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Production of lactose-hydrolyzed milk using ethanol permeabilized yeast cells

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Abstract

To overcome the problem of enzyme extraction and poor permeability of cell membrane to lactose, experimentation was carried to permeabilize *Kluyveromyces marxianus* NCIM 3465 cells for their subsequent use for the production of lactose-hydrolyzed milk. Different process parameters, such as biomass load, temperature, agitation and treatment time, were optimized for maximum lactose hydrolysis in skim milk using these cells. The ethanol-permeabilized yeast cells gave 89% hydrolysis of milk lactose under optimized conditions.

Keywords: Yeast; Permeabilization; Skim milk; Lactose hydrolysis

1. Introduction

Lactose, the main carbohydrate present in milk, is a disaccharide with a low relative sweetness and solubility, which is not easily digested by a significant fraction of the global population. Furthermore, lactose is a hygroscopic sugar and has a strong tendency to absorb flavours and odours and causes many defects in refrigerated foods such as crystallization in dairy foods, development of sandy or gritty texture and deposit formation (Carrara & Rubiolo, 1994). Treatment of milk and milk products with β-D-galactosidase to reduce their lactose content is the appropriate method for increasing their potential uses and to deal with the problems of lactose insolubility and lack of sweetness (Mahoney, 1997). Moreover, this treatment can make milk, a most suitable food, available to a large number of adults and children that are intolerant to lactose.

β-D-Galactosidase can be obtained from a wide variety of sources, such as microorganisms, plants and animals, however, according to the source, their properties differ markedly. The use of microbial lactases offers several advantages over other sources. The most widely used microbial sources are *Kluyveromyces* sp. and *Aspergillus* sp. Fungal enzymes generally have acidic pH optima in the range of 2.5–5.4, whereas yeast lactases are most active at pH values of 6.0–7.0. Consequently, fungal enzymes are most effective in acidic products such as, whey, and the yeast enzymes are most useful in treating products near to a neutral pH such as milk (Finocchiaro, Olson, & Richardson, 1980; Joshi, Gowda, Katwa, & Bhat, 1989).

It has been established that the industrial application of β-D-galactosidase has been hampered by the difficulty and expense of releasing active enzyme in good yield from the cells, and further, the cost of the purification processes, especially in the case of bacteria and yeast. Thus, the use of whole cells as a source of β-D-galactosidase has been found as an interesting alternative, which has not been fully

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explored. However, a major drawback in the use of whole cells is the poor permeability of the cell membrane to lactose (Joshi, Gowda, Katwa, & Bhat, 1987). The use of permeabilization technology, however, can overcome this problem and be helpful in the development of a low-cost technology for lactose hydrolysis. The present work was therefore carried out to apply permeabilization technology for the production of lactose-hydrolyzed milk using yeast cells.

2. Materials and methods

2.1. Microorganism

Kluyveromyces marxianus NCIM 3465 was procured from the National collection of Industrial Microorganisms, National Chemical Laboratory, Pune (India).

2.2. Maintenance and cultivation of the culture

The culture was revived on maintenance medium containing (w/v) malt extract (0.3%), yeast extract (0.3%), peptone (0.5%) and glucose (1.0%). The culture was incubated at 30 °C for 48 h and maintained for fortnightly intervals on agar slants at 4 °C. The yeast was cultivated for the production of enzyme on the fermentation media composed of lactose (5%), peptone (0.5%), yeast extract (0.3%), ammonium sulphate (0.2%) and potassium dihydrogen orthophosphate (0.1%). The 50 ml fermentation media, contained in a 250 ml flask, were inoculated with 20 h old inoculum, incubated at 30 °C temperature for 24 h under shaking conditions (100 rpm).

2.3. Permeabilization of yeast cells

The permeabilization of yeast cells was carried out using ethanol (15 min treatment time), following the method of Joshi et al. (1989).

2.4. Production of lactose-hydrolyzed milk

The permeabilized yeast cells were used for the lactose hydrolysis in 10% (w/v) skim milk at shake flask level. The boiled milk samples (50 ml of skim milk in 250 ml capacity conical flasks) were, after cooling, inoculated with a known weight of permeabilized yeast cells. The flasks were incubated at 30 °C under shaking conditions (100 rpm) for 3 h (unless otherwise specified). The samples were taken at specific time intervals and analyzed for lactose content. All the experiments were performed in triplicate and the mean values are reported.

2.5. Optimization of process parameters

The various process parameters, such as biomass load, temperature, agitation and treatment time were optimized by varying the respective parameters.

2.6. Enzyme assay

The assay for measurement of enzyme activity was followed by the method of Miller (1972). One unit of enzyme activity is defined as one micromole (μmol) of 2-nitrophenol liberated per min under standard assay conditions. All the enzyme assays were performed in triplicate and the mean values are reported.

2.7. Lactose estimation

The lactose estimation was carried out by following the procedure of Nickerson, Vujicic, and Lin (1976). To the 5 ml of prepared milk sample (treatment with zinc acetate–phosphotungstic acid reagent and sodium hydroxide) were added 5 ml of glycine–NaOH buffer and 0.5 ml each of methylamine–HCl and sodium sulfite solution. The sample mixture was thoroughly mixed and kept at 65 °C in a water bath for 25 min. After cooling, the sample mixture, immediately, in an ice-water bath for 2 min, the absorbance of the sample was taken at 540 nm on a spectrophotometer.

3. Results and discussion

3.1. General

The effect of the following process parameters was monitored to optimize the lactose hydrolysis in skim milk during the course of the present investigation.

3.2. Permeabilization of yeast cells

To find out the effectiveness of ethanol as a permeabilizing agent, yeast cells were treated with different ethanol concentrations (20–70%, v/v). The results (Fig. 1) showed a progressive increase in the enzyme activity up to 50% (v/v); however, a decrease in enzyme activity was observed with further increase in the concentration. The ethanol concentration of 50% (v/v) displayed maximum enzyme activity (1.54 IU/mg DW). However, low enzyme activity was recorded with other ethanol concentrations used. It has been observed that permeabilization increases with the chemical concentration up to a critical value, where a maximum enzyme activity can be observed. At higher concentrations of the agent, the enzyme activity decreases, which may be attributed to the leakage of the enzyme from the cells or cell lysis. At low concentrations, the lower enzyme activity may be due to an insufficient amount of the agent for effective permeabilization.

Kluyveromyces cells are known to possess a lactose carrier protein (lactose permease) on their cell membrane that mediates the transport of lactose across the cell membrane (Dickson & Barr, 1983). Yet, availability of substrate seems to be the limiting factor in expressing the full enzymatic activity of whole cells. In permeabilization, the cell envelope is altered to allow small molecules, such as substrates, products, or coenzymes, to cross freely. The permeabilizing