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Preparation of soya bean meal protein hydrolysate by agarose-entrapped *Bacillus subtilis* cells

S. K. Khare, Krishna Jha & A. P. Gandhi

Soya bean Processing and Utilisation Project, Central Institute of Agricultural Engineering, Nabibagh, Bhopal-462018, India

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Bacillus subtilis cells entrapped in an agarose gel matrix were used for the hydrolysis of soya meal protein. A maximum hydrolysis in terms of tyrosine content (31·8 mg tyrosine) was observed at pH 7·0–7·5 and temperature 55°C in 4 h with a cell concentration of 25% (w/v). The hydrolysate consisted of 3·8% (w/v) solid content.

INTRODUCTION

Soya meal is a protein-rich defatted cake left after oil extraction from soya bean. It contains as much as 50% lysine-rich protein (Gandhi *et al.*, 1992). So far its main utilisation has been as animal feed, however, considering the existing protein–calorie malnutrition problem in most of the developing countries including India, its potential utilisation as a protein source, in the form of protein isolates, concentrates and hydrolysates, is ever increasing (Meyer, 1970; Kinsella, 1979; Barraquio & Vande, 1988; Gandhi, 1992). These are mainly used as protein supplements or as food ingredients. Isolates and concentrates contain antinutritional factors, namely trypsin inhibitor and soya lectins; however, hydrolysates, wherein these are hydrolysed to amino acids and peptides, are generally regarded as safe to use (Delvalle, 1981). Hydrolysates are mainly used as seasoning agents (soya sauce), flavouring agents, and to a lesser extent for providing functional properties (Dubois & Hoover, 1981). Hitherto, hydrolysates were prepared by chemical hydrolysis (Hsich, 1980). With increasing demand for protein hydrolysate, much attention is being paid to the development of a viable enzymatic process for preparing protein hydrolysate from soya meal (Alder-Nissen, 1982). The present investigation has therefore been undertaken to explore the possibility and viability of using immobilised *Bacillus subtilis* cells, a known high protease producer, for this purpose.

MATERIALS AND METHODS

Pure culture of *Bacillus subtilis* (NRRL-B 14201) was procured from the Northern Regional Research Laboratory (Peoria IL, USA). All the chemicals used in this investigation were of analytical grade.

Culture

The active culture was prepared according to the method described by Coller (1957). A loopful of spores was aseptically transferred to 3·0 ml of sterilised distilled water and heat-shocked for 30 min at 80 ± 1°C. One ml of this was then grown in nutrient broth at 37 ± 1°C in a shaker water bath at a speed of 200 rpm. At an approximate OD of 0·3, a 1·0 ml aliquot was then transferred to fresh medium. It was again allowed to grow for 12 h. The third and final transfer was done again with a 1·0 ml aliquot and the cells were allowed to grow until the OD reached 0·4–0·5. The active cells (vegetative stage) were then harvested by centrifugation at 10 000 rpm for 15 min in a refrigerated centrifuge. The cells were washed with sterilized distilled water at least twice and re-centrifuged. Finally, the pellets were collected and suspended in phosphate buffer (0·1 M, pH 7·0) for further experimentation.

Entrapment of cells in agarose gel matrix

The active cells of *Bacillus subtilis* were entrapped in an agarose gel matrix following the method described by Kanasowud *et al.* (1989). Agarose solution (2·0%, w/v) was prepared in Tris buffer (0·1 M, pH 8·0). Two grammes of frozen cell paste were suspended in 8·0 ml of agarose solution and the mixture was heated to 50–55°C. This heated mixture was dropped into 40 ml of constantly shaking paraffin oil with the help of a sterilised hypodermic syringe. The mixture was cooled in ice and washed with buffer. Droplets of cells

immobilised with the agarose matrix were formed which were collected by centrifugation at 10 000 rpm for 15 min. Cells were extensively washed with phosphate buffer (0·1 M, pH 7·0) and finally suspended in an equal volume of this buffer for further study.

Hydrolysis of soya meal protein

One gramme of soya meal, suspended in 10 ml of water (pH 7·5), was incubated with different concentrations of immobilised cells. The temperature was maintained at 50°C and the mixture was shaken constantly. Samples were withdrawn at various intervals and the extent of protein hydrolysis was determined by the tyrosine value and expressed as mg of tyrosine formed per ml of the solution (Lowry *et al.*, 1951).

RESULTS AND DISCUSSION

Bacillus subtilis cells were immobilised in an agarose gel matrix under optimised conditions. Free and entrapped cells were used for soya meal protein hydrolysis and the extent of hydrolysis was monitored in terms of tyrosine formation. In each case the same quantities of cells showed similar ranges of proteolytic activity, resulting in 24 mg tyrosine formation from 5% (w/v) soya meal slurry (Fig. 1). This level of hydrolysis was achieved in 4 h, beyond which there was no further increase in tyrosine formation. These results indicate that entrapment did not cause any change in the proteolytic activity of cells. *Bacillus subtilis* is known to be a rich source of proteases and these proteases have previous been utilised for efficient hydrolysis of casein by many workers (Choi *et al.*, 1991; Haque & Mozaffer, 1992). In the case of soya meal, Pivtsaeva *et al.* (1989) have successfully used free proto-subtilin to achieve 60% protein hydrolysis. In the present study the protein hydrolysis is comparatively low, but with an added advantage of re-usability of the system.

