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MAXIMIZATION OF PRODUCTION OF PROTEIN HYDROLYSATES BY USING IMMOBILIZED PAPAIN

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ABSTRACT

Maximization of the production protein hydrolysates was studied by subjecting various cereals for hydolysis by immbilized papain at varying reaction conditions. The degree of hydrolysis was determined by the trichloroacetic acid (TCA) assay method. The optimum conditions for maximizing the production of protein hydrolysate are 2% concentrated solution of sodium alginate, an incubation time of 24 hours, five grams of alginate beads, 30 mL of enzyme loading volume and usage of beads up to 7 cycles. Among the different substrates, the enzyme exhibited the highest activity on the red gram. For the maximum production of protein hydrolysates, the concentration of red gram was optimized to 2%, at pH 5, the time of incubation 60 minutes R. P. M. 120 and the temperature 50^0C.

Key words: Papain, Immobilized enzyme, Alginate beads, Cereals, Degree of hydrolysis.

INTRODUCTION

From the past few years, protein hydrolysates are widely used for obtaining value added products from dietary proteins because of the improvement in nutritional and functional characteristics, retardation of deterioration, and removal of toxic or inhibitory ingredients. They are often used in the preparation of different nutritional formulations, such as supplementation of drinks to enhance their nutritional and functional properties, or special medical diets. Against what appears to be an increasing incidence of many allergic disorders, the production of safe and effective hypoallergenic diets at a commercial scale has become of increasing interest. Although elemental diets with free amino acids are allergen free, and can be used with success in therapy, protein hydrolysates are applied in hypoallergenic formulas for feeding food-allergic or allergy-prone infants. They show a reduced or null antigenic activity and because peptides are less hypertonic than free amino acid mixtures, their use as a nitrogen source in enteral feeding improves absorption efficiency and reduces

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osmotic problems. Several investigators have evaluated the immunogenicity and/ or allergenicity of enzymatically hydrolyzed proteins for potential use in formulas for feeding food-allergic individuals. Thus, the effect of the degree of hydrolysis (DH) on antigenicity has been studied in whey proteins and casein. It was reported that the most effective reduction of antigenicity in whey proteins was obtained by treatment with pepsin and chymotrypsin, successively.

Papain is a protein-cleaving enzyme derived from papaya and certain other plants[1]. It is usually produced as a crude, dried material by collecting the latex from the fruit of the papaya tree. It consists of 212 amino acids stabilised by 3 disulfide bridges[2]. Papain by far is the most widely studied of the cysteine enzymes because of its commercial value. Besides being used as a meat tenderizer[3], other uses of papain include fibrinating wounds, clotting of milk, shrink proofing of wool, reduce viscosity and increase palatability and in low doses, it can be used as an indigestion medicine. Based on its proteolytic activity, it is used to produce the protein hydrolysis, which is most useful in the pediatric and geriatrics diet. The activity of papain is expressed in papain units (PU). The assay of papain activity is based on the hydrolysis of casein. As already mentioned that papain can cleave the peptide bonds and can convert complex proteins to simpler[3]. The effect is pronounced in the immobilized form of enzyme[4].

The present study was aimed to maximize the protein hydrolysates production by using the papain as a proteolytic enzyme. It was done in the two stage process. Initially, the papain was immobilized in alginate beads and optimization of the immobilization parameters for the better papain activity was done. Second step was protein hydrolysates production by using the cereal powders and optimization of the various process parameters like substrate concentration, rpm, reaction temperature and incubation time for enhancement of the rate of hydrolysis.

EXPERIMENTAL

Materials and methods

Chemicals: Papain, sodium alginate, calcium chloride, casein, red gram powder, green gram powder and trichloroacetic acid were purchased from the Sigma-Aldrich Laboratories.

Papain was immobilized by matrix entrapment method. After cooling the hot solution of sodium alginate to room temperature, the enzyme papain is added to the gel and mixed well. The enzyme and sodium alginate mixture is poured drop by drop into a 1%

solution of calcium chloride by using pipette. Then enzyme was entrapped and immobilized in the calcium alginate beads.

Optimization of immobilization parameters

Initially sodium alginate concentration was optimized at varying concentrations of 0.5%, 1.0%, 1.5%, 2.0% and 2.5%. The curing time is also optimized by keeping the beads at various time intervals in $CaCl_2$ solution. The enzyme loading is also optimized. The operational stability was studied by checking the number of batches the beads without losing significant enzyme activity.

