

Research Article

Purification, Immobilization, Stabilization and Characterization of Commercial Extract with β -galactosidase Activity

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Abstract

From a commercial enzyme extract juice with purpose to stabilize it in the industry, has been able to extract an extract rich in beta-galactosidase activity. It has been designed to extract and purification protocol based on polyethyleneimine SUPPORTS agarose activated with next purification yields 80%. Subsequently it has been immobilized in different SUPPORTS, with yields of 95% in mobilization. To estabilizar dimensional structure of the enzyme in the supports by multipoint covalent immobilization, are subsequently incubated at pH 10. Both groups activated SUPPORTS glyoxyl, has remained almost unchanged initial enzyme activity, whereas in the derivative BrCN activity was not observed after this process. Once completely inactivated derivatives, these have been thoroughly washed with water, and then placed in reaction initial conditions (pH, ionic strength) and all derivatives, have managed to recover more than 80% of the initial activity, and reused again for these new cycles reaction.

Introduction

The enzyme β -galactosidase is widely distributed in nature in different organisms like animals or plants [1]. In nature, these enzymes mainly catalyze the hydrolysis of lactose to give their corresponding monosaccharides (glucose and galactose) through the recognition of galactose. But also it has been seen that also hydrolyze other substrates that are in its structure galactose residues coupled by a link β 1-4 to other compounds or even some of them can hydrolyze or synthesize other covalent bonds as the β 1-6 or β 1-2, more rarely [1]. Thus, these enzymes can also transfer galactosyl residues to other compounds with hydroxyl, sulfoxy or amine groups [2].

The main application of this enzyme is the hydrolysis of lactose in milk and several dairy products. In addition most of them are able to catalyse the production of some galactosyl-oligosaccharides. In these synthetic reactions, the use of lactases may be improved adding organic solvents or co-solvents to the reaction medium, or using moderately high temperatures. Normally, these conditions increase

the solubility of substrates or products [3,4], or shift thermodynamic equilibrium [5-7], improve enzyme properties [8,9], or prevent microbial contamination. However, enzymes may be readily inactivated in the presence of high concentrations of co-solvents or high temperature by different mechanisms [10-12].

The use of immobilized enzymes on solid supports (e.g., glyoxyl-agarose), may prevent some of these inactivation causes [13]. Immobilized enzymes cannot aggregate or suffer any other kind of intermolecular interaction. If the enzyme molecules are bonded to the supports by multipoint covalent attachment, these immobilized preparations are more resistant to any kind of distortion such this promoted by the action of organic solvents or heating [14]. However, even highly stabilized enzyme preparations become inactivated after incubation in the presence of organic solvents or high temperatures for long periods of time, reducing the operational life of the biocatalyst. In these cases it would be interesting if the recovery of the catalytic activity could be performed. In this way the situation will be different depending of the inactivation cause. At pH 7 and low temperatures, for instance, the primary structure of the enzyme hardly would be chemically modified. Thus, the main cause for inactivation of immobilized monomeric enzymes should be the distortion of their three-dimensional structure that can drive to an incorrect and inactive conformation of the enzyme [15,16]. In this way, if this incorrect conformation may be reversed toward the active form, the recovery of the enzyme activity may be achieved. When the enzyme inactivation is produced by its exposure to the high temperature, beside to the promotion of incorrect enzyme conformations, it is also possible that some chemical modifications of the enzyme may occur [12,13,17-19]. These chemical modifications may produce some difficulties or even the impossibility to recover the enzyme activity. In these processes the derivatives prepared through multipoint covalent attachments may play a double role; it may oppose more difficulties to the movements of the polypeptide chains because it keeps unaltered the points through the immobilization is produced, and these enzyme-support bonds may behave as reference points allowing the easier recovery of the catalytic activity.

In this paper, the study of the recovered activity, final stability of different immobilized preparations of Pectinex SP Ultra β -galactosidase immobilized on glyoxyl supports is studied. Finally the possibility of reactivation of the different derivatives will also be studied. For this proposal inactivation in aqueous medium at high temperature will be compared with the inactivation at lower temperatures and in the presence of high concentration of organic solvents. The effect of the previous incubation in saturated guanidine hydrochloride of the enzyme to destroy any incorrect but stable β -galactosidase structure will also be studied.

