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Hydrolysis of flatulence-causing galacto-oligosaccharides by agarose-entrapped *Aspergillus oryzae* cells

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Aspergillus oryzae cells were entrapped within agarose beads. These entrapped cells, having α-galactosidase activity of 0·72 IU/ml, were used for hydrolyzing flatulence-causing galacto-oligosaccharides present in soymilk. The total of 52% raffinose, 70% stachyose, and 66·3% total oligosaccharides of soymilk was observed to be hydrolyzed in 8 h at 50°C by these immobilized cells.

INTRODUCTION

Soymilk is considered a suitable economical substitute for dairy milk and an ideal nutritional supplement for lactose-intolerant populations (Biranjan *et al.*, 1983; Khare *et al.*, 1992). However, the flatulence and gastric discomfort associated with the consumption of soymilk restrict its popularization and diminish its nutritive value (Hellendorn, 1969; Rackis *et al.*, 1970; Calloway *et al.*, 1971). The flatulence is caused by the presence of certain galacto-oligosaccharides, i.e. raffinose and stachyose, in soymilk (Cristofaro *et al.*, 1974; Jha, 1981). There have been a number of attempts to utilize α-galactosidases from various sources for hydrolyzing these galactosides and to obtain flatulence-free soymilk (Sugimoto & Van Buren, 1970; Cruz *et al.*, 1981). However, the search still continues for a more viable process.

Aspergillus oryzae has been shown to be a potential source of α-galactosidase (Park *et al.*, 1979). Cruz and Park (1982) have successfully demonstrated significant oligosaccharides removal by pretreatment of soymilk with crude extracts of α-galactosidase obtained from *Aspergillus oryzae* culture. Their results indicate a possibility of using whole cells of *A. oryzae* in immobilized form, which may lead to a more viable process with the added advantages of reusability and cost-effectiveness. The present investigation was therefore undertaken with a view towards utilizing immobilized/entrapped *A. oryzae* cells for pretreatment of soymilk and to study the extent of oligosaccharide removal and the feasibility of the process.

MATERIALS AND METHODS

Micro-organisms

Aspergilllus oryzae (NRRL, 1989) was maintained on mycological agar and preserved under refrigeration at 4°C. The culture was grown on mycological agar for one week at 30°C until sufficient sporulation was obtained. The spore suspension was prepared by washing the surface with 10 ml of sterilized distilled water.

Production of active cells

The active culture was produced through submerged cultivation of the *A. oryzae* in a chemically defined medium as described by Cruz *et al.* (1981). Sterilized medium (100 ml) was inoculated with 1 ml of spore suspension in a 500-ml Erlenmeyer flask. It was then incubated at 30°C for five days on a shaker water bath with agitation of 250 r/min. The active cells were harvested by centrifugation at 10 000 r/min for 15 min.

Entrapment of *A. oryzae* cells in agarose

A. oryzae cells harvested after five days were entrapped within agarose by the method described by Nilsson *et al.* (1983). The entrapped cells were suspended in an equal volume of citrate—phosphate buffer (0·5M, pH 4·0) for further studies.

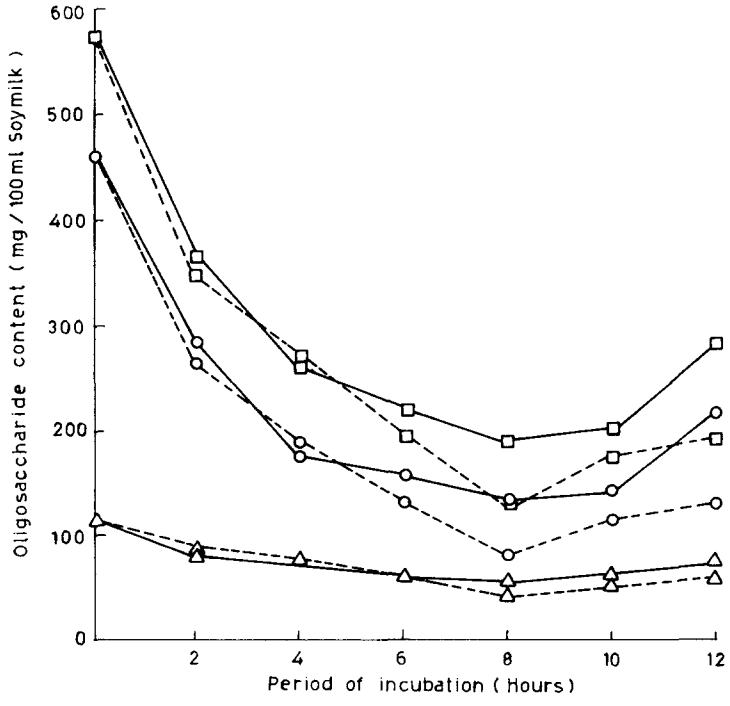

Fig. 1. Hydrolysis of galacto-oligosaccharides by agarose-entrapped *Aspergillus oryzae* cells. - - - - Free cells; —— immobilized cells; Δ raffinose; ○ stachyose; □ total oligosaccharides.