Production of protein hydrolysates

One percent solutions of red gram, black gram, green gram, soya bean and casein were prepared and the pH, temperature were adjusted depending upon the experimental conditions. The reaction was initiated by the addition of the immobilized enzyme to give a final enzyme-to-substrate ratio of 1 : 100 (w/w). After the reaction period, the mixture was cooled, adjusted to pH 7.0 with 4M NaOH and heated at 90^0C for 15 minutes to inactivate the enzyme. Then the mixture was centrifuged at 10,000 rpm for 20 minutes at 4^0C in a REMI cooling centrifuge. The supernatant was used for the estimation of the degree of hydrolysis of proteins.

Degree of hydrolysis (DH) determination

The DH was determined by the trichloroacetic acid (TCA) assay method. DH was calculated by the ratio of the percentage of 10% TCA-soluble nitrogen to total nitrogen in the sample[5]. Aliquots were removed at the final time required and mixed with 20% TCA to create 10% TCA-soluble and TCA-insoluble fractions. After 30 min, the mixture was centrifuged at 3000 rpm and the supernatants were analyzed for nitrogen by the semimicro-Kjeldahl method.

Optimization of protein hydrolysates production

Effect of temperature

5 g of beads and 2% of substrate mixture was kept for incubation in shaker incubator at different temperatures of 30°C, 45°C, 60°C and 75°C. Then the samples were estimated for the protein hydrolysates.

Effect of time of incubation

To check the effect of time of incubation on the production of protein hydrolysates at

different time intervals of incubation were maintained. Substrate and immobilized enzyme kept for incubation at different time intervals (24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs.), After incubation, the samples were drawn and analyzed the degree of hydrolysis.

RESULTS AND DISCUSSION

The following aspects were studied concerning the production of protein hydrolysates.

Effect of sodium alginate concentration and curing time on immobilized papain activity

It has been reported that the porosity of the calcium alginate beads depends upon the alginate type and the gelling agent concentration[6]. So various concentrations of sodium alginate beads, in order to vary the relative degree of cross linking, which would create different pore size, are prepared. The immobilization efficiency was found to be highest (80%) for a concentration of 2% (w/v) sodium alginate (Fig. 1).

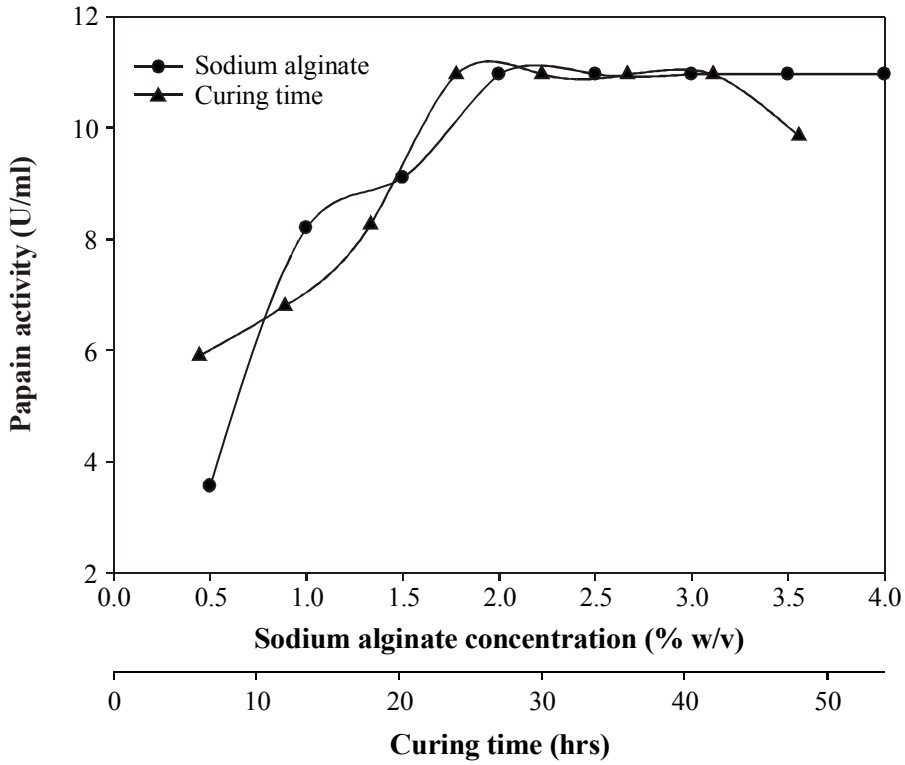

Fig 1: Influence of sodium alginate concentration and curing time on papain activity

The lower immobilization efficiency in case of lower percentage of sodium alginate solution might be due to large pore size and consequently, greater leakage of the enzyme from the matrix.

