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PURIFICATION AND CHARACTERIZATION OF α-GALACTOSIDASE FROM SOYBEAN

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Abstract:-The α-galactosidase from germinated soyabean seeds is well optimized for various physico-chemical parameters such as incubation period, pH, and temperature. The partial purification was carried out with cold acetone precipitation. The enzyme showed optimal α-galactosidase activity with the phosphate buffer molarity of 25 mM/l having pH 7.4 and with temperature optima of 40°C. Enzyme shows maximum activity towards the raffinose family sugars such as melibiose, and it was minimum for guar gum, but increases with time. Purification of α-galactosidase by ion-exchange chromatography shown that α-galactosidase from soybean was present in two sub units.

Keywords:α-galactosidase; soybean; germination; gaur gum; purification; raffinose.

INTRODUCTION:

α-galactosidase (alpha-d-galactosidase galactohydrolase E.C.3.2.1.22) is an exoglycosidase which catalyses hydrolysis of terminal α-1, 6 linked galactosyl residue from a wide range of substrates including oligosaccharides of raffinose family sugars such as raffinose, stachyose, melibiose, verbascose [1]. Moreover the galactooligosaccharides are hydrolyzed by this enzyme due to which nutritional quality of legumes was increased [2]. Enzyme is widely distributed in nature among plants, animals and micro-organisms [3].There is a considerable interest in enzymes that catalyzes hydrolysis of glycosidic bonds, due to their extensive industrial and biochemical applications. The enzyme α-galactosidase is ubiquitous in legume seeds and for the first time it was reported from sweet almond [4]. Enzyme is involved in the hydrolysis of galactolipids; the deficiency of this enzyme has been implicated in the manifestation of a disorder Fabry's disease in humans [5]. α- galactosidase activity is also present in human saliva [6]. Soy is good source of protein because it contains significant amount of all the essential amino acids for humans. Soybeans are the primary ingredient in many processed food, including dairy products [7].Legumes such as beans contains large amount of anti-nutritional factors, mainly, galacto-oligosaccharides which are responsible for flatulence in humans. Hence, α-galactosidase is used in processing of legume based foods. The nutritive value of diets is increased by direct addition of enzyme and also the enzyme is being used to improve digestibility in animal feed [8]. α- galactosidase also has interesting application in human Xenotransplantation and in blood group transformation [9]. α-galactosidase present in multiple forms, particularly in plants however there was no difference in α-galactosidase activity according to gender, blood type, and secretor status. [10].Different methods can be used in the purification of α-galactosidase enzyme. The enzyme from Aspergillus niger is used in beet sugar industry and in food and feed processing [11]. In beet sugar industry, α-galactosidase is used in elimination of D-raffinose and thus, facilitates sugar crystallization and consequently improves yield of sucrose [12]. Among micro-organisms, α-galactosidase activity was first detected in Brewer's yeast.

MATERIALS AND METHODS:

Plant foods JS-335 variety of soybean used as experimental material were collected from local market and used as a source of α-galactosidase. 1 gm of soybean seed was taken in muslin cloth and washed with tap water. After washings seeds are treated with 70% alcohol for 2 min. and then decant the alcohol. Again the seeds are treated with 0.1% mercury chloride for 2 min. In sterile laminar flow, decant the chemical sterilant and washed the seeds thoroughly five times with sterile distilled water. After surface sterilization of seeds, they are kept in sterilized conical flask containing sterilized wet cotton for germination [13]. Enzyme was extracted by using 25mM phosphate buffer having pH 7.4 in precooled mortar and a pestle.

This extract was centrifuged by cold centrifugation at 7000 rpm for 20 min. The supernatant obtained was then precipitated by cold acetone in 1:1 proportion and precipited enzyme dissolved in same buffer which is then used as an enzyme source for further study. Concentration of protein in enzyme has been estimated according to Lowry method by using BSA as standard [14].

