Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 784–787
Food Chemistry
www.elsevier.com/locate/foodchem

Rapid communication

Hydrolysis of sucrose by invertase entrapped in polyvinyl alcohol hydrogel capsules

M. Rebroš *, M. Rosenberg, Z. Mlichová, Ľ. Krištofíková

Department of Biotechnology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 81237 Bratislava, Slovakia

Received 23 January 2006; received in revised form 11 April 2006; accepted 8 June 2006

Abstract

Invertase was immobilised into polyvinyl alcohol capsules (LentiKats®). The enzyme retained 86% of its original activity after the immobilisation process. Kinetic constants, pH and temperature profiles, and stability were determined, for both free and immobilised enzyme. Entrapped invertase was used for 45 repeated batch hydrolyses and no decrease in enzymatic activity was observed. The immobilised enzyme retained 78% of its initial activity after 8 months of storage.

Keywords: Invertase; Immobilisation; LentiKats®; Poly(vinylalcohol); PVA gel

1. Introduction

Invertase is an enzyme mainly used for the production of invert sugar in the food industry (Tanriseven & Dogan, 2001). Invert sugar mixture has the advantage of being colourless, in contrast to the coloured products obtained by acid hydrolysis (Monsan & Combes, 1984) and has a lower crystallinity than sucrose at the high concentrations employed (Işik, Alkan, Toppare, Cianga, & Yagci, 2003).

The immobilisation of invertase is often reported as a method for saving operational costs in invert sugar production (Tümtürk, Arslan, Disli, & Tufan, 2000). In addition, immobilisation increases the stability of the enzyme, by protecting the active material from deactivation, it enables repeated use, facilitates easy separation, and speeds up recovery of the enzyme (Akgöl, Kacar, Denizli, & Arica, 2001). One of the recently developed immobilisation techniques are lens-shaped capsules known as LentiKats®, developed by Ding and Vorlop (1995), made of PVA (polyvinyl alcohol) hydrogel. Compared to other immobilisation systems LentiKats® offers several advantages, such as low matrix cost, inexpensive and simple gel preparation, uncomplicated separation from the reaction mixture (diameter: 3–4 mm), low diffusion limits (thickness: 200–400 μm). In addition, this matrix has excellent mechanical stability and is virtually non-degradable (Ding & Vorlop, 1995; Rebroš, Rosenberg, Mlichová, Krištofíková, & Paluch, 2006).

In this study, invertase was immobilised into LentiKats®. The kinetic parameters, pH and temperature profiles of immobilised and free invertase were compared. The long-term stability of immobilised enzyme was tested in a repeated batch mode of hydrolysis.

2. Materials and methods

2.1. Materials

Invertase from bakers' yeasts (Sigma, St Louis, MO, USA) was used in the experiments. In this work, 1 unit was defined as the amount of enzyme able to form 1 g h^{-1} of glucose from sucrose in acetate buffer (pH 4.5, 10 mM) at 30 °C.

* Corresponding author. Tel/fax: +421 2 5296 7085.
E-mail address: martin.rebros@stuba.sk (M. Rebroš).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.06.020

2.2. Invertase immobilisation

Immobilised invertase was prepared on pilot-scale equipment of the MEGA company (MEGA a.s., Prague, Czech Republic), according to a patent (Ding & Vorlop, 1995). The enzyme (50 ml, 100 g/l) was mixed with 1 l of PVA gel (PVA 17–99), in liquid form (10% w/v), extruded through thin nozzles onto a hard surface and dried down in a laminar airflow cabinet to 30% of the initial mass. Solid gel particles (LentiKats®) were swollen in the stabilising solution according to the manufacturer. The immobilised invertase was stored at 4 °C, in acetate buffer (pH 4.5, 10 mM).

