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EMULSIFIED ENTRAPMENT OF *Glycine Max* B-AMYLASE INTO CHEMICALLY MODIFIED BOVINE SERUM ALBUMIN AND STUDY ITS APPLICATIONS IN DETERGENTS

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ABSTRACT

β-Amylase is converted the starch into maltose, digesting it off from the non-reducing end and thus producing limit dextrins. This property of β-Amylase has wide applications in food, pharmaceutical, paper, leather, detergent and textile industries too. In present work, *Glycine max* β-Amylase was entrapped by emulsification and through covalent coupling by glutaraldehyde which is an excellent cross linking agent. Biodegradation of emulsified entrapped *Glycine max* β-Amylase into chemically modified bovine serum albuminwas carried out by the alkaline protease which is proteolytic enzyme. Successful sustained release of bound enzyme from emulsified chemically modified bovine serum albumin widens its applications by increased stability and sustained usablity. Starch forms a major constituent of foods and is major component of stains during spilling of foods on cloths. Further, this emulsified entrapped *Glycine max* β-Amylase into chemically modified bovine serum albumin along with alkaline protease were used in detergents that work on starchy stains on stained cloths that lead to vanish of these strains and clear them by digesting starch during washing.

KEYWORDS: *Glycine max* β-Amylase, bovine serum albumin, Glutaraldehyde, Entrapment, Emulsification

INTRODUCTION

Emulsified entrapment was previously used for immobilization of enzymes into microspheres which are spherical particles that may be in the form of microcapsules in which the active gradient is dispersed in a hydrophilic or non-polymer layer. Hence, this type of entrapment was responsible for controlled release of active ingredient. These entrapped microspheres have been used as carriers in the delivery of drugs, antigens, hormones, enzymes and genes [12,11].

Albumin is biodegradable, biocompatible and non-toxic in nature. Due to these exploitable properties, it was commonly chosen as a matrix material for the preparation of the microspheres and other chemically modified entrapment or immobilization. Encapsulation in albumin microspheres has been seen an extensive development during the past 20 years. These were generally prepared by the formation of a water-in-oil emulsion and subsequent stabilization of the protein droplets by using

chemical cross-linking agents such as glutaraldehyde, formaldehyde or 2,3-butadione, or by thermal denaturation of the protein. Glutaraldehyde, a linear, 5-carbon dialdehyde, is a clear, colourless to pale straw-coloured, pungent oily liquid that is soluble in all proportions in water and alcohol, as well as in organic solvents [3] The stability of an enzyme (protein) can typically be increased by crosslinking because intra- and intermolecular crosslinks lead to a more rigid molecule that can resist conformational changes. Earlier, β-Amylase has been immobilized on polymeric materials; poly(acrylamide–acrylic acid) resin [P(AM-AAc)] and poly(acrylamide–acrylic acid–diallylamine–HCl) resin [P(P(AM-AAc-DAA-HCl) [3] ,alkylamine porous silica, gelatin film through crosslinking,phenylboronate-agarose [13]. The titanium-chelation method was been used to immobilize β-amylase on to Spheron [2]. In our present work, bovine serum albumin was used as matrix for immobilization of *Glycine max* β-Amylase which was chemically modified by emulsification with coconut oil and treatment with glutaradehyde. Biodegradation of emulsified entrapped enzyme was done with alkaline protease for controlled and steady release of bound enzyme as well as used for washing of stained cloth for destaining.

MATERIALS &METHODS

Extraction Pulses Amylase

Cotyledons from 3-day old seedlings from germinating seeds of *Glycine max* were homogenized using pestle mortar in 0.05M sodium phosphate buffer (pH 7.0). 4-6 ml of buffer was added per gram of fresh weight. Enzyme extract was filtered through two layers of cloth and centrifuged for 15min at 4°C. The supernatant was collected which contained crude enzyme and stored at 4°C and used for entrapment by emulsification and chemical modification of bovine serum albumin.

Enzyme assay

Amylase was assayed according to the procedure followed by Bernfeld, 1955[1]. The activity of extracellular amylase was estimated by determining the amount of reducing sugars released from starch. The starch solution was prepared from 1% (w/v) soluble starch in distilled water. 0.5 ml of the enzyme extracts were added to 1 ml of the starch solution and the mixture incubated at 37ºC for 20 min. After that 2 ml of DNS was added to terminate the reaction and the reaction mixture was boiled at 100ºC for 5 minutes. The amount of reducing sugars in the final mixture was determined spectrophotometrically at 570 nm [7]. One unit of enzymatic activity is defined as the amount of enzyme that produces 1 µmol of maltose per minute (Robert, R B & Evan R K, 2003 and Garen, A. & Levinthal C, 1960).

