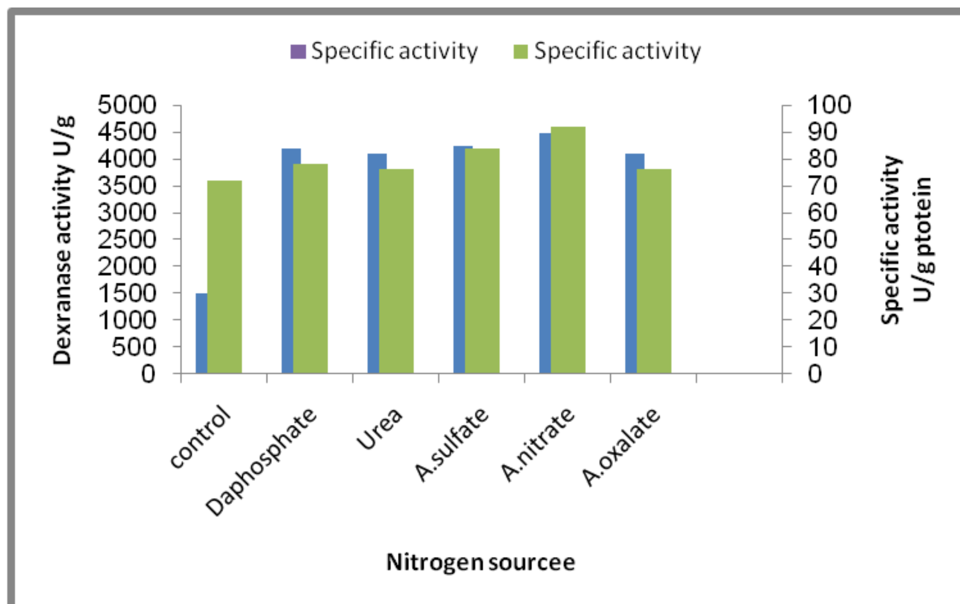

Figure 4: Effect of nitrogen source on dextranase production by *Aspergillus awamori* F-234.

Effect of nitrogen source on dextranase production by *Aspergillus awamori* F-234

Figure (4) illustrated that dextranase production and specific activity were upgraded by supplementation inorganic nitrogen sources as reasonable nitrogen sources to the fermentation medium.

ISSN: 0975-8585

Ammonium nitrate took after by ammonium sulphate were more suitable than other tried nitrogen sources to obtain high enzyme production. In examination of the acquired results those reported [8] that utilizing a blend of sodium nitrate and yeast extract as nitrogen source produced the highest dextranase activity by *P. aculeatum* compared with utilizing of ammonium sulfate.

Enzyme precipitation

The solubility of proteins fluctuates according to the ionic strength of the solution, to the salt saturation since proteins contrast notably in their solubility at a high ionic strength, salting-out is an exceptionally helpful method to assist in the purification of a given protein. Ammonium sulfate is commonly technique used to purify proteins by altering their saturation, as it is exceptionally water- soluble. **Figure (5)** illustrates that there is no linear correlation between's the percent of protein recovered by ammonium sulfate and the percent of enzyme recovered activity. At 60% ammonium sulfate saturation about 43% of total protein was recovered containing 72% of total dextranase activity.

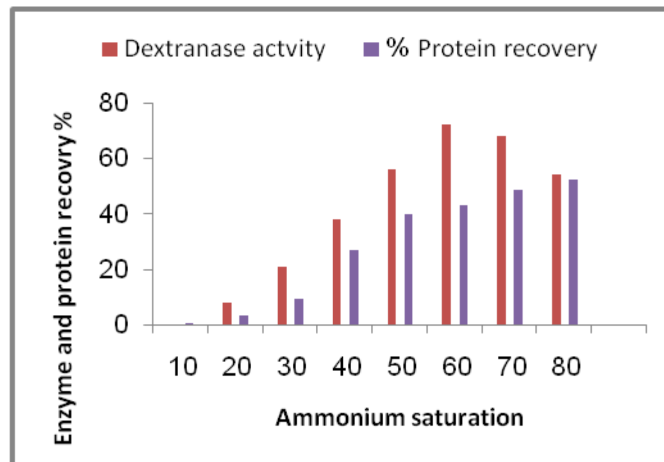

Figure 5: Enzyme recovery by ammonium sulphate precipitation.