2.3. Determination of activity and kinetic parameters of invertase

Experiments were performed at the desired pH (3.2–6) and temperature (25–75 °C), in sucrose (10% w/v) dissolved in 50 ml of acetate buffer (10 mM), containing 2 g of immobilised or 0.1 ml of free invertase. The reaction mixture was agitated (200 rpm) with a magnetic stirrer. Samples (0.5 ml, taken 15 min after the initiation of the enzyme reaction) were boiled for 2 min to inactivate the enzyme. Activities were calculated from the amount of the glucose formed in the course of the enzyme hydrolysis (15 min). Sucrose, glucose, and fructose concentrations were determined by HPLC with an ion exchange column (Watrex 250 × 8 mm, Polymer IEX 8 μm H form; Watrex, Prague Czech Republic) and 9 mM H_2SO_4 as a mobile phase, at 25 °C and the flow rate 0.7 ml min^{-1}, with RI detector K-2301 (Knauer, Berlin Germany). The parameters K_M and V_m were determined in 50 ml of acetate buffer (pH 4.5, 10 mM). Sucrose (Mikrochem, Pezinok Slovakia) concentration varied between 50 and 300 mM. The kinetic constants were estimated from Lineweaver–Burk plots. Experiments were duplicated.

2.4. Repeated batch hydrolysis

Reactions were performed in a magnetically stirred reactor at 30 °C in 50 ml of 10 mM acetate buffer (pH 4.5) containing 10% (w/v) of sucrose. All reactions were initiated by the addition of 6 g of LentiKats® with immobilised invertase. The activity was determined as described above. After each cycle (100% hydrolysis), immobilisates were recovered by a simple filtration step, washed with sterile distilled water and used for the next batch run. Experiments were duplicated.

3. Results and discussions

3.1. Effect of pH on enzyme activity

The influence of pH on the initial activity of free and immobilised invertase was studied in the pH range 3.2–6.0 at 30 °C (Fig. 1). The optimum pH for sucrose hydrolysis

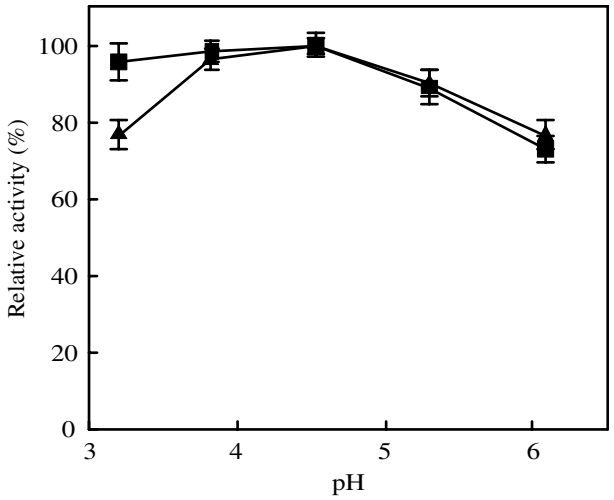

Fig. 1. pH profiles of free (▲) and immobilised (■) invertase. Enzymes were incubated in 10 mM acetate buffer at various pH values (3.2–6) at 30 °C.

was determined to be 4.5 for both free and immobilised enzyme. It is significant that the immobilisation of the enzyme broadens the pH optimum. At pH 3.2 the immobilised enzyme retained 95% of its maximum activity, whereas the activity of the free enzyme was only 76%. The broadening of the invertase pH optimum was also observed after its entrapment (Emregul, Sungur, & Akbulut, 2006; Ohmori & Kurokawa, 1994; Tanriseven & Dogan, 2001) or covalent immobilisation (Bayramoğlu, Akgol, Bulut, Denizli, & Yakup Arica, 2003; Chen, Kang, Neoh, & Tan, 2000). However, this effect was not observed when the invertase was adsorbed on poly(acrylamide/maleic acid) hydrogel (Arslan, Tumturk, Caykara, Sen, & Guven, 2000) or magnetic polyvinyl alcohol microspheres (Akgöl et al., 2001). This effect is the consequence of secondary interactions between the enzyme and polymer matrix during the immobilisation process. The broadened pH range can be an advantage in the application of immobilised enzyme at lower pH, which can eliminate the possibility of microbial contamination during long-term operation.

