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Partial Purification of β-Galactosidase Extracted from Apricots (Prunus armeniaca kaisa)

Chemistry

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ABSTRACT

β Galactosidases are enzymes which has an increasing biotechnological and medicinal application for lactose hydrolysis and oligosaccharides synthesis. The β-galactosidase produced from apricot seeds was extracted and purified by precipitation with ammonium sulphate (30-70%) and dialysis was carried out at 4 degree C for 24hr. The crude extract had a higher specific activity (6.19 U/mg proteins) which indicates a high enzyme yield. Purification of crude extract which was done with ammonium sulphate (30-70%) increased the specific activity to 18.49 U/mg proteins which was improved by dialysis to 21.61 U/mg proteins. The enzyme presented an optimum activity at pH 8.5 and 55 degree C. The enzyme activity obtained was almost stable between pH 5.0 and 6.5.

KEYWORDS:

β-Galactosidase, Lactose hydrolysis, Apricots, Extraction, Enzyme

INTRODUCTION

β-Galactosidase or β-D-galactoside-galactohydrolase (EC. 3.2.1.23) is an important enzyme industrially used for the hydrolysis of lactose from milk and milk whey for several applications. It is also important for the lactose intolerant community as it is responsible for making lactose-free milk and other dairy products.

Lactose, a major component of milk and whey, used in food products, is somewhat limited due to its low solubility and in digestibility in many individuals. Lactose cannot be absorbed as such, but it must be hydrolysed into its component saccharides (which are easily absorbed from the intestine) by the action of intestinal β-galactosidase (1). A significant number of the adult population (almost 75 %) is unable to digest lactose due to a genetic deficiency of this galactosidase/lactase enzyme – lactase deficiency (2,3). Limited digestion of lactose can cause unpleasant gastrointestinal symptoms of varying severity, termed lactose intolerance (4). To the clinical symptoms of lactose intolerance belongs: nausea, vomiting, abdominal distension, cramps, flatulence, flatus, diarrhea and abdominal pain.

Individuals with these symptoms often begin to avoid milk and dairy products thus leads to a lower calcium intake (5), which is associated with reduced bone mineral density and an increased risk of developing osteoporosis (6). Therefore individuals with lactose intolerance can consume free milk and dairy lactose products. Enzymatic hydrolysis of lactose by β-galactosidase is one of the most important technologies to produce lactose reduced/free milk and related dairy products for consumption by lactose intolerant people (7-11).

Lactase (β-D-galactosidase EC 3.2.1.23) is a hydrolytic enzyme that has two enzymatic activities; one is responsible for the hydrolysis of lactose and cleaves cellotetrose and cellotriose and the second activity associated with splitting β-glycosides. β-galactosidase hydrolyzes lactose into glucose and galactose, which can be readily absorbed and hence caters to lactose intolerant people. β-Galactosidases are widely distributed in microorganisms (bacteria, fungi, yeasts), plants especially in almonds, peaches, apricots, apples and animal organs (12-14). Extraction and purification of the β-galactosidases from many microorganisms for the preparation of de-lactosed milk had already been reported (15-16).

Keeping in view, the aim of the present work was the extraction of β-galactosidase from apricots followed by partial purification and to obtain high enzyme yield.

EXPERIMENTAL

Materials

Apricot (β-galactosidase) in packed form of 250g were procured from Agro Ted India LLP, Vashi, Navi Mumbai . O-nitrophenyl-ß-D-galacto pyranoside (ONPG), Bovine serum albumin, Folin reagent and Dialysis bag were obtained from Himedia,Mumbai.

Methods

Enzyme extraction

Fresh apricot seeds (20 g) were grounded by pestle and mortar. Homogenization (Remi motor, India) was done for 15 min at 4°C with 100 ml of 0.02 M acetate buffer (pH 5) followed by sonication (Lark Ultrasonic Sound Detector),for 60 minutes. The homogenate was filtered and centrifuged (8400 rpm) for 15 min and supernatant so referred to as crude extract.

Partial purification

For preliminary assay; 50 ml of crude enzyme extract were used to determine the appropriate ammonium sulfate concentration for partial purification. The ammonium sulphate concentration (30-70%) was used to purify the remaining initial extract through two steps: first, the solution was brought to 30% concentration then centrifuged at 8400 rpm for 10 min and the precipitate discarded. Secondly, the enzyme solution was brought to 70% saturation and the enzyme precipitate retained and diluted to 50 ml of 0.02 M acetate buffer (pH 5). The content was kept in the dialysis bag (length-6 inch). The system was placed in an ice cooled bath on a magnetic stirrer and dialyzed against the same buffer for 24 hr at 4°C with changing the dialysis buffer thrice.

Enzyme activity assay

One unit of ß-galactosidase activity is defined as the amount of enzyme required to release 1 µmol of o-nitrophenol (ONP) per min under the assay condition. The activity of ß-galactosidase was assayed (17) using ONPG as a substrate. The reaction mixture consisted of 0.4 ml of ONPG, 1.4 ml of 0.02 M acetate buffer (pH 5) and 0.2 ml of ß-galactosidase extract. The reaction mixture was incubated at optimum temperature for 30 min. The reaction was terminated by the addition of 1ml of 1M sodium carbonate. Yellow colour was developed.The product (ONP) was estimated spectro-photometrically (Model No.308 spectrophotometer) at 410 nm.