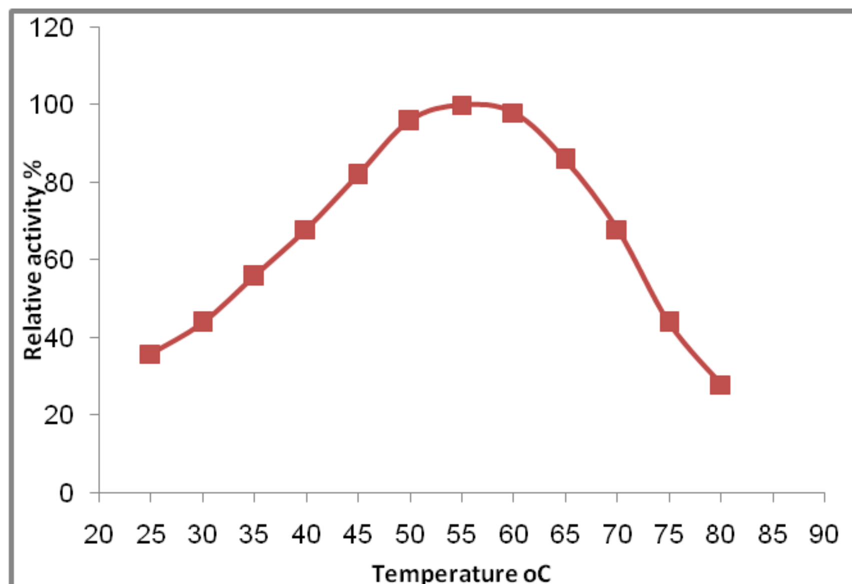

Figure 6: Effect of incubation temperature on dextranase activity produced by *Aspergillus awamori* F-234.

Determination of optimum temperature of the crude enzyme

Figure (6) demonstrated that the vast majority of the enzyme activities were observed in the range between 50-60°C and the highest activity of dextranase has happened at 55°C. Thereafter a decrease

reduction in activity was observed as the temperature was above 60ºC.Similar results were observed [8]. In another study by [2], an optimum Temperature for dextranase activity was at 37 ºC.

Determination of optimum pH activity of the crude enzyme

Figure (7) illustrated that the optimum activity was found at pH 6.0. A noteworthy abatement in the activity occurred above pH 6.5. These results are in concurrence with that acquired by [8] who indicated that the maximum dextranase production by *Penicillium ilacinum* and *P. aculeatum NRRL*-896 was at pH 6.0.