3.2. Effect of temperature on enzyme activity

The maximum activity of free enzyme was observed at 55 °C (Fig. 2). The activity of the immobilised invertase was investigated in the range between 25 and 55 °C at pH 4.5. The activity of the immobilised enzyme also reached its maximum at 55 °C. However, at temperatures higher than 55 °C the LentiKats® were found to be mechanically unstable and started to melt. Because of this 55 °C was estimated as the maximum temperature for sucrose hydrolysis with invertase immobilised in LentiKats®. This temperature seems to be the maximum for the application of this type of capsule, since 55 °C was also the maximum temperature for hydrolysis by glucoamylase immobilised in LentiKats® (Rebroš et al., 2006).

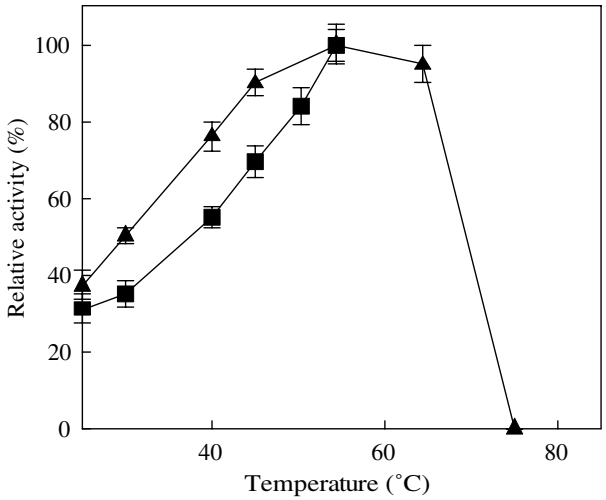

Fig. 2. Temperature profiles of free (▲) and immobilised (■) invertase. Enzymes were incubated in 10 mM acetate buffer (pH 4.5) at various temperatures (25–75 °C).

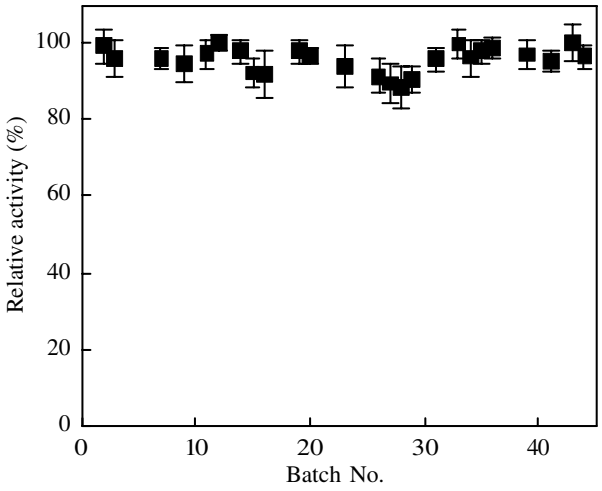

Fig. 3. Repeated batch hydrolysis with invertase immobilised in LentiKats®. Reactions were performed at 30 °C in 10 mM acetate buffer (pH 4.5) containing 10% (w/v) of sucrose.

3.3. Kinetic constants

The kinetic constants were estimated at pH 4.5 and 30 °C. The K_M for immobilised enzyme was 3.6-fold higher than that of the free enzyme (Table 1). The increase in K_M value can be a consequence of either structural changes of the enzyme, introduced by the immobilisation procedure, or lower accessibility of the substrate to the active site of the immobilised enzyme (Arslan et al., 2000; Emregul et al., 2006; Selampinar, Akbulut, Ozden, & Toppare, 1997). The V_m value of enzyme increased after immobilisation, by about 17% compared to that of the free invertase. The increase in V_m was also observed after invertase immobilisation on nylon-6 microbeads (Amaya-Delgado, Hidalgo-Lara, & Montes-Horcasitas, 2006) and after the adsorption of invertase on poly(acrylamide/maleic acid) hydrogels (Arslan et al., 2000).