Enzyme assay

α-Galactosidase activity was determined by the method of Harpaz *et al.* (1974) by using *p*-nitrophenol α-D-galactopyranoside (PNPG) as substrate. One unit of α-galactosidase activity is defined as the amount of enzyme required to liberate 1 μmole of *p*-nitrophenol per minute.

Pretreatment of soymilk with agarose-entrapped cells

Soymilk samples (10 ml) (obtained from the Takai tofu plant, located at the Central Institute of Agricultural Engineering, Bhopal) were incubated with 4 ml of entrapped *A. oryzae* cells (0·72 unit/ml) at 50°C. Aliquots (1 ml) were withdrawn at various intervals, and the protein was precipitated with $Ba(OH)_2$ and $ZnSO_4$ as described by Cruz and Park (1982). The supernatant was used for determination of the oligosaccharide content.

Determination of oligosaccharide content

TMS derivatives were prepared by the method of Sweeley *et al.* (1963) and analysed by gas chromatography by using an OV-1 column with nitrogen as carrier at a flow rate of 30 ml/min. The injection and oven temperatures were 268°C and 150–300°C (10°C increase per min), respectively.

RESULTS AND DISCUSSION

Aspergillus oryzae harvested on the fifth day showed 1·58 unit α-galactosidase activity per ml of culture medium. Cruz and Park (1973) have also reported a similar range of α-galactosidase activity in their study on *A. oryzae* grown under similar conditions. Entrapment of these cells in an agarose matrix resulted in 54·4% loss of α-galactosidase activity (Table 1). Khare and Gupta (1988) have also observed a similar loss in β-galactosidase activity on entrapment in polyacrylamide gel. According to Zabrosky (1973), the entrapment of the cells generally results in 35–40% decrease in enzyme activity. the slightly greater loss in the present study may be due to the exposure of cell–agarose solution to 55°C for 2–3 min during polymerization. However, the range of recovery seems to be good and, overall, 0·72/unit/ml activity in immobilized cells appears to be reasonable enough for oligosaccharide hydrolysis, since enzymes with similar specific activity have been successfully used for this purpose in other cases. There was no change in the optimum temperature of 50°C and optimum pH of 4·0 for *A. oryzae* α-galactosidase as a result of entrapment (Table 1). These values are in agreement with those reported earlier (Cruz & Park, 1982). Entrapment of β-galactosidase in polyacrylamide gel has also been reported not to cause any change in these parameters (Khare & Gupta, 1988).

The oligosaccharide hydrolyses in soymilk by these cells were performed at these optima. Figure 1 shows oligosaccharide hydrolysis by *A. oryzae* cells. Soymilk

Table 1. α-Galactosidase activity of *Aspergillus oryzae* cells

	Free cells	Agarose-entrapped cells
Enzyme activity (unit/ml)	1·58	0·72
Optimum pH	4·0	4·0
Optimum (°C) temperature	50	50

samples initially contained 570 mg of total oligosaccharides per 100 ml, out of which 457 mg stachyose and 113 mg raffinose were the main constituents. Free cells hydrolysed 77% oligosaccharides in 8 h at 50°C (among which, 57% raffinose and 82% stachyose were removed individually) whereas immobilized cells hydrolysed 66·3% oligosaccharides, 52% raffinose, and 70% stachyose under similar conditions. Incubation beyond 8 h does not increase the extent of hydrolysis. Slightly higher oligosaccharide hydrolysis by free cells may occur because of better access of the substrate to the cells, which may otherwise meet diffusion constraints in the case of immobilized cells. Almost complete removal of oligosaccharides has been reported by using purified α-galctosidase from *Aspergillus saitoi* (Sugimoto & Van Buren, 1970) and *Aspergillus oryzae* (Cruz & Park, 1982) but these free-enzyme preparations were not reusable, which made the process economically unviable. The only report so far available on the use of immobilized cells for this purpose is by Thananunkul *et al.* (1976). They used polyacrylamide-entrapped *Mortierella vinacea* cells and achieved a 50% decrease by free cells and a 40% decrease by immobilized cells in total oligosaccharides in 12 h at 50°C in a batch process. Besides giving slightly more efficient oligosaccharide hydrolysis over *Mortierella* cells, the use of *Aspergillus oryzae* in the present study is more advantageous from the viewpoint of this being an acceptable source of food-grade enzyme (Reichelt, 1983). Thus 66·43% oligosaccharide hydrolysis with potentially reusable *A. oryzae* cells seems to be a promising approach for reducing flatulence-causing galacto-oligosaccharides and for obtaining low-oligosaccharide soymilk. The efficiency of this process may be further improved if used in a packed column in which it may form the basis of a continuous process for obtaining oligosaccharide-free soymilk. However, more studies would be required in terms of column size, flow rate, and the number of reusable cycles.