Time required for the gel to set is an important step in immobilization as it affects the stability of the resulting calcium alginate beads. The effect of curing time on the activity of the beads was evaluated. The treatment beads in calcium chloride batch for 24 hours gave an activity of 80% (Fig. 1). Prolonged curing of beads with calcium chloride solution did not improve the activity and structural stability of beads.

Effect of amount of enzyme loaded in immobilized beads

Different volumes of papain solutions were mixed with the sodium alginate and the degree of catalysis was assayed. The reaction rate increased along with the increasing the enzyme concentration in the immobilized beads. The highest activity was achieved by preparing the beads by mixing of 30 mL of papain in the sodium alginate solution (Fig. 2). On further increasing the volume, the activity was not increased.

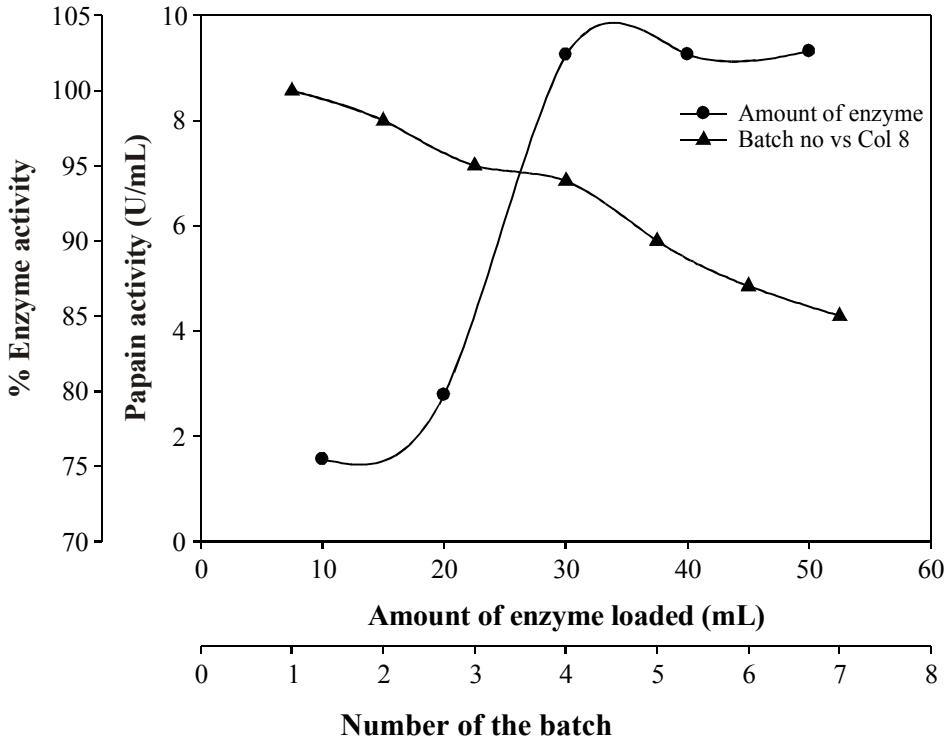

Fig 2: Effect of enzyme loading and life cycle of beads

Operational stability

The operational stability of immobilized enzymes is one of the most important factors effecting the utilization of an immobilized enzyme system. The operational stability of the papain was evaluated in the batch process. The results (Fig. 2) indicated that on repeated use of the immobilized papain, 85% of the initial activity was retained up to seven cycles. After seven cycles, there was loss of enzyme and denaturation and due to physical loss of enzyme from the carrier.

Protein hydrolysates production and optimization

Rate of hydrolysis on natural substrates

The substrate specificity of the immobilized papain was tested on various substrates and results are shown in Fig. 3. The immobilized catalyst exhibited an appreciable hydrolytic capability in presence of proteins obtained from red gram, black gram, green gram and soya bean powder, which was comparable to casein. It may be inferred that the present entrapment system did not pose any diffusional limitations in case red gram powder.