Materials and Methods

β -galactosidase from commercial enzyme preparation Pectinex Ultra SP-L produced by *Aspergillus aculeantus* (Novo Nordisk Ferment AG, Denmark, [20]). *o*-nitrophenyl- β -D-galactopyranoside, (*o*-NPG), ethylenediamine (EDA), sodium borohydride, 1,4-butanediol diglycidyl ether, glycidol, and DTT (dithiothreitol), were from Sigma (St. Louis, MO, USA). Coomassie (Bradford)

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protein assay kit was purchased from Pierce (USA). Reagents for electrophoresis were purchased from Sigma Chemical Co. (St. Louis, USA). Sodium periodate was from Merck, (Darmstadt, Germany) Cyanogen bromide (CNBr-activated Sepharose 4BCL), Carboxy-Methyl Sepharose (CM-Sepharose Fast Flow) and the rest of sepharose supports were purchased from GE Healthcare (Biosciences AB, Uppsala-Sweden). X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) was from Roche Diagnostic (Mannheim, Germany). Coomassie (Bradford) protein assay kit was purchased from Pierce (USA) Reagents for electrophoresis were purchased from Sigma Chemical Co. (St. Louis, USA) and all other reagents were of analytical grade.

Standard enzymatic activity assay

β -galactosidase activity: β -galactosidase activity was determined spectrophotometrically using *o*-NPG as substrate. The reaction medium contained 13.3 mM *o*-NPG dissolved in 50 mM sodium phosphate buffer of pH 7.0. To initialize the reaction, a sample of enzymatic solution or derivative suspension (2-3 U/mL) was added to a cuvette containing 2 mL of substrate solution. The activity was determined by recording the increment of absorbance at 405 nm due to the release of *o*-nitrophenol (molar extinction coefficient of *o*-nitrophenol ($3100 \text{ M}^{-1} \text{ cm}^{-1}$). One β -galactosidase unit was defined as the amount of enzyme which liberates 1 μmol of *o*-NP per min under the described conditions. All the assays were performed at 25°C in thermostated spectrophotometric cells using magnetic stirrers. The gels in suspension did not interfere with spectrophotometric measurements.

Determination of protein content

Protein concentration was determined according to the procedure of Bradford [21] using bovine serum albumin as protein standard.

Preparation of different glyoxyl supports

Glyoxyl-Agarose supports were prepared following the methodology described by Guisán [22].

Glyoxyl-amine-epoxy supports: First the sepharose supports were activated with epoxy groups. For this proposal a solution composed by 440 mL of water, 160 mL of acetone, 32.8 g of NaOH and 2 g of NaBH_4 and 110 mL of epichlorohydrine (temperature was not exceed to 25°C) was mixed with 100 g of sepharose 4BCL. The suspension was left under mechanical and gently stirring in an open beaker during 16 hours. After this time, the support was washed with an excess of water and then dried under vacuum. Then support was oxidised using 35 mL of a solution 100 mM of sodium metaperiodate dissolved in 1375 mL of water. The suspension was mildly stirred during 90 minutes. After this time the supports were washed with water to remove the excess of oxidant. Finally, the oxidized support was treated with 1 L of a solution 2% of ethylenediamine at pH 7.5 during 1 hour. Then the support was washed with an excess of water. When specified, supports were used after being reduced with a solution 0.1 M sodium bicarbonate and 1 mg/mL of sodium borohydride at pH 10 during 30 minutes; or treated with a solution 5% of mercaptoethanol at pH 8.7 overnight. In all cases the relation (V_s/V_T) was (1/10).