Immobilization of enzyme into chemically modified albumin

Glutaraldehyde was used as a crosslinking agent and coconut oil was used as emulsifier. The oil bath was prepared by adding 0.5ml of 25% glutaraldehyde and 2.6ml of n-Butanol to 50ml of coconut oil. 50U of enzyme was mixed with bovine serum albumin. This solution filled in an 18-guage syringe and dispersed in the prepared oil bath [6,11]. It was continuous stirred overnight in an incubator shaker at 37°C. Next day, the oil bath was centrifuged at 5000rpm at 4°C for 20mins and the supernatant was removed. The pellet was collected which contained emulsified entrapped *Glycine max* β-Amylase. The pellets are washed with cold diethyl ether and chilled acetone (6: 2 ratios). It was stored in dry conditions at 4°C.The emulsification with coconut oil was increased the stability of bound enzyme into chemically modified bovine serum albumin which allowed the controlled and sustained release.

Biodegradation of entrapped *Glycine max* β-Amylase

Entrapped *Glycine max* β-Amylase was incubated at 37°C with different concentrations of Alkaline Protease (5U, 10U, 15U, 20U, 25U, 30U). The enzyme assay of entrapped enzyme was performed for consecutive eight days by dinitrosalicylic reagent method [1].

Study of Kinetic Parameters of *Glycine max* β amylase

Various kinetic parameters was carried out such as effect of pH, time of incubation, temperature,

substrate concentration and effect of $CaCl_2$ concentration were studied for entrapped enzyme.

Preparation of test samples

Buffers with pH 1.5- pH 11.5 were prepared with sodium acetate buffer, sodium phosphate buffer and sodium carbonate buffer. Assay of entrapped *Glycine max* β-Amylase was done by diluting it with alkaline protease buffer mixture (1: 1 ratio) and then added 1ml of 1 %(w/v) starch solution. Effect of time of incubation was varied from 5-25 minutes, temperature (20-60°C), starch concentration (0.25-1.50%). Metal ions act as inhibitors of β-amylase and bind at the active site of enzyme but in the presence of calcium ion, the activity of amylase was increased [6]. A range of concentrations from 2% - 10% were prepared to study the effect of $CaCl_2$. The enzyme assay of entrapped enzyme was performed by dinitrosalicylic reagent method for studying the various kinetic parameters by dinitrosalicylic acid method at 570 nm.

Study of applications of entrapped *Glycine max* β amylase in destaining of stained fabrics

Three different samples of washing powders (Ariel, Surf excel and Tide) were chosen and three samples of stained fabrics were used for study. Pieces of chosen fabric (1"× 1") were stained with different sources of strains (dried strains of cornflour curry, soil and potato curry) and dipped in a beaker containing 2mg of entrapped enzyme with 30U alkaline protease solution along with 1 ml of detergent solution. Each sample of stained fabrics were tested with all three samples of washing powder.

RESULTS & DISCUSSION

Glycine max β-Amylase is entrapped in chemically modified bovine serum albumin by emulsification with coconut oil and covalently coupled by using glutaraldehyde. 85% of immobilization was achieved in this emulsified entrapment of *Glycine max* β-Amylase into chemically modified bovine serum albumin. Biodegradation study for controlled and sustained release of entrapped enzyme from chemically modified bovine serum albumin was performed by incubating 2mg of entrapped enzyme with alkaline protease solution (5U, 10U, 15U, 20U, 25U, 30U) overnight at 4ºC. This study was carried our in all incubation mixtures for 1-8 days. The released studies were showed that 30U of alkaline protease lead to excellent controlled and sustained release of entrapped enzyme. The pattern of biodegradation for each dilution was showed an increase from 1^{st} to 4^{th} day, the highest being at 5^{th} day and remained constant till 8^{th} day (Fig 1). pH study was done for optimum pH over the range pH 1.5-pH 11.5 which was showed that the entrapped *Glycine max* β amylase had maximum activity at pH 6.5 which was lower than that free enzyme (8.5) [8,9]. Optimum time of incubation was 25 minutes which is also higher than that of free enzyme (15 minutes) [10,5]. The entrapped β-amylase was studied for its optimum temperature for maximal activity over 20°C to 60°C which showed increased enzyme activity at 40°C. The thermal Stability of an entrapped enzyme was 50°C which was same as that of free enzyme [4,9]. The effect of substrate concentration at different starch concentrations (0.75- 1.25%) was studied which showed increases activity at 1.25% and afterwards it was fairly constant to confirmed the state of saturation of entrapped enzyme for optimal starch concentration. The 2-3% of $CaCl^2$ concentration was pretty enough for maximal activity for entrapped enzyme. The entrapped enzyme was lost only 40% of its activity after 6 month when stored in 0.1 M KCL solution at 0°C to 4°C which was pretty similar to earlier report [11]. The present study was showed increased storage stability after the immobilization (Table 1).

