Journal of Biotechnology 158 (2012) 137–143

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Immobilization of glucosyltransferase from *Erwinia* sp. using two different techniques

Fabiano Jares Contesini[a,*], Carolina Ibarguren[b], Carlos Raimundo Ferreira Grosso[c], Patrícia de Oliveira Carvalho[d], Hélia Harumi Sato[a]

[a] *Laboratory of Food Biochemistry, Department of Food Science, College of Food Engineering, State University of Campinas (UNICAMP), Monteiro Lobato Street, 80, Zip Code 13083-862, P.O. Box 6121, Campinas, SP, Brazil*
[b] *INIQUI-CONICET, Universidad Nacional de Salta, Av Bolivia, 5150, A4408FVY, Salta, Argentina*
[c] *Department of Food Planning and Nutrition, College of Food Engineering, State University of Campinas (UNICAMP), Monteiro Lobato Street, 80, Zip Code 13083-862, P.O. Box 6121, Campinas, Brazil*
[d] *Pharmacy course, University São Francisco, São Francisco de Assis Av, 218, Zip Code 12916-900, Bragança Paulista, SP, Brazil*

ARTICLE INFO

Article history:
Received 18 October 2011
Received in revised form 10 January 2012
Accepted 12 January 2012
Available online 24 January 2012

Keywords:
Glucosyltransferase
Isomaltulose
Trehalulose
Erwinia sp.
Immobilization

ABSTRACT

Two different techniques of glucosyltransferase immobilization were studied for the conversion of sucrose into isomaltulose. The optimum conditions for immobilization of *Erwinia* sp. glucosyltransferase onto Celite 545, determined using response surface methodology, was pH 4.0 and 170 U of glucosyltransferase/g of Celite 545. Using this conditions more than 60% conversion of sucrose into isomaltulose can be obtained. The immobilization of glucosyltransferase was also studied by its entrapment in microcapsules of low-methoxyl pectin and fat (butter and oleic acid). The non-lyophilized microcapsules of pectin, containing the enzyme and fat, showed higher glucosyltransferase activity, compared with lyophilized microcapsules containing enzyme plus fat, and also lyophilized microcapsules containing enzyme without fat addition. The non-lyophilized microcapsules of pectin containing the glucosyltransferase and fat, converted 30% of sucrose into isomaltulose in the first batch. However the conversion decreased to 5% at the 10th batch, indicating inactivation of the enzyme.

1. Introduction

Isomaltulose, also called Palatinose® and Lylose® is a reducing sugar and structural isomer of sucrose. This sugar is an interesting substitute for sucrose, in the food industry, considering that it is 50% as sweet as sucrose (Takazoe, 1989), non-carcinogenic (Sasaki et al., 1985) and has low glycemic index (Achten et al., 2007). The insulin release after isomaltulose ingestion is correspondingly reduced as compared to other sugars, creating the possibility of its application in diabetic and sports food and drinks. Considering that isomaltulose has no toxicity, this sugar is commercially used as a sucrose substitute in foods, soft drinks and medicines (Lina et al., 2002). Isomaltulose can be converted into sugar alcohol, Isomalt®, which has other useful properties for foods.

Isomaltulose is naturally present in very small quantities in honey, sugarcane extract and other sugar-rich fluids (Huang et al., 1998). A few bacterial species are known to have the ability to produce glucosyltransferase, which converts sucrose into isomaltulose. These microorganisms include *Erwinia* sp. D12 (Kawaguti et al., 2010), *Serratia plymuthica* (Kawaguti and Sato, 2010), *Klebsiella* sp. k18 (Orsi et al., 2009). For industrial purposes, the immobilization of the biocatalyst (enzyme and/or cells) offers several advantages, including reusability, easy product separation, enhancement of enzyme stability and continuous operation (Oliveira et al., 1997). Adsorption and entrapment are generally simple and cheap methods previously well-studied for other biocatalysts. Moreover, the adsorption technique using Celite as the support is reported as being very interesting for other enzymes such as lipases (Khare and Nakajimab, 2000; Silva et al., 2008).