Purification of the enzyme

The commercial enzyme preparation Pectinex Ultra SP-L was purified prior to use. The used strategy was the ionic adsorption

on an anionic exchange support. Firstly the commercial enzyme preparation was dialyzed against distilled water using a membrane with a size-cut of 12÷14 kDa (three changes with a ratio 1/50 v/v) at room temperature. After the dialysis, the enzyme was adsorbed on PEI-agarose 4 BCL under continuous stirring (1 g support/10 mL of enzyme solution). Periodically, samples of the suspensions and supernatant were taken and their activity was measured by using the *o*-NPG assay. After enzyme adsorption, β -galactosidase immobilized preparation was filtered under vacuum using a sintered glass funnel and washed with 5 mM sodium phosphate buffer pH 7.0, containing 50 mM NaCl. β -galactosidase was desorbed from PEI-agarose by suspending the immobilized enzyme (in a relation 1/10 w/v) in 5 mM sodium phosphate buffer pH 7.0 containing 200 mM NaCl during one hour at room temperature. Periodically, the activity of suspensions and supernatants was measured by using the *o*-NPG assay described above. The suspension was vacuum filtered in order to separate the supernatant solution, containing the β -galactosidase, from support. Finally the solution was dialyzed against 5 mM sodium phosphate buffer pH 7.0 (three changes with a ratio 1/50 v/v) at room temperature. The purified and dialyzed solution was stored at 4°C.

Preparation of pectinex β -galactosidase derivatives

Enzyme solution at the indicated pH and conditions was mixed with the specified amount of different supports. At different times, samples of the supernatant, the support-enzyme suspension, and an enzyme solution incubated in the presence of the inert support were withdrawn and the activity was assayed. All the experiments were performed using less than 4 U/mL of support in order to avoid diffusional problems that could alter the apparent enzyme stability. Typically, in a standard experiment a relation of 1:10 (g of support/mL of solution) was used. All the immobilization processes were performed at room temperature, unless where otherwise stated.

Diferent glyoxyl derivatives

Immobilization on glyoxyl-agarose at pH 10.0: Purified solution of β -galactosidase was diluted 1:1 with 100 mM sodium carbonate buffer pH 10.0 containing 200 mM D-galactose and 50% v/v of glycerine. The resulting solution was offered to glyoxyl-agarose 4 BCL. The pH was adjusted to 10.0. During the immobilization the suspension was maintained under continuous gentle stirring at room temperature. Finally, the formed Schiff's bases were reduced using 1 mg/mL sodium borohydride.

Immobilization on glyoxyl-agarose at pH 8.5 (DTT): Purified solution of β -galactosidase was diluted 1:1 with 100 mM sodium carbonate buffer pH 8.5 containing 100 mM DTT, 200 mM D-galactose and 50% v/v of glycerine. The resulting solution was offered to glyoxyl-agarose 4 BCL. After enzyme immobilization, the derivative was filtered under vacuum on a sintered glass funnel and incubated in 100 mM sodium bicarbonate buffer pH 10.0 containing 200 mM D-galactose and 50% v/v of glycerine, in order to get an intense multipoint covalent attachment between the enzyme and the support. Finally, the Schiff's bases were reduced using 1 mg/mL of sodium borohydride.

CNBR-agarose-derivative

2 g of washed CNBr-activated Sepharose 4B was prepared as instructed by the supplier (Pharmacia-Sweden), and it was incubated with 5 mL of 5 mM of sodium phosphate buffer at pH 7 and 10 mL of β -galactosidase solution (0.2 mg/mL), for 1 hour at 4°C temperature

under gentle stirring. After that, the immobilized preparation was filtered and washed with 0.1 M sodium bicarbonate buffer supplemented with 0.5 M NaCl at pH 8.4. Afterwards, the remaining reactive groups were blocked with 1 M of ethanolamine, and pH 8, (4 ml/g of support) at room temperature for 2 hours under gentle stirring. Finally, the derivative was washed alternatively with 0.1 L of sodium acetate buffer 0.1 M at pH 4.0 supplemented with 0.5 M NaCl, and 0.1 L TRIS-HCl buffer 0.1 M, pH 8 supplemented with 0.5 M NaCl.

Thermal inactivation of the soluble enzyme and different enzymes preparations

The different enzyme preparations (soluble or immobilized enzyme) were incubated at the indicated conditions. Samples were withdrawn periodically using a pipette with a cut-tip and under vigorous stirring in order to have a homogeneous biocatalyst suspension. The activity was measured using the *o*-NPG assay described above. The experiments were carried out by triplicate and the standard error was under 5%. In each case, the initial activity was considered to be the 100% value.

About 3 U/mL of soluble β -galactosidase enzyme were incubated in 5 mM sodium phosphate buffer at pH 7 and 50-60°C. Samples were periodically withdrawn using a pipette with a cut-tip and under vigorous stirring to have a homogenous biocatalyst suspension, and their remaining activities were determined as described above.