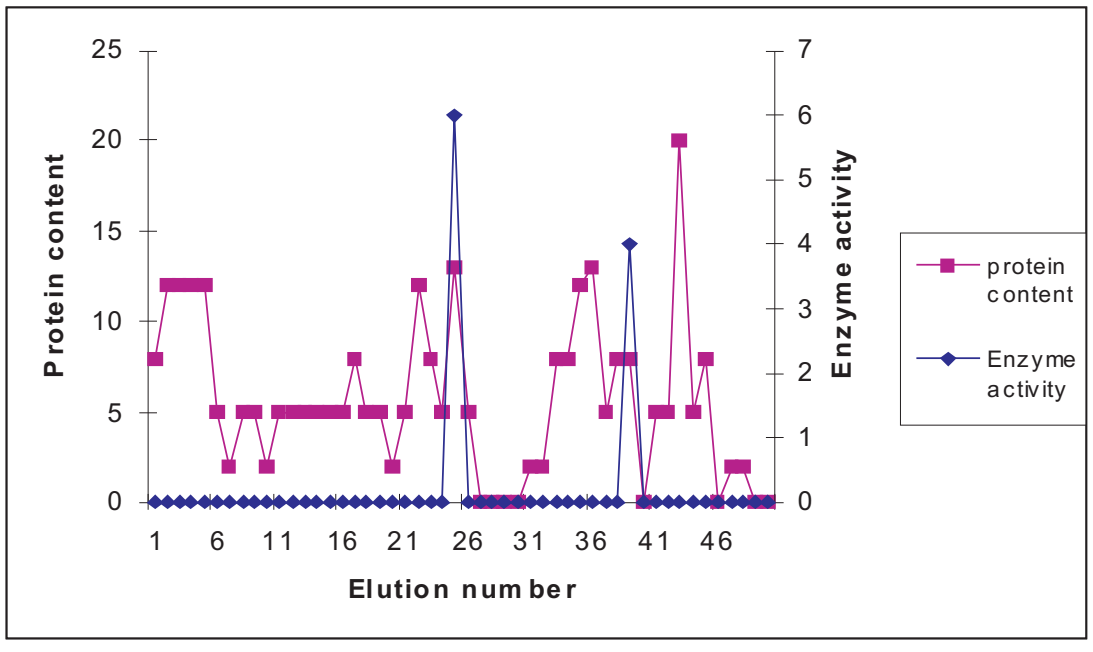
ENZYME ASSAY

Assay of a-galactosidase was carried out by DNSA method with some modifications [15]. Reaction mixture contains 0.5 ml of enzyme solution, 0.5 ml of melibiose substrate dissolved in sodium phosphate buffer and 1.5 ml sodium phosphate buffer having pH 6.5 The mixture was incubated for 10 minutes at 50oC and then 0.5 ml reaction mixture was taken add 2.5 ml of DNSA kept in boiling water bath for 10 min.Then quantity of reducing sugar ultimately the enzyme activity was measured by the absorbance at 530 nm spectrophotometerically. The enzyme assay was carried out after 24, 48 and 72 hrs germination of soybean. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µg of glucose per min per ml of enzyme under assay conditions. The specific activity is expressed as units of enzyme activity per microgram of protein content in the enzyme source.

PURIFICATION OF ENZYME

Germinated soybean seeds produce an α-galactosidase which has been partially purified by cold acetone precipitation. After centrifugation at 7000 rpm. for 20 min. the pellet obtained was dissolved in 25mM phosphate buffer having pH 7.4 and was used for further study. The enzyme was purified on DEAE-Cellulose ion-exchange chromatography column.

Fig.1: Purification of α- galactosidase by ion exchange chromatography.



CHARACTERIZATION OF ENZYME

The different parameters of enzyme such as effect of incubation period, pH and temperature on the enzyme activity were estimated. The effect of incubation period of gaur gum on enzyme activity was also evaluated.

Fig.2: Effect of Germination Period on Enzyme Activity.