3.4. Operational stability

Operational stability is the main parameter which determines the possible application of immobilised enzyme in large scale processes. This parameter was studied in repeated batch hydrolysis. Each batch cycle was performed until sucrose was completely converted into glucose and fructose. The operating temperature was set at 30 °C because it was observed that the stability of invertase decreases with increasing temperature (Akgöl et al., 2001). No decrease of the initial activity was observed during 45 repeated batch runs (Fig. 3), which confirmed that invertase immobilised in LentiKats® is very stable, without any treatment of the enzyme before immobilisation. The advantage of this method, compared to the entrapment of invertase into calcium alginate gel, is that there is no need for enzyme cross-linking before immobilisation into LentiKats® (Tanaka, Kurosawa, Kokufuta, & Veliky, 1984; Tanriseven & Dogan, 2001). The entrapment of this enzyme into the structure of PVA gel seems to be more effective than covalent bonding onto this matrix. Invertase immobilised onto magnetic polyvinyl alcohol microspheres lost 17% of its initial activity at 30 °C, after 40 h of operation in a packed-bed reactor (Akgöl et al., 2001). Chitosan-modified invertase immobilised on alginate-coated chitin support lost 20% of its initial activity after 50 h of continuous operation at 30 °C (Gómez, Ramirez, Villalonga, Hernandez, & Villalonga, 2006). Also a fibre-entrapped invertase, using a cellulose acetate-titanium–isopropoxide composite as gel matrix, lost almost 50% of its initial activity after 10 runs (Ohmori & Kurokawa, 1994). Compared to these results, invertase immobilised in LentiKats® seems to be a very stable immobilised system. Another advantage of this immobilised system is that its production is available at pilot–plant scale (www.mega.cz).

Table 1
The K_M and V_m values of free and immobilised invertase

	K_M (mM)	V_m (U mg^{-1})	Recovered activity (%)
Free	44.8	0.41	100
Immobilised	160.5	0.48	86

3.5. Storage stability

Enzymes are generally not stable during storage in solution and their activities decrease gradually over time. Immobilised enzymes are more stable than free enzymes (Amaya-Delgado et al., 2006; Tümtürk et al., 2000). The immobilised invertase was stored in acetate buffer (10 mM, pH 4.5) at 4 °C. After 8 months the enzyme retained 78% of its initial activity. Compared to covalent binding onto magnetic polyvinyl alcohol microspheres, entrapment seems to make the enzyme more stable, since covalently bound enzyme retained only 25% of its initial activity after 50 days of storage (Akgöl et al., 2001).

4. Conclusions

Invertase was successfully immobilised into polyvinylalcohol gel in the form of LentiKats®. The immobilisation significantly broadened the pH range of the enzyme, which allows the application of immobilised enzyme at lower pH values. The activity of free and immobilised enzyme reached its maximum at 55 °C. However, this temperature seems to be critical for application of these capsules, because LentiKats® lost their mechanical strength at these conditions. The immobilised enzyme was employed in 45 repeated batch hydrolyses. No decrease in enzymatic activity was observed, suggesting the potential application of LentiKats® in large scale sucrose hydrolysis. In addition, enzyme immobilised in LentiKats® showed excellent storage stability, since it retained 78% of its initial activity after 8 months of storage.

Acknowledgements

This work was supported by company MEGA a.s (www.mega.cz) and by VEGA (The Grant Agency of Ministry of the Education of the Slovak Republic) 1/2391/05 and 1/2390/05. The authors thank Dr. Juraj Švitel and Dr. Dušan Slugeň for critically reading this manuscript.