Inactivation of different β -galactosidase immobilized preparations in the presence of organic co-solvent

The different enzyme immobilized preparations were incubated in 25 mM Tris-HCl buffer pH 7.0 containing 70% v/v of 1,4-dioxane at room temperature. Samples were withdrawn periodically using a pipette with a cut-tip and under vigorous stirring in order to have a homogeneous biocatalyst suspension. The activity was measured using the *o*-NPG assay described above. The experiments were carried out by triplicate and the standard error was under 5%. In each case, the initial activity was considered to be the 100% value.

Incubation of pectinex β -galactosidase on chaotropic agent solutions

Firstly, the different β -galactosidase immobilized preparations were suspended in 5 mM sodium phosphate buffer pH 7.0 and the initial activity was measured. Then, they were vacuum filtered and incubated in 25 mM sodium phosphate buffer solution containing 8 M of guanidine at pH 7.0 and room temperature. The addition of guanidine led to the complete inactivation of all derivatives. Thus, they were vacuum filtered, abundantly washed with distilled water in order to remove guanidine and finally re-suspended in 5 mM sodium phosphate buffer pH 7.0 at room temperature. The activity was determined along the time using the *o*-NPG assay described above. When a constant value of residual activity was achieved, this was considered the maximum recovered activity. Several consecutive cycles of inactivation/reactivation of immobilized β -galactosidase were performed. In each case, the initial activity was considered to be the 100% value.

Reactivation experiments

The different enzyme immobilized preparations were inactivated by incubating in 5 mM sodium carbonate buffer pH 9.0 at 50°C. Samples were withdrawn periodically using a pipette with a cut-

tip and under vigorous stirring in order to have a homogeneous biocatalyst suspension. The activity was measured using the *o*-NPG assay described above. When the residual activity was under the 50%, the corresponding derivative was vacuum filtered and re-suspended in 5 mM sodium phosphate buffer pH 7.0 at room temperature. The reactivation process was followed along the time determining the enzymatic activity using the *o*-NPG assay described above. The experiments were carried out by triplicate and the standard error was under 5%. In each case, the initial activity was considered to be the 100% value.

Sds-page of the free and immobilized enzyme

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed according to Laemmli [23] in a separation zone of 9 cm \times 6 cm and a concentration zone of 5% polyacrylamide. Samples were analyzed in 12% polyacrylamide PAGE gels and stained using Coomassie blue. Low molecular weight markers from GE Healthcare were used (14-94 kDa).

Results and Discussion

Purification of β -galactosidase from Pectinex Ultra SP-L extract

Purification of the β -galactosidase from the commercial extract is shown in table 1. This purification was performed in only one step using a polyanionic exchanger as the agarose activated with polyethyleneimine. The lactase activity was adsorbed on this support. Finally, the enzyme was desorbed from this support after incubation with 150 mM of NaCl. After this process the lactase activity was almost 85% and a purification factor of 8.5 was obtained. The enzyme was characterized using electrophoresis techniques. As it can be seen in figure 1A and 1B in lane 3 there is only a single band corresponding to around 43 KD. This enzyme was confirmed to be the lactase when electrophoresis was performed in native conditions and stained with activity test. This result also confirms that the enzyme is a monomer in the active form.

Table 1: Purification of β -galactosidase contained in the commercial preparation Pectinex Ultra SP-L.

Step	Activity (U/ml)	Protien (mg/ml)	Recovery (%)	Sp.Activ. (U/mg)	Purification factor
Crude Extract	1.7	5	100	0.34	1
PEI-Ag Desorbed	1.44	0.5	84.7	2.88	8.5