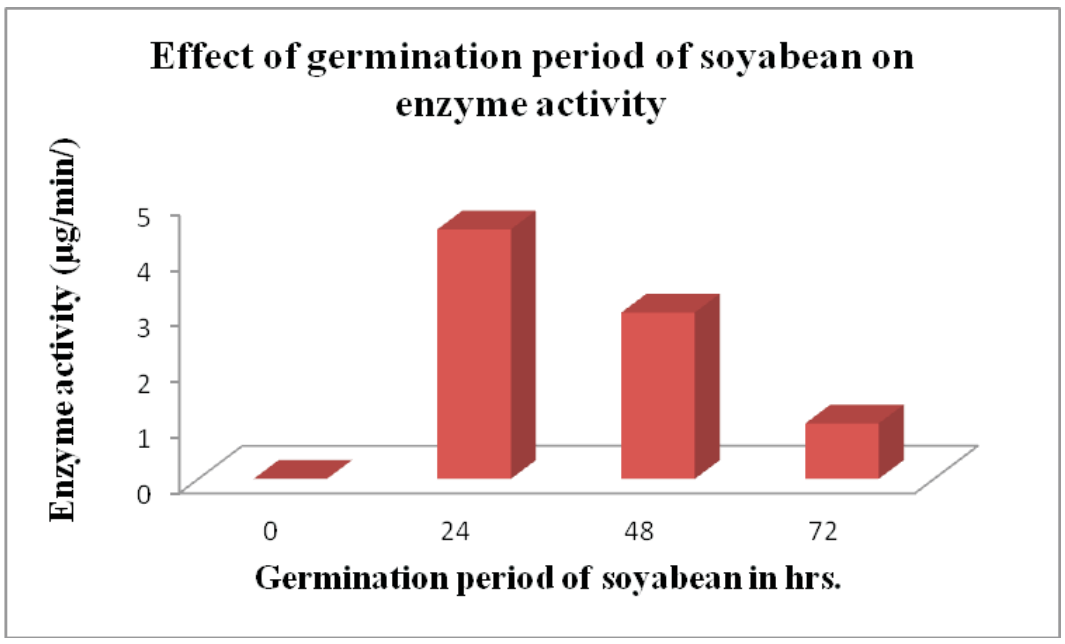


Fig.3: Effect of pH on Enzyme Activity.

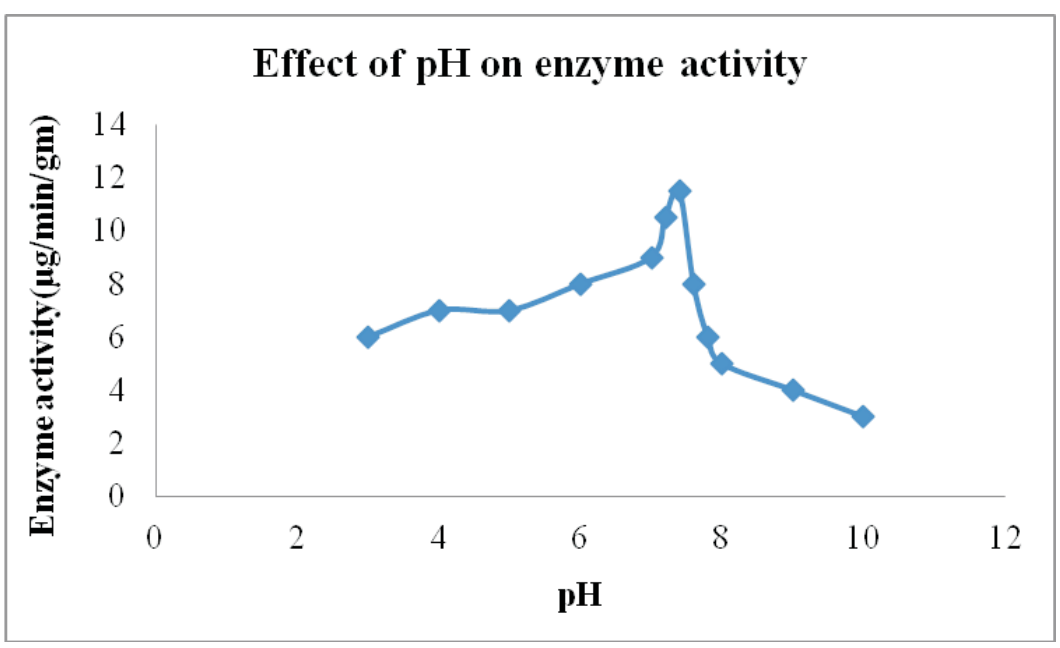


Fig.4: Effect of Temperature on Enzyme activity.