There are several works in the literature reporting bacterial cell immobilization in isomaltulose production (Kawaguti et al., 2006; Oliva-Neto and Menão, 2009). However, few studies are focused on the immobilization of extracted glucosyltransferase, which converts sucrose into isomaltulose. The immobilization of the enzyme presents some advantages compared to cell immobilization, such as lower risk of microbial contamination of the product, the former prevents the risk of unwanted catalytic activity; whole cells bring along further resistance to mass transfer due to the presence of the cell wall, which drastically reduces reaction rates (Chen, 2007). Thus, this work aimed to immobilize the glucosyltransferase from *Erwinia* sp. D12, in two different supports by adsorption (Celite) and entrapment (low-methoxyl pectin

* Corresponding author. Tel.: +55 19 35212175; fax: +55 19 32892832.
E-mail address: fabiano.contesini@gmail.com (F.J. Contesini).

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2012.01.012

microcapsules), considering they are simple and cheap techniques. Afterward, the conversion of sucrose into isomaltulose catalyzed by the immobilized preparations was evaluated. This *Erwinia* sp. strain has been studied by our research group and presented interesting applications in the production of isomaltulose (Kawaguti et al., 2006, 2007; Kawaguti and Sato, 2010). Initially, response surface methodology (RSM) was used to evaluate the influence of pH value in the adsorption of glucosyltransferase onto Celite 545, the concentration of enzyme adsorbed onto the support and the addition of glutaraldehyde to retain the enzyme. RSM has never been used for the study of the immobilization of glucosyltransferase. This technique is used to identify the relationships between the variables and the response, generally resulting in the optimization of the process and simultaneously limiting the number of experiments. In the second part of this study, the glucosyltransferase was immobilized in microcapsules of low-methoxyl pectin with the addition of fat material (butter and oleic acid). The morphology of the microcapsules was examined using optical microscopy and scanning electron microscopy. The immobilized glucosyltransferase in low methoxyl pectin was used in different batches for the conversion of sucrose into isomaltulose. It must be highlighted that those two supports have never been studied for the immobilization of glucosyltransferase that converts sucrose into isomaltulose.

2. Material and methods

2.1. Microorganism

The *Erwinia* sp. sp D12 strain that produces glucosyltransferase, that converts sucrose into isomaltulose, was previously isolated and obtained from the Food Biochemistry Laboratory, College of Food Engineering – UNICAMP and maintained on nutrient agar, at 4 °C.

2.2. Glucosyltransferase production

For the preparation of the pre-inoculum, a 15 h culture of the *Erwinia* sp. strain was inoculated in 250 mL Erlenmeyer flasks containing 50 mL of the fermentation medium previously optimized by Kawaguti et al. (2007). The medium was composed of sugar cane molasses (Companhia Energética Santa Eliza, BR) (150 g/L) Milhocina® (corn steep liquor) (Corn Products do Brasil, BR) (20 g/L), Yeast Extract Prodex Lac SD® (Produtos Especiais para Alimentos S/A, BR) (15 g/L), and the pH adjusted to 7.5. The flasks were incubated at 200 rpm, 30 °C, for 15 h. A 300 mL aliquot of this pre-inoculum and 3 mL of Dow Corning® FG-10 antifoam (D'altomare Química, São Paulo, SP, BR) were aseptically added to a New Brunswick Bioflo IIc 6.6-L fermenter (New Brunswick Scientific, Edison, NJ, USA) containing 2700 mL of culture environment with the same composition. Agitation and aeration were kept at 200 rpm and 1 vvm, respectively. After 8 h of fermentation at 27 °C, cell mass was recovered by centrifugation at 9600 × *g* for 15 min at 5 °C and washed twice with distilled water. A cell suspension of 10% (m/v) containing wet cells, in distilled water, was prepared. Cells were disrupted by sonicator treatment (Labline Instruments, Inc., IL, USA), for 20 s at 180–200 W, and this step was repeated 15 times. The suspension was then centrifuged at 9600 × *g* for 15 min at 5 °C and the supernatant was used as crude glucosyltransferase. The glucosyltransferase activity was assayed by measuring the reducing sugars formed from the sucrose using the Somogyi method (1945) with glucose as the standard. A mixture of 450 μL of a 10% (m/v) sucrose solution in 0.05 M citrate-phosphate buffer, pH 6.3, and 50 μL of enzyme solution (23 U/mL) was incubated for 25 min at 33 °C. One unit of glucosyltransferase activity (U) was defined as the amount of enzyme that releases 1 μmol of reducing sugars from sucrose per minute under the above assay conditions.