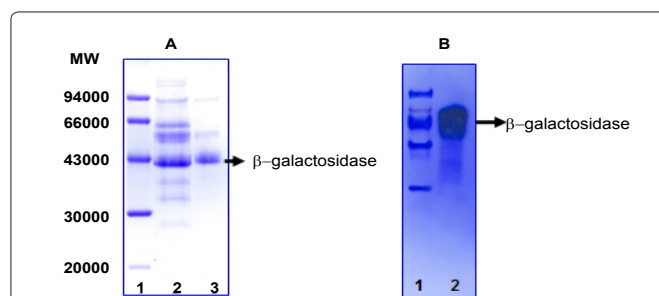


Figure 1: Analysis by electrophoresis of the purification of β -galactosidase from Pectinex Ultra SP. (A): SDS-PAGE studies: Lane 1: Low molecular weight marker, Lane 2: Crude extract from PECTINEX ULTRA; Lane 3: Purified enzyme by adsorption/desorption on polyethyleneimine support. (B): Non denaturing electrophoresis of the enzyme. Lane 1: Molecular weight markers; Lane 2: Purified enzyme stained with X-Gal and Coomassie brilliant blue R-250.

Study of the stability of soluble pectinex β -galactosidase at alkaline pH

The immobilization of enzymes on glyoxyl supports generally is able to promote a high stabilization of the enzymes [13]. The immobilization of enzymes on this support is done by a multipoint mechanism being necessary the immobilization through more than one point simultaneously [13]. This generally makes the enzyme immobilization on this type of support do not occur at neutral pH because at this pH only would be reactive the amine groups with the lower pK that normally are the terminal ones, while the rest of lysines are protonated and therefore not reactive. The only exception to this occurs in the case of multimeric enzymes with several amine terminals in the same plane of the surface and thus able to interact simultaneously with several glyoxyl groups on the support. In addition recently it has been developed a new strategy for immobilizing on glyoxyl supports at neutral pH consisting in stabilizing the Schiff bases formed using thiolated compounds [24]. Due to this normal mechanism the enzyme immobilization on glyoxyl supports should be performed at alkaline pH in which lysines are mostly deprotonated, and therefore reactive, so it is of great interest to study the stability of the enzyme under these relatively drastic conditions [13].

When the enzyme was incubated at pH 10 immediately lost the catalytic activity being impossible their measure. So it was studied the effect of the addition of various additives as sugars such as glycerol or galactose as a competitive inhibitor which is generally described for other lactases to have a large protective effect. Besides this, the rest of the experiments were always at 4°C to minimize inactivation by the temperature. In [figure 2A](#) it can be seen the stability obtained

during the incubation at alkaline pH of the enzyme after the addition of the different protective agents. The stability was improved when glycerol and galactose were added resulting in a similar stabilization. Interestingly, the effect of both preservatives was additive resulting in a higher stabilization respect of the separate stabilizing agents. In the same figure, it can be seen as the maximum stabilization was achieved when the enzyme was incubated in the presence of galactose and with 25% glycerol. Moreover, it was optimized the concentration of glycerol in the presence of a fixed concentration of galactose. The concentration of glycerol in which maximum stability was obtained was 25% decreasing the stability when glycerol concentration was increased ([Figure 2B](#)). These are the optimal conditions required for working at alkaline pH during the immobilization processes.

Immobilization of purified β -galactosidase from pectinex Ultra SP-L, on different supports

Purified β -galactosidase could be readily covalently immobilized on BrCN and glyoxyl-agarose supports in different conditions (glyoxyl at pH10 and glyoxyl+DTT at pH 7) In all cases the activity retained just after immobilization was almost unaltered ([Table 2](#)).

The enzyme was immobilized on commercial supports as CNBr sepharose and on glyoxyl agarose supports under two different conditions and thus through different mechanisms.

First, the immobilization was performed at pH 10 where the enzyme is immobilized through the richest place in lysines. Subsequently the immobilization was done at neutral pH that although is described that the immobilization of monomeric enzymes are not produced it was also described when adding some thiolated compounds such as mercaptoethanol or DTT this support is capable to immobilize monomeric proteins in these conditions [24,25]. The use of DTT allowed the immobilization of almost all the offered enzyme to the supports ([Table 2](#)). Subsequently the different derivatives previously immobilized on glyoxyl supports were incubated at alkaline pH to facilitate the interaction between the lysines of the protein surface and the aldehyde groups on the support. However, usually this increase of the multiinteraction comes with a decrease of the catalytic activity and this is also very often correlated with an increase of the stability of the obtained preparations [13,22]. The final activity of the derivatives after incubation at alkaline pH was around 50-60% ([Table 2](#)).