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REFERENCES

Biranjan, J. R., Ingole, D. R. & Sanyal, S. R. (1983). Utility of soymilk in dietotherapy of undernourished children. *Indian Med. Gas.*, **9**, 313–16.

Collaway, D. H., Hickey, C. A. & Murphy, E. L. (1971). Reduction of intestinal gas-forming properties of legumes by traditional and experimental food processing. *J. Food Sci.*, **36**, 251–5.

Cristofaro, E., Mottu, F. & Wuhrmann, J. J. (1974). Involvement of raffinose family of oligosaccharides in flatulence. In *Sugars in Nutrition*, ed. H. L. Sipple & K. McNutt. Academic Press, NY, USA, p. 314.

Cruz, R. & Park, Y. K. (1982). Production of fungal α-galactisidase and its applications to the hydrolysis of galacto-oligosaccharides in soybean milk. *J. Food Sci.*, **47**, 1973–5.

Cruz, R., Batistela, J. C. & Wosiacki, G. (1981). Microbial α-galactosidase for soymilk processing. *J. Food Sci.*, **46**, 1196–1200.

Harpaz, N., Harold, M., Flower, M. & Sharon, N. (1974). Purification of coffee bean α-galactosidase by affinity chromatography. *Biochim. Biophys. Acta*, **341**, 213–20.

Hellendorn, E. W. (1969). Intestinal effect following injection of beans. *Food Technol.*, **23**, 795–800.

Jha, Krishna (1981). Studies on α-galactosidase of intestinal bacteria in relation to flatulence. Ph.D. thesis. G. B. Pant University of Agriculture & Technology, Pantnagar, India.

Khare, S. K. & Gupta, M. N. (1988). Immobilization of *E. coli* β-galactosidase and its derivatives by polyacrylamide gel. *Biotechnol. Bioengng.*, **31**, 829–33.

Khare, S. K., Joshi, K. C., Gandhi, A. P. & Jha, Krishna (1992). Soybean: a nutritive and remedial diet. *FLCG Newsl.*, **20**, 11–14.

Nilsson, K., Birnbaum, S., Flygare, S., Linse, L., Schroder, U., Jeppson, U., Larsson, P.-O., Mosbach, K. & Brodelius, P. (1983). A general method for immobilization of cells with preserved viability. *Eur. J. Appl. Microbiol. Biotechnol.*, **17**, 319–26.

Park, Y. K., DeSanti, M. S. S. & Pastore, G. M. (1979). Production and characterization of α-galactosidase from *Aspergillus oryzae*. *J. Food Sci.*, **44**, 100–3.

Rackis, J. J., Sessa, D. J., Steggerda, F. R., Shimizu, J., Anderson, J. & Pearl, S. L. (1970). Soybean factors related to gas production by intestinal bacteria. *J. Food Sci.*, **35**, 634–9.

Reichelt, J. (1983). Toxicology. In *Industrial Enzymologyz* ed. T. Godfrey & J. Reichelt. Nature Press, NY, USA, p. 148.

Sugimoto, H. & Van Buren, J. P. (1970). Removal of oligosaccharide from soymilk by an enzyme from *Aspergillus saitoi*. *J. Food Sci.*, **35**, 656–60.

Sweeley, C. C., Bentley, R., Makita, M. & Well, W. W. (1963). Gas chromatography of TMS of sugars and related compounds. *J. Am. Chem. Soc.*, **85**, 2497–503.

Thananunkul, D., Tanaka, M., Chichester, C. O. & Lee, T.-C. (1976). Degradation of raffinose and stachyose in soybean milk by α-galactosidase from *Mortierella vinacea*. *J. Food Sci.*, **41**, 173–5.

Zabrosky, O. R. (1973). In *Immobilized Enzymes*. CRC Press, Cleveland, OH, USA, p. 83.