The application of the entrapped enzyme with detergents showed that the entrapped enzyme into chemically modified *Glycine max* β-amylase was carried out. Among the three samples of detergent solutions, Arial detergent with entrapped enzyme solution was worked fairly well as compared to others (Fig 2. a & b). Hence, emulsified entrapped β-Amylase into chemically modified bovine serum albumin with detergent was efficient to destain the stained fabrics after washing to enhance the efficiency of washing powders to wash off the hard and dried strains of

food stuffs from the fabrics which was not reported earlier.

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Figures:

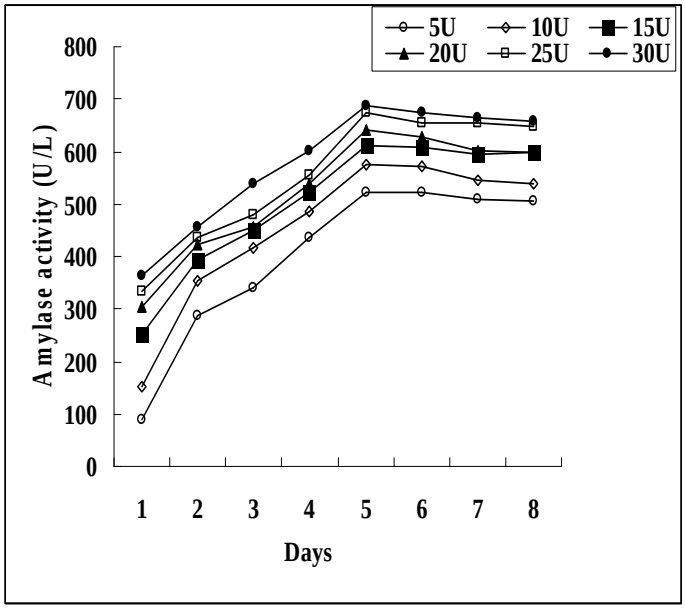

Fig 1. Biodegradation of emulsified entrapped *Glycine max* β-amylase into chemically modified bovine serum albumin by using different concentration of alkaline proteases.

Fig: (a)

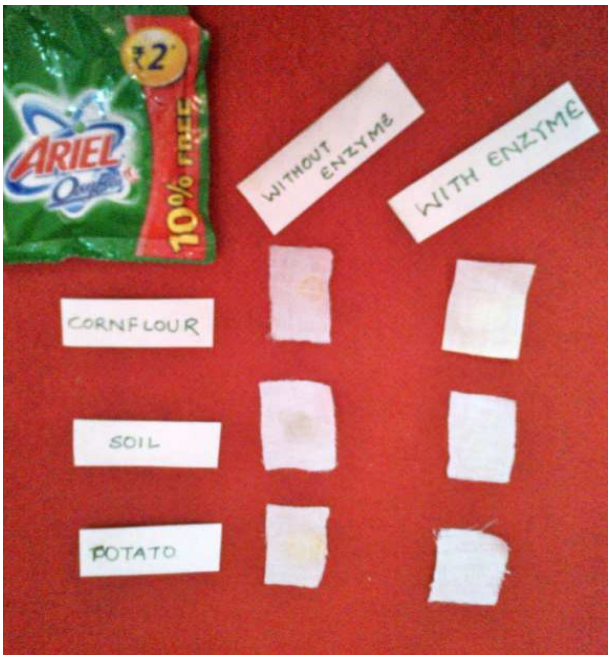

Fig: (b)

Fig 2. Washing results of two types of detergents with and without emulsified entrapped *Glycine max* β-amylase into chemically modified bovine serum albumin on stained samples of fabrics [(a) with surf excel & (b) Arial].

Table 1:

Kinetic Parameters	*Free Glycine max* β-amylase	*Entrapped Glycine max* β-amylase
pH	8.5	5.5
Temperature	50 ºC	50 ºC
Time of Incubation	15 minutes	25 minutes
Substrate concentration/	1%	1.25%
$CaCl_2$ concentration	6%	2-3%
Storage stability	7-15 days	6 months

Table 1: Kinetic parameters of free and emulsified entrapped *Glycine max* β-amylase into chemically modified bovine serum albumin.