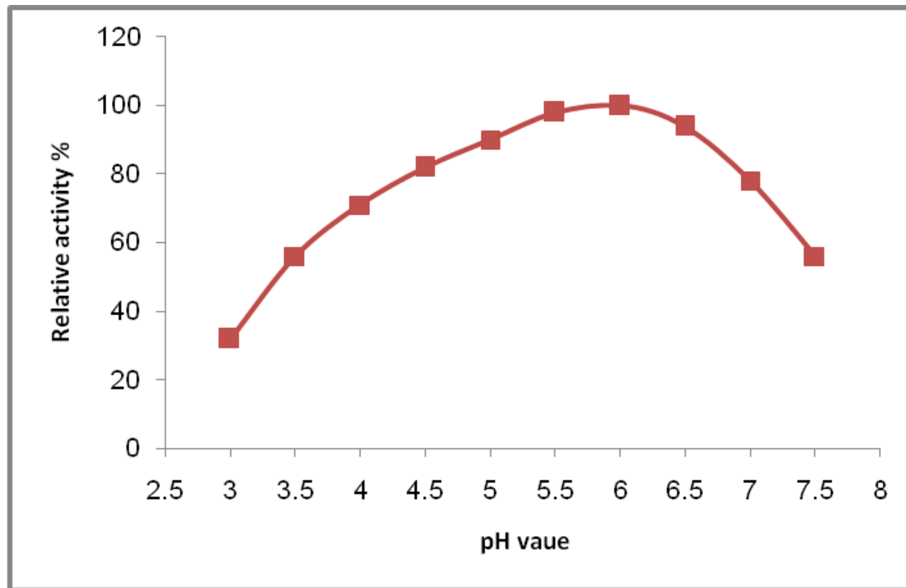

Figure 7: Effect of incubation pH on crude dextranase activity produced by *Aspergillus awamori* F-234.

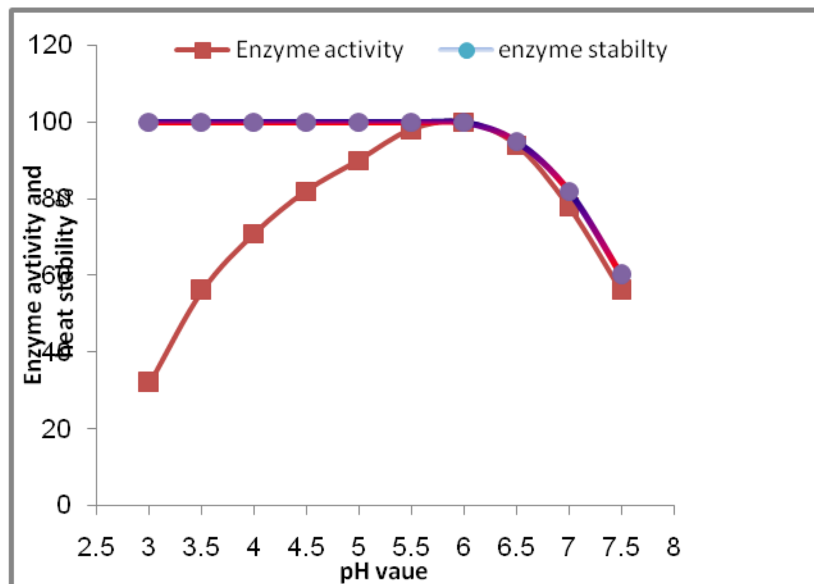

Figure 8: Effect of pH value on crude dextranase activity and stability produced by *Aspergillus awamori* F-234.

Effect of pH value on crude dextranase activity and stability

Figure (8) exhibited that the produced dextranase was stable for 90 minutes in the range of pH 3.0-6.0 in the scope of pH 3.0-6.0 and retention of 96 % of its activity was at pH 6.5.while the retention of 78 and

56 % were at pH 7.0-.7.5.Where the level of inactivation extend from minor conformational changes to irreversible inactivation depending on the incubation conditions [20].

Effect of temperature on crude dextranase activity and stability

Thermal stability is maybe the most regularly experienced and generally altogether, **figure (9)** outlines that the produced dextranase was stable for an hour and a half at temperature 60°C then diminished to be 86, 68, 44 and 28% at temperatures 65, 70, 75and 80 respectively It is obvious that the enzyme is stable within its active range and this determines the optimal conditions of the reaction. These outcomes offer ideal conditions for industrial usage [21].