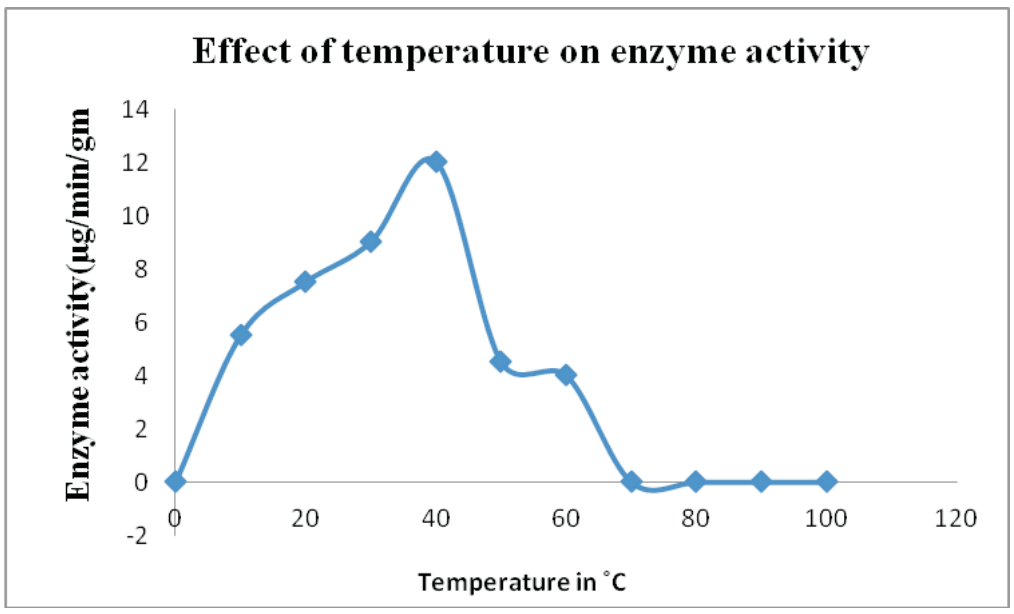


Fig.5: Effect of enzyme activity in guar gum at various incubation times.

