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Selection of the Optimal Medium for Adsorption of Plant Proteases †

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Abstract: Immobilized enzymes are the most sought preparations on the world market. They are used in medicine, veterinary medicine, food industry, winemaking and brewing. The simplest method for immobilizing biocatalysts on insoluble carriers is the simple adsorption method. Its advantage is to preserve the natural conformation of the enzyme, which slightly reduces its catalytic ability compared to the native form. In our study, we carried out the selection of optimal conditions for adsorption immobilization of acid-soluble chitosan (Mr = 350 kDa) enzymes of plant origin (ficin, papain and bromelain) on a matrix. Ficin (EC 3.4.22.3), papain (EC 3.4.22.2), bromelain (EC 3.4.22.4) (Sigma) were chosen as objects of study, azocasein (Sigma) was used as a substrate for hydrolysis, and an acid-soluble high molecular weight chitosan (350 kDa) was used as an immobilization matrix synthesized by Bioprogress CJSC. Suitable buffer systems for immobilization were identified by the optimal ratio of protein content, total and specific activity. Ficin is immobilized on a chitosan matrix using glycine buffer with a pH of 8.6. Glycine buffer with a pH of 8.6–10.5 is optimal medium for sorption of papain on chitosan. Bromelain is immobilized on a chitosan matrix under Tris-glycine buffer with pH 8.5 conditions.

Keywords: ficin; papain; bromelain; chitosan; immobilization

1. Introduction

Immobilized enzymes are the most sought preparations on the world market. They are used in medicine, veterinary medicine, food industry, winemaking and brewing. The simplest method for immobilizing biocatalysts on insoluble carriers is the simple adsorption method. Its advantage is to preserve the natural conformation of the enzyme, which slightly reduces its catalytic ability compared to the native form [1].

Cysteine proteolytic enzymes have particular interest (ficin, bromelain, and papain) due to their broad substrate specificity and the possibility of their use in all industries and production [2].

Chitosan is produced by deacetylation of chitin. It is a straight-chain polymer formed by β- (1,4)-linked glucosamine monomers; hydroxyl and amino groups are targets for chemical modifications aimed at obtaining suitable materials for various purposes [3,4].

The aim of the work was the selection of optimal conditions for adsorption immobilization of acid-soluble