Stability of the different β -galactosidase preparations against the pH

Previous work by other authors have reported that the optimal pH of the enzyme is about 5.4 and the optimal temperature is around 55-60°C [20]. Although this optimal pH would make to the enzyme a good candidate for hydrolysis-synthesis in acid media such as in the case of hydrolysis of lactose in acid whey, it would also be very

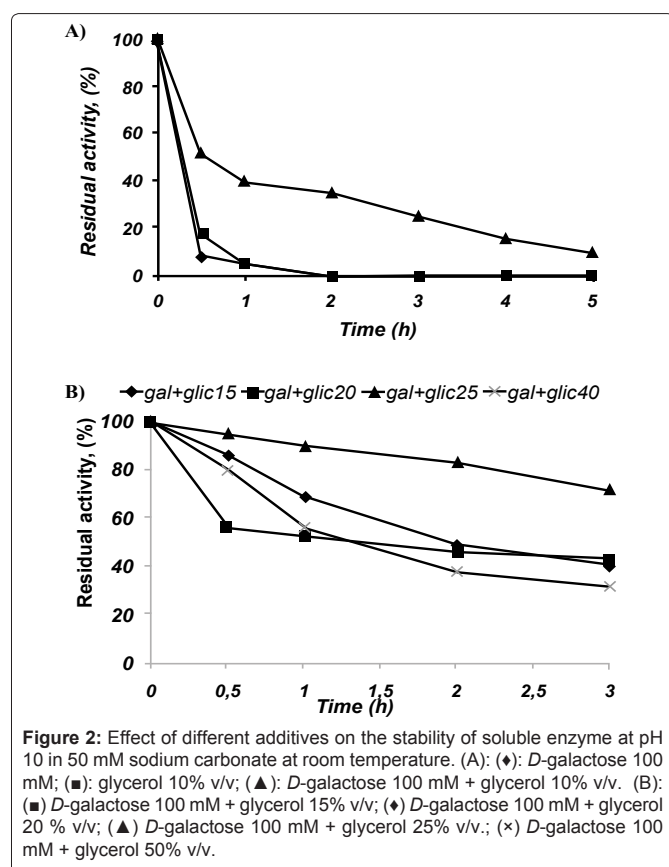


Table 2: Immobilization yield and expressed activity of the different immobilized preparations of β -galactosidase from Pectinex ULTRA SP-L. Yield is defined as the percentage of immobilized enzyme. Expressed activity is defined as the ratio between the observed activity and the expected activity.

Support	Yield (%)	Expressed Activity (%)	Expressed activity after incubation at pH 10 (%)
BrCN-activated sepharose	30	95	Nd
Glyoxyl-agarose pH 10.0	>95	95	53
Glyoxyl-agarose+ DTT	>95	93	61

interesting to study the option of using catalysts of this enzyme in other conditions as neutral pH (very useful for the hydrolysis of lactose in milk). The stability of different enzyme preparations previously described (Figure 3 A and 3B) was studied and as it can be observed the derivative performed in the presence of DTT was rather more stable than the derivative CNBr that has similar characteristics to those of soluble enzyme. The resulting most stable in all checked conditions was that immobilized through the area richest in lysines. This is logical considering that in principle the most favourable area for multipoint covalent immobilization processes is the area that has more lysines on its surface.

Effect of the incubation at alkaline pH and reactivation of different enzyme preparations

It is in many cases of great importance the use of alkaline pH that in some cases may favour some processes especially in the synthesis of oligosaccharides from lactose. Thus the different preparations were incubated at pH 9 and 50°C obtaining the rapid inactivation of all derivatives incubated in these conditions and this was faster in the case of BrCN derivative (Figure 4).