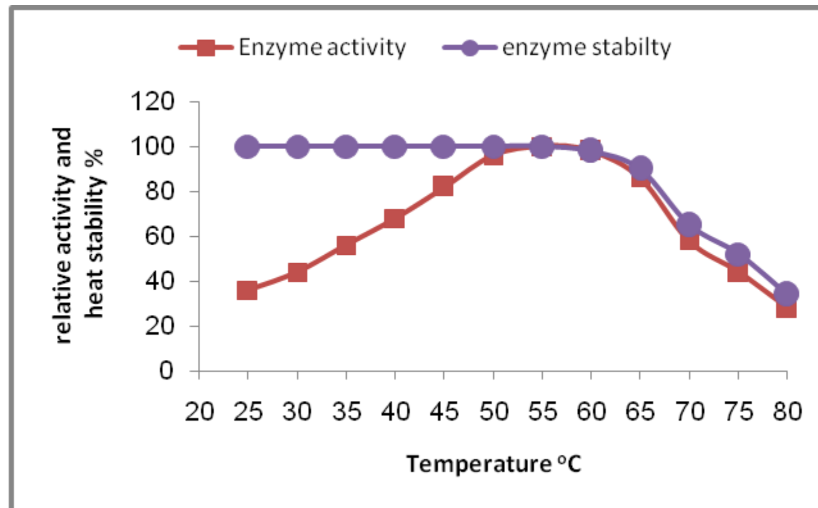

Figure 9: Effect of temperature on crude dextranase activity and stability produced by *Aspergillus awamori* F-234.

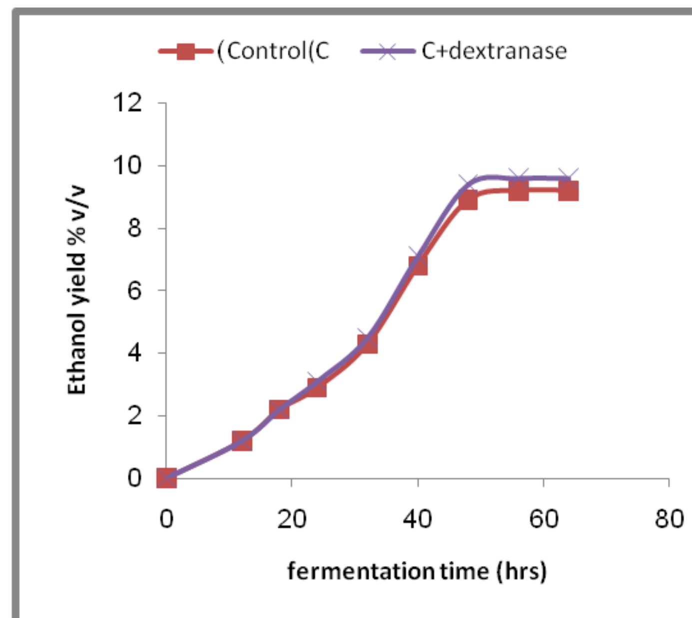

Figure 10: Effect of dextranase as an additive on ethanol yield from sugar cane molasses fermentation by *S. cerevisiae.*

Application of dextranase in alcoholic fermentation

The main applicable strategy today in the sugar industry is the enzymatic hydrolysis of dextrans. The use of microorganisms and/or enzyme is ordinarily considered as environment friendly. **Fig:(10)** outlines that the addition of dextranase formulated in enzymatic preparations has been effectively utilized for hydrolyzing dextrans found as a part of molasses to glucose which can fermented by *Saccharomyces cerevisiae* to ethanol

bringing about fermentation efficiency subsequently expanding of ethanol yield. Where ethanol can be delivered by using any lignocellulosic material with the assistance of microorganisms [22] in this way, the efficiency and specificity of the medium additives are a gainful point of view to convey concentrated on targeted products like bioethanol. Various examinations suggested a minimization of dextran levels in the sugar processing plant by different methodologies even with the enzyme addition [8].

CONCLUSION

In the present study, the point was to optimize dextranase production conditions by *A. awamori* F-234. Thermostability and pH stability of the produced enzyme had been also examined.In like way, to assess the effect of dextranase on ethanol yield under sugar cane molasses fermentation.

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