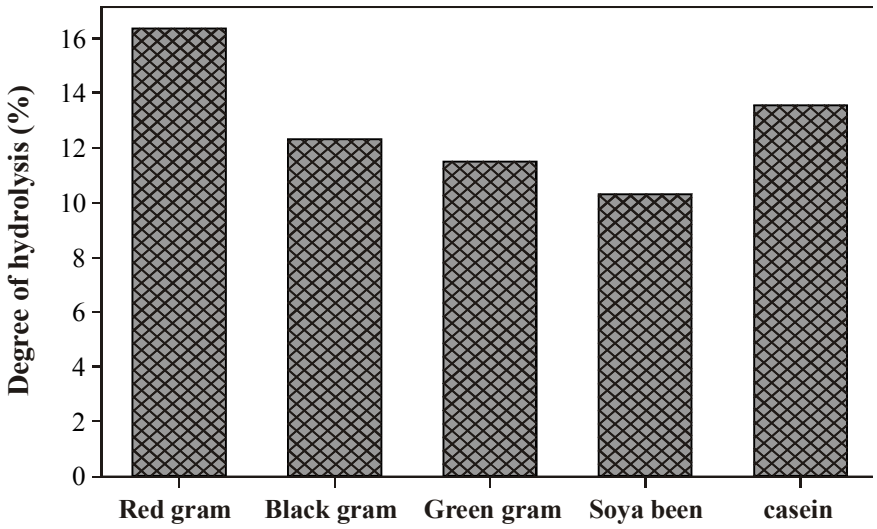

Fig. 3: Effect of various substrates for hydrolysates production

Effect of substrate concentration on enzyme activity

The effect of substrate on enzyme activity was well documented. At the optimum concentration, it shows the grater activity. To determine the optimum concentration, different concentrations of red gram powder solutions were taken (1%, 1.5%, 2%, 2.5% and

3%). The 2% solution shows the optimum activity. On further increasing the concentration, a decrease in the degree of hydrolysis was observed. This state was attained due to substrate inhibition at the high concentration (Fig. 4).

Effect of amount of beads on enzyme activity

Different amounts of papain-immobilized beads were introduced into the reaction medium and degree of catalysis was assayed. The rate of hydrolysis increased with the increase in amount of immobilized catalyst and maximum activity was reached with 5 g of beads (Fig 4). However, on further increasing beads volume, no significant improvement in the hydrolysis rate was observed.

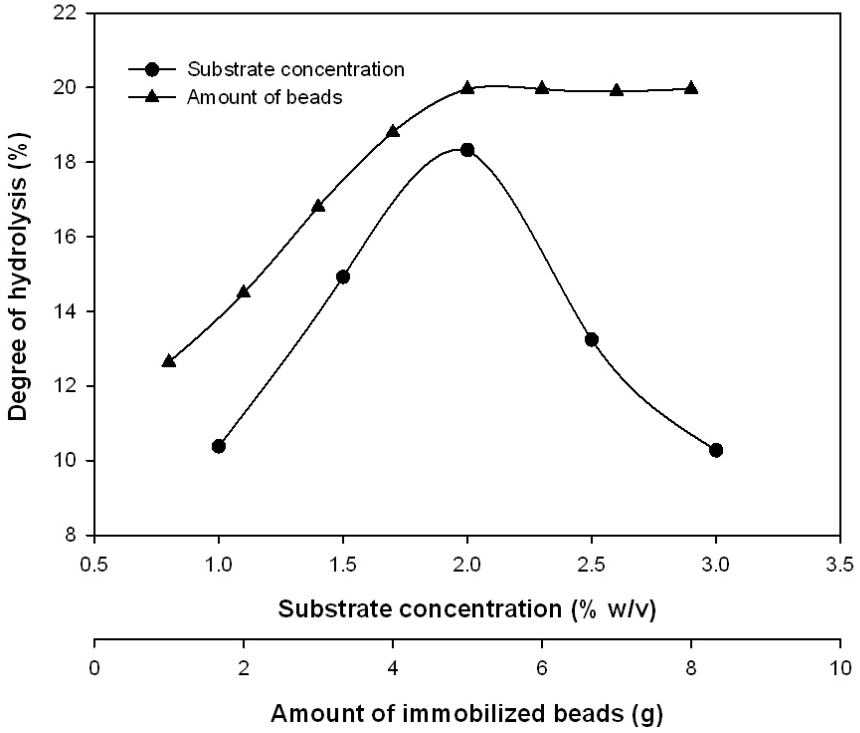

Fig 4: Influence of substrate concentration and enzyme concentration for hydrolysates production

Effect of rpm, temperature and incubation time on enzyme activity

Mass transfer resistance is one of the important limiting factors for the immobilized enzymes activity. To over come this problem, proper mixing of the contents is necessary. At the conical flasks level, mixing can be attained by keeping the reaction mixer and beads on

orbital shaker. At low speed, the mixing is not proper and at high speed, the contents at the reaction centre may escape. Due to this reason, the reaction may not take place. To test the optimum rpm, the reaction mixer is kept at different rpm speeds. Fig. 5 shows the optimum rpm was 120. After this, on increasing the speed also, there is no change in the rate of hydrolysis.