Protein assay

Protein content was determined by the method described by Lowry et al.(18) with bovine serum albumin as standard.

Specific activity of enzyme was calculated as following:

Specific activity of enzyme (unit / mg) = Total activity(unit)) / Total protein(mg)

Enzyme kinetic methods

P^H and Temperature optima

The optimal pH of β-galactosidase activity was determined in the assay mixture with different pH ranging from 4-8 using acetate buffer (4.0-5.5), phosphate buffer (6-8). The optimal temperature of enzyme activity was determined by incubating the assay mixture at pH 5 for 30 min at a temperature ranging from 40 to 80 degree C.

RESULTS AND DISCUSSION

Partial purification of ß-galactosidase

The purification steps, protein concentration and specific activity of apricot seeds ß-galactosidase are shown in Table 1. The crude extract had a high specific activity (6.19 U/mg protein) which indicates a high enzyme yield as reported for other sources, e.g. mung bean

(10).Purification of crude extract with ammonium sulphate (30-70%) improved the specific activity to 18.49 U/mg protein. Furthermore, the collected precipitate from the ammonium sulphate precipitation step was dissolved in acetate buffer pH 5 and dialyzed for 24 hr. against the same buffer. The dialysis step increased the specific activity to 21.61 U/mg proteins.

Table-1. Purification steps of apricot seeds β-galactosidase with total activity

Purification Steps	Total activity(u)	Total Protein (mg)	Specific activity/mg protein
Crude extract	10568	1705	6.19
Ammonium sulphate(30-70%)	8654	468	18.49
Dialysis	7436	344	21.61

Optimum pH

The activity of apricot ß-galactosidase was found to vary with pH values (Fig.1). The optimum pH of the enzyme activity was 5.0. The activity of the enzyme decreased gradually at pH values higher or lower than the optimum pH value, retaining more than 50% of its maximum activity at pH range of 4-6. The enzyme activity decreased drastically with the increasing of the pH over 7. These results agree with the observation reported earlier (19) that the pH optimum of plant ß-galactosidase lies in the acidic range while those derived from bacteria are in the neutral range. Gulzar and Amim (20) extracted three isoenzymes of ß-galactosidase from apricots and they found that the optimum pH was found between 4.0 and 6.0.

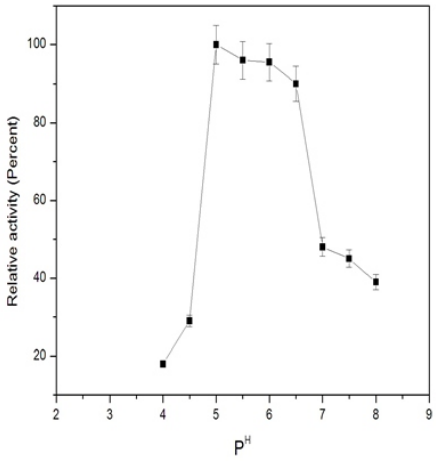

Figure1. Effect of P^H on the Beta-galactosidase activity

Optimum temperature

The activity of apricot ß-galactosidase increased with increasing the temperature up to reaching maximum activity at 55°C (Figure.2). On further increase in temperature, the enzyme activity declined gradually and at 80°C, enzyme exhibited 11% of the maximum activity. The loss of activity of the enzyme at higher temperatures could be attributed to its unfolding and subsequent loss of active site (14).

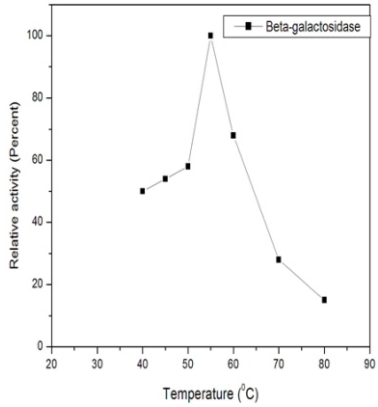

Figure2. Effect of Temperature on Beta-galactosidase activity

CONCLUSION

β-galactosidase is an important enzyme in the dairy industry to obtain low lactose products, ideal for intolerant consumers. Besides microorganisms, plants are also the efficient sources for enzyme production. In this study, β-galactosidase was extracted effectively from the Apricot seeds, purified partially by ammonium sulphate (30-70%) and dialyzed for 24 hours. Partial Purification of crude extract with ammonium sulphate increased the specific activity and it was further improved by dialysis. The enzyme presented an optimum activity at pH 5 and 55°C. It demonstrated the ability of β-galactosidase production & was evaluated under different optimal conditions like pH, temperature.

SIGNIFICANCE

The commercial importance of galactosidase is its application in the dairy industry to obtain low lactose products, ideal for intolerant consumers. The present investigation will be an aid to explore the possibility of preparation of free lactose milk for preparing free lactose cheese suitable for lactose intolerance.

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