Because of this rapid inactivation it would be useful to recover the activity of the catalysts for reuse in more reaction cycles and thereby increasing the productivity of different immobilized catalysts. The possibility of recovering the enzyme activity of the previously inactivated derivatives during different industrial processes is a point of great interest in the use of biocatalysts in chemical industry. In order to try to reactivate the different immobilized derivatives they were resuspended in conditions of neutral pH and room temperature, conditions that may allow the refolding of different enzymes. In the

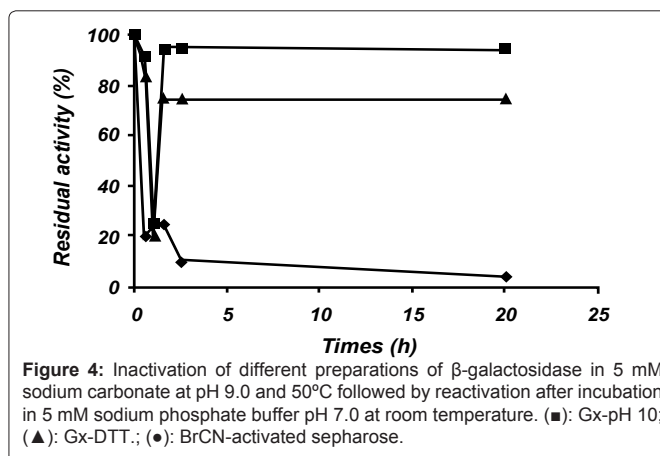


Figure 4: Inactivation of different preparations of β -galactosidase in 5 mM sodium carbonate at pH 9.0 and 50°C followed by reactivation after incubation in 5 mM sodium phosphate buffer pH 7.0 at room temperature. (■): Gx-pH 10; (▲): Gx-DTT.; (●): BrCN-activated sepharose.

figure it can be seen how the two derivatives immobilized on glyoxyl support could recover much of its initial activity. Especially good was the result obtained with the glyoxyl derivative immobilized at pH 10 where the recovering was almost all of the initial activity. In the case of the BrCN derivative, the right refolding of the enzyme structure was not produced becoming in an irreversible lost of the activity.

Moreover it was also studied the use of agents capable of folding the protein structure such as concentrate guanidine. For this proposal the most stable preparation and the reference preparation (BrCN sepharose) were incubated in saturated guanidine. Both preparations were rapidly and completely inactivated. Once produced the inactivation caused by the unfolding of the protein structure, the two derivatives were incubated in aqueous conditions and neutral pH. In these conditions the glyoxyl derivative was able to recover over 80% of the initial activity (Figure 5). This experiment was performed during several cycles of reaction yielding a recovery over 40% of the initial activity after 4 cycles of reaction in the presence of guanidine in high concentrations.

In both cases it has been found how in the case of the immobilized preparation through many points of attachment to the support has been possible the right refolding of the structure of the enzyme. This may be because when an enzyme is covalently bound on a support through many points the groups attached to the support must keep their relative positions during any conformational change, and it also seems to remain in the right relative positions even after incubation with saturated solutions of chaotropic reagents.

Effect of immobilization on the enzymatic stability in the presence of organic solvents

The different immobilized derivatives were incubated in the presence of organic solvent to check the stability in conditions, in some cases may be advantageous as in the case of the synthetic processes in which a decrease in the amount of water present in the reaction increases the thermodynamic efficiency of it. Under these conditions the immobilized derivatives incubated at pH 10 were more stable than the derivative BrCN immobilized on BrCN agarose (Figure 6).

When the different derivatives were incubated in the presence of 70% dioxane, the stabilities obtained of the derivatives immobilized on glyoxyl supports were similar and better than that obtained after