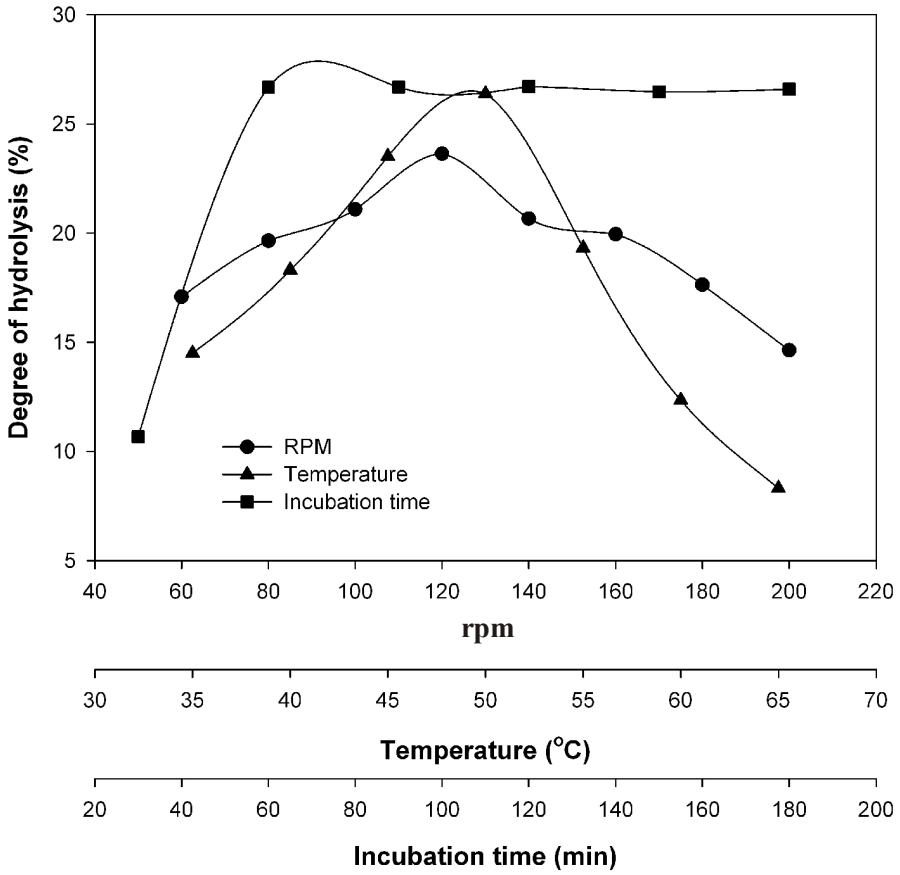

Fig. 5: Influence of rpm, temperature and incubation on protein hydrolysates production

The effects of temperature on enzymes were well documented. To find the optimum temperature, we incubated the reaction mixer and immobilized enzyme at different temperatures in orbital shaker. The optimum activity of the enzyme was found at a temperature of 50^0C (Fig. 5).

To check the effect of incubation time on the enzyme activity, the reaction mixer and the immobilized beads were kept in orbital shaker and at particular time intervals, samples

were withdrawn from time to time and they were analyzed for the product. It was observed that the maximum yield was obtained at 60 minutes (Fig. 5). Further increase in the time of incubation does not increase the yield of the product. This stage is attained due to the end product inhibition or saturation of the enzyme.

CONCLUSION

The optimum conditions for maximizing the production of protein hydrolysate are summarized as follows: 2 % Concentrated solution of sodium alginate, an incubation time of 24 hours, five grams of beads, 30 mL of enzyme loading volume and usage of beads up to 7 cycles. Among the different substrates, the enzyme shows the greatest activity on the red gram. For the maximum production of protein hydrolysates, the concentration of red gram was optimized to 2%, at pH 5, the time of incubation was 60 minutes and rpm was 120, while maitaining the temperature at 50^{o}C.

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