References

Akgöl, S., Kacar, Y., Denizli, A., & Arica, M. Y. (2001). Hydrolysis of sucrose by invertase immobilized onto novel magnetic polyvinylalcohol microspheres. *Food Chemistry, 74*, 281–288.

Amaya-Delgado, L., Hidalgo-Lara, M. E., & Montes-Horcasitas, M. C. (2006). Hydrolysis of sucrose by invertase immobilized on nylon-6 microbeads. *Food Chemistry, 99*, 299–304.

Arslan, F., Tumturk, H., Caykara, T., Sen, M., & Guven, O. (2000). The effect of gel composition on the adsorption of invertase on poly(acrylamide/maleic acid) hydrogels. *Food Chemistry, 70*, 33–38.

Bayramoğlu, G., Akgol, S., Bulut, A., Denizli, A., & Yakup Arica, M. (2003). Covalent immobilisation of invertase onto a reactive film composed of 2-hydroxyethyl methacrylate and glycidyl methacrylate: properties and application in a continuous flow system. *Biochemical Engineering Journal, 14*, 117–126.

Chen, Y., Kang, E. T., Neoh, K. G., & Tan, K. L. (2000). Covalent immobilization of invertase onto the surface-modified polyaniline from graft copolymerization with acrylic acid. *European Polymer Journal, 36*, 2095–2103.

Ding, W. A., & Vorlop, K. D. (1995). Gel aus Polyvinylalcohol und Verfahren zu seiner Herstellung. *Patent DE*, 4327923.

Emregul, E., Sungur, S., & Akbulut, U. (2006). Polyacrylamide-gelatine carrier system used for invertase immobilisation. *Food Chemistry, 97*, 591–597.

Gómez, L., Ramirez, H. L., Villalonga, M. L., Hernandez, J., & Villalonga, R. (2006). Immobilization of chitosan-modified invertase on alginate-coated chitin support via polyelectrolyte complex formation. *Enzyme and Microbial Technology, 38*, 22–27.

Işik, S., Alkan, S., Toppare, L., Cianga, I., & Yagci, Y. (2003). Immobilization of invertase and glucose oxidase in poly 2-methylbutyl-2-(3-thienyl) acetate/polypyrrole matrices. *European Polymer Journal, 39*, 2375–2381.

Monsan, P., & Combes, D. (1984). Application of immobilized invertase to continuous hydrolysis of concentrated sucrose solutions. *Biotechnology and Bioengineering, 26*, 347–351.

Ohmori, Y., & Kurokawa, Y. (1994). Preparation of fibre-entrapped enzyme using cellulose acetate-titanium–iso-propoxide composite as gel matrix. *Journal of Biotechnology, 33*, 205–209.

Rebroš, M., Rosenberg, M., Mlichová, Z., Krištofíková, L'., & Paluch, M. (2006). A simple entrapment of glucoamylase into LentiKats® as an efficient catalyst for maltodextrin hydrolysis. *Enzyme and Microbial Technology, 39*, 800–804.

Selampinar, F., Akbulut, U., Ozden, M. Y., & Toppare, L. (1997). Immobilization of invertase in conducting polymer matrices. *Biomaterials, 18*, 1163–1168.

Tanaka, H., Kurosawa, H., Kokufuta, E., & Veliky, I. A. (1984). Preparation of immobilized glucomylase using ca-alginate gel coated with partially quaterized poly(ethleneimine). *Biotechnology and Bioengineering, 26*, 1393–1394.

Tanriseven, A., & Dogan, S. (2001). Immobilization of invertase within calcium alginate gel capsules. *Process Biochemistry, 36*, 1081–1083.

Tümtürk, H., Arslan, F., Disli, A., & Tufan, Y. (2000). Immobilization of invertase attached to a granular dimer acid-co-alkyl polyamine. *Food Chemistry, 69*, 5–9.