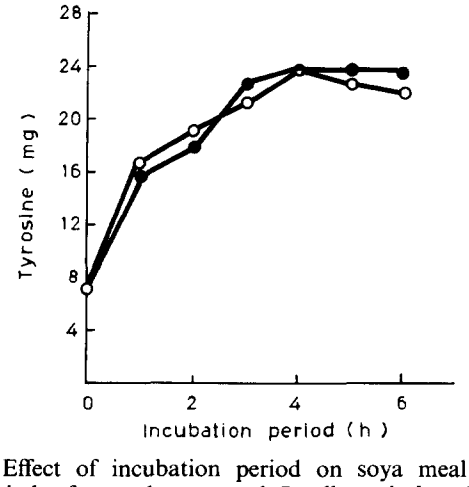

Fig. 1. Effect of incubation period on soya meal protein hydrolysis by free and entrapped *Bacillus subtilis* cells. Cells (10%, w/v) were incubated with 100 ml of 5% soya meal slurry at 30°C and pH 7·0. Tyrosine content was estimated in aliquots withdrawn at various intervals of time. (○) Free cells; (●) immobilised cells.

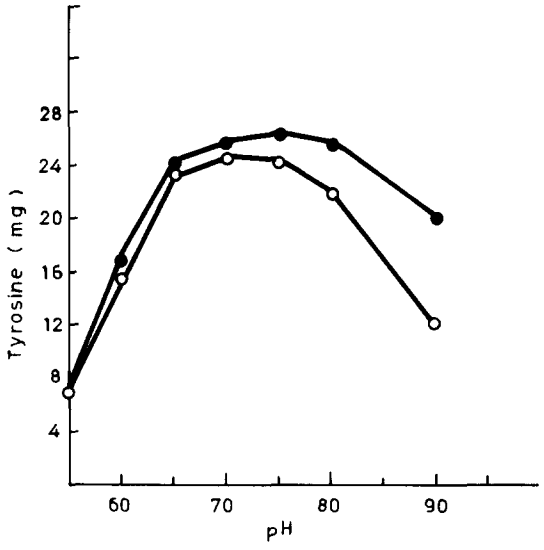

Fig. 2. Effect of pH on soya meal protein hydrolysis by free and entrapped *Bacillus subtilis* cells. 10% cell (w/v) were incubated at 30°C with 100 ml of 5% soya meal slurry adjusted to various pH. Tyrosine formation was determined after 4 h in each case. (○) Free cells; (●) immobilised cells.

The conditions for protein hydrolysis by free and immobilised cells were further optimised. The effect of pH on the extent of hydrolysis is shown in Fig. 2. The maximum hydrolysis in native as well as immobilised cells was observed from pH 7·0 to 7·5. The optimum temperature in both the cases was found to be 55°C (Fig. 3). Thus, there was no change in pH and temperature optima of cells as a result of entrapment. Agarose matrices are neutral in charge and have been reported not to cause any alternation in these enzymatic properties (Porath & Axen, 1976).

Enzyme concentration is considered to affect the reaction rate and end-product formation significantly. With this viewpoint the hydrolysis was carried out using different concentrations of cells (Fig. 4). 25%

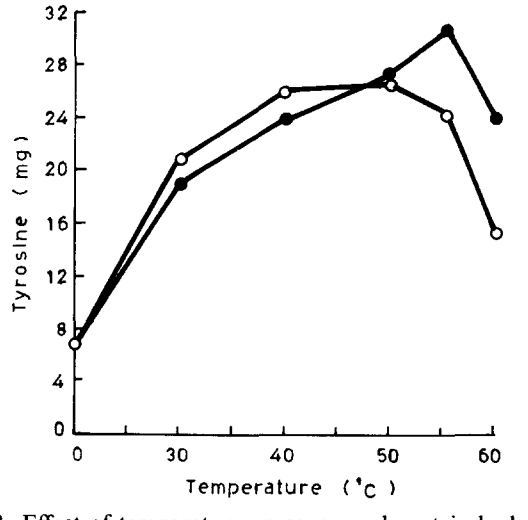

Fig. 3. Effect of temperature on soya meal protein hydrolysis by free and entrapped *Bacillus subtilis* cells. 10% cells (w/v) were incubated with 100 ml of 5% soya meal slurry (pH 7·5) for 4 h. The samples were incubated at different temperatures and tyrosine formation was determined in each case. (○) Free cells; (●) immobilised cells.

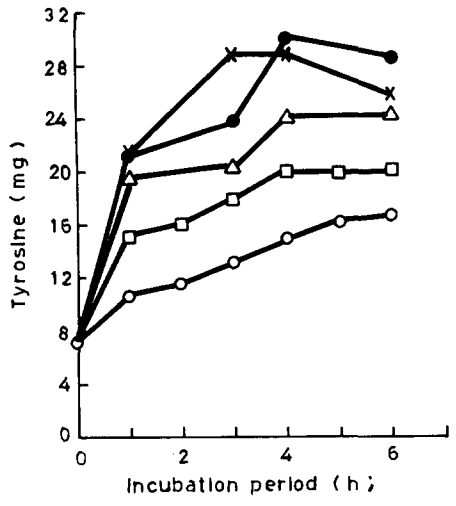

Fig. 4. The rate of hydrolysis of soya meal protein by different concentrations of cells. Immobilized cells of different concentrations were incubated at 55°C with 100 ml of 5% soya meal slurry adjusted to pH 7·5. Aliquots were withdrawn at various intervals of time and tyrosine concentration was monitored. (○) 10% (w/v) cells; (□) 15% (w/v) cells; (Δ) 20% (w/v) cells; (●) 25% (w/v) cells; (×) 30% (w/v) cells.

agarose-entrapped cells showed maximum protein hydrolysis resulting in 31·8 mg tyrosine formation. The hydrolysate obtained under optimised conditions was clear and milky with a solid content of 3·8% (w/v).

Thus, entrapped *Bacillus subtilis*, having an advantage of re-usability, could efficiently hydrolyse soya meal protein. Though the chemical composition of hydrolysate remains yet to be worked out, it may still find application as an amino acid and protein supplement in various food systems.

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