2.3. Optimization of the immobilization conditions of glucosyltransferase onto Celite 545

For the immobilization of glucosyltransferase onto Celite 545 (Merck, Darmstadt, Germany with particle size of 0.02–0.1 mm), Erlenmeyers flasks (250 mL) containing 40 mL of 0.1 M buffer (citrate-phosphate pH 4.0–5.6, phosphate pH 6.5–8.0), 10 g of Celite 545 and different concentrations of the enzyme (14.1–246.2 U/g of Celite 545) were incubated for 12 h, 130 rpm, at 5 °C. The samples of immobilized glucosyltransferase onto Celite 545 were filtered using qualitative paper, washed with distilled water and transferred to Erlenmeyer flasks (250 mL) containing 40 mL of different concentrations of glutaraldehyde solutions. The flasks were incubated for 1 h, at 130 rpm, at 5 °C, and then the samples of immobilized enzyme were filtered using qualitative paper and washed with distilled water. The Erlenmeyers flasks containing 10 g of immobilized glucosyltransferase preparation and 50 mL of 10% (m/v) of sucrose solution in distilled water were incubated for 2.5 h, at 33 °C and 130 rpm. The sucrose solution was changed three times and the carbohydrates were analyzed as described in Section 2.5.

Two sequential central composite designs were employed to optimize the immobilization of glucosyltransferase onto Celite 545. The first design was a 2^3 five level central composition design (2^3-CCD) consisted of 2 axial points (+/−) at a distance of $\alpha = 1.68$, with four replicates at the center point. A total of 17 runs were performed in each experiment to study the independent variables. The independent variables were pH value for the adsorption of glucosyltransferase onto Celite 545, concentration of enzyme and glutaraldehyde, while the conversion of sucrose into isomaltulose was the dependent variable.

The second design (2^2-CCD) was carried out, fixing glutaraldehyde concentration for all the assays as 0.1%; pH value and enzyme concentration were varied at different levels. The immobilization process and the system reaction were carried out as described earlier and the conversion of sucrose into isomaltulose (dependent variable) was determined.

The variables were coded according to Eq. (1):

$$X_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

where X_i is the dimensionless coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable (X_i) at the center point and ΔX_i is the step change value. The variables and levels are shown in Tables 1 and 2. The system behavior was determined by a second-order polynomial equation, based on the equation below

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \quad (2)$$

where Y is the predicted value for the response, β_0 is the offset term, β_i is the linear effect coefficient, β_{ii} is the squared effect coefficient and β_{ij} is the interaction effect. $x_i x_j$ represents the interaction between different coded values, where i is one parameter and j is other.

The experimental results were considered only for the first batch and they were fitted to the second-order polynomial function. Student's *t*-tests were used to determine the statistical significance of the regression coefficients and analysis of variance (ANOVA) was performed to evaluate the statistical significance of the model. The response model was expressed in terms of coded variables, ignoring the statistically non-significant terms. Variables with a confidence level greater than 90% were considered to have a significant influence on the response (%). All data were treated with the aid of the Statistica® software 7.0 (Statsoft, Inc., Tulsa, USA).