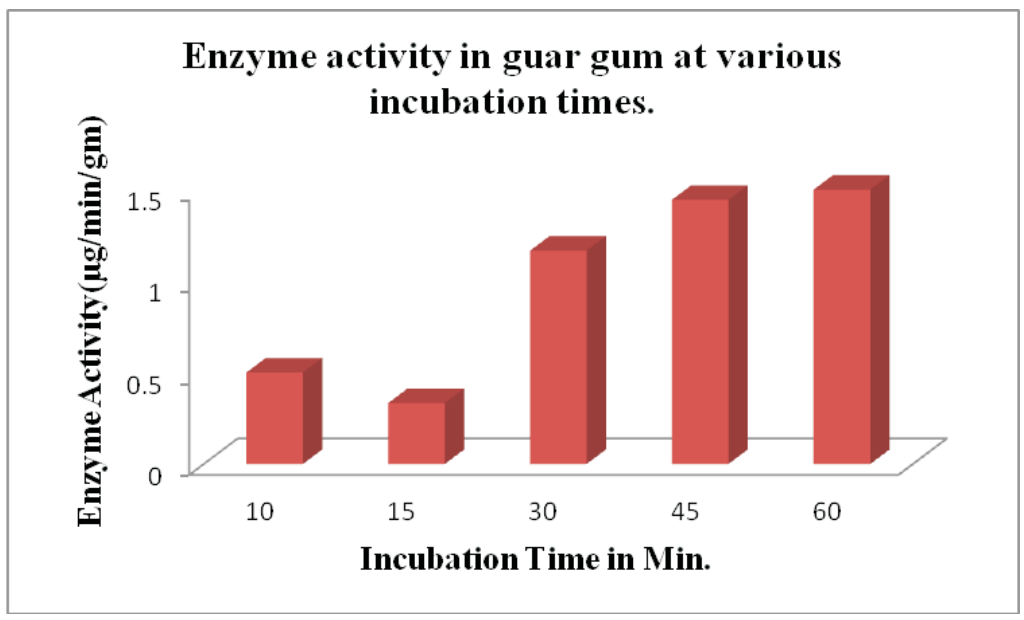
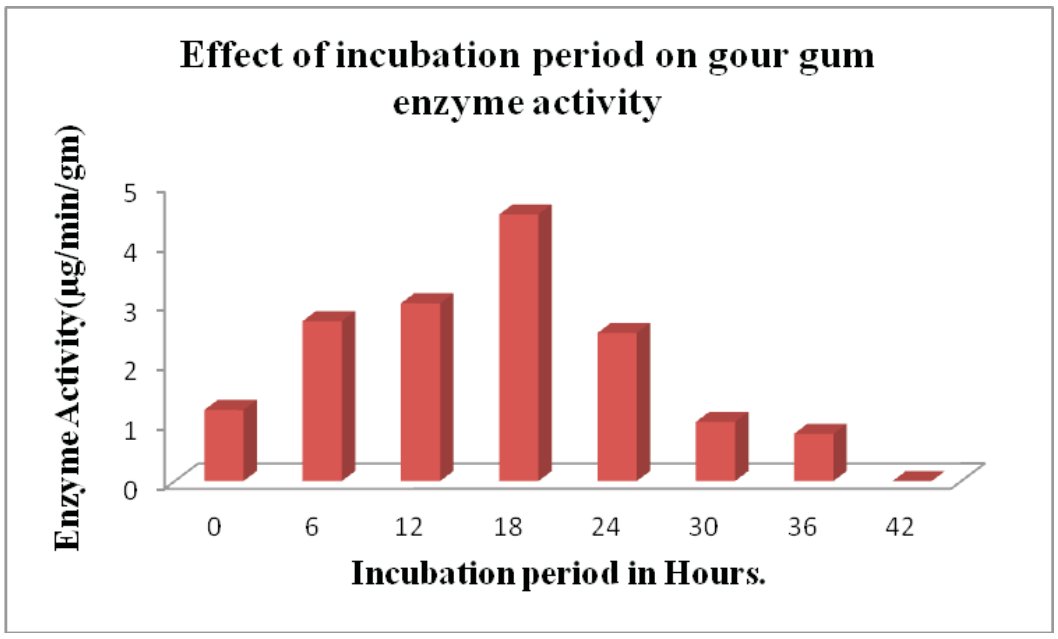


Fig.6: Effect of incubation period on enzyme activity in guar gum.



RESULT AND DISCUSSION

In present study purification of α- galactosidase from soy bean was carried out, it was found that the enzyme α-galactosidase was not a constitutive enzyme; it was synthesized during germination i.e. an inducible enzyme. The enzyme activity was decreased as germination period was increased. The enzyme was stable at broad range of pH which is its important characteristics for its application in various fields. The broad substrate specificity of enzyme it makes applicable for the guar gum processing. As reported in several cases the biosynthesis of α-galactosidase could be induced in presence of sugars namely raffinose, melibiose and Galactose. Soybean was germinated within 24 hrs, when it was soaked in the distilled water but it has also be seen that the addition of 25mM phosphate buffer rather than the sterilized distilled water in wet cotton then germination period of soybean seed get reduced. The enzyme activity was seen at 0, 24, 48, 72 hrs as 0, 4.5, 3.0 and 1.0 µg/min/gm of seed respectively. It has been seen that 0 hrs to 24 hrs the enzyme activity was increased, but it was reduced after 24 hrs of germination time. Most of the plant sources α-galactosidase has optimum pH range between 6.0-8.0. α-galactosidase showed higher activity at pH 7. 4 though it has pH range 7.0-8.0. i.e. neutral to slightly basic and was stable at broad pH range 3.0-10.0 In plants α- galactosidase was present in multiple forms, while soy bean α-galactosidase was present in two subunit forms. The enzyme was stable at temperature range between 10ºC to 60ºC. It was inactivated at temperature 65ºC within 10 min. It was observed maximum activity between 30º to 40ºC. When guar gum used as substrate rate of enzyme activity was increased with time up to 48 hrs. then it is significantly decreased with the time. . All data was analyzed by using MS excel computer program and results are obtained in Mean ± SD manner.

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