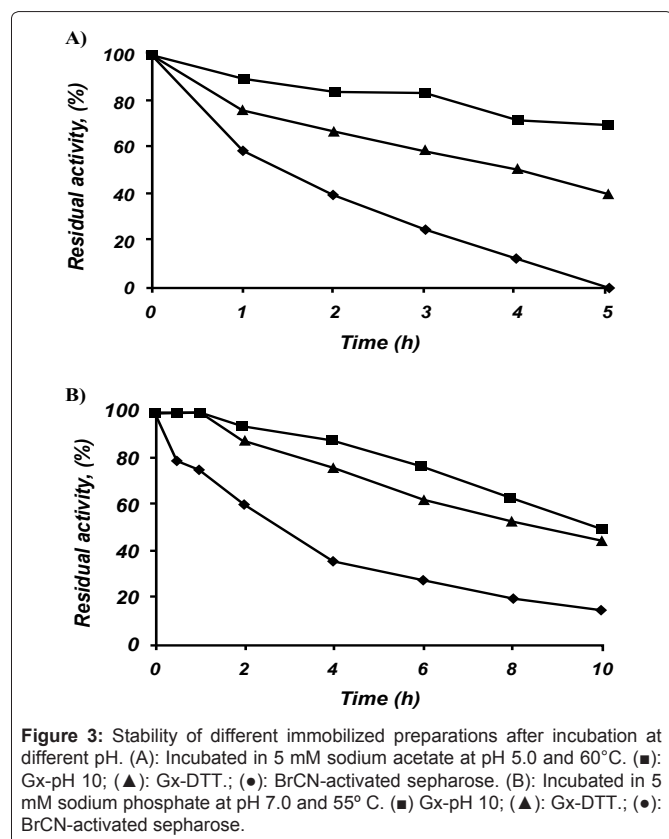


Figure 3: Stability of different immobilized preparations after incubation at different pH. (A): Incubated in 5 mM sodium acetate at pH 5.0 and 60°C. (■): Gx-pH 10; (▲): Gx-DTT.; (●): BrCN-activated sepharose. (B): Incubated in 5 mM sodium phosphate at pH 7.0 and 55°C. (■) Gx-pH 10; (▲): Gx-DTT.; (●): BrCN-activated sepharose.

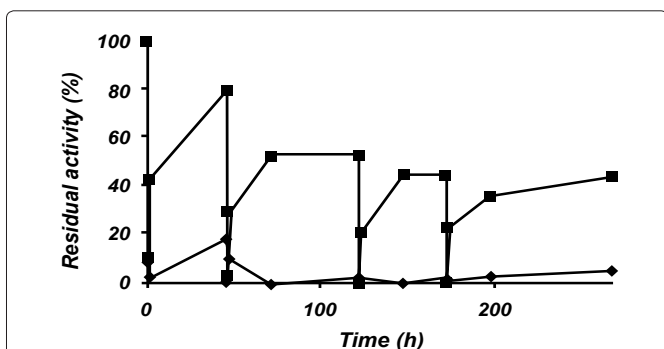


Figure 5: Inactivation-reactivation curves of different immobilized preparations of β -galactosidase. Inactivation was performed by incubation in 9 M guanidine and reactivation was done after incubation in 5 mM sodium phosphate pH 7.0. Figure show four cycles. (■): Gx-pH 10; (●): BrCN-activated sepharose.

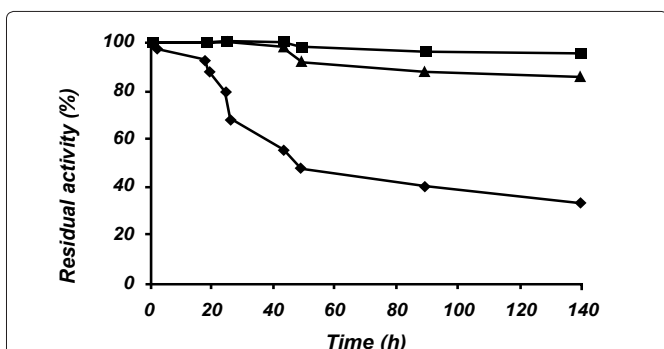


Figure 6: Stability of different immobilized preparations after incubation in 25 mM Tris-HCl and dioxane 70% at pH 7.0 and 25°C. (■): Gx-pH 10; (▲): Gx-DTT.; (●): BrCN-activated sepharose.

immobilization on activated agarose with cyanogen bromide. In fact the best derivative kept completely unaltered its catalytic activity after more than 140 hours.

In addition, as in the case of inactivation by pH or temperature the derivative immobilize on glyoxyl support at pH 10 recovered almost unaltered its catalytic activity (data not shown).

Conclusions

In this paper there is disclosed a method which has allowed us to purify, immobilizing, and even reactivate stabilize lactase enzyme derived from crude extracts of Pectinex Ultra SP-L. It has also been extracted from a commercial extract prepared to stabilize fruit chief, a beta galactosidase to hydrolyze lactose excellent properties and prepare Galactooligosaccharides (GOS), with good properties as alimentary additives.

Furthermore, good catalysts were prepared from the immobilized enzymes, biochemical properties with excellent, stability, activity. Once inactivated derivatives, these have been able to reactivated and restore almost 100% of the initial enzyme activity, and thus be reused again in successive cycles reaction.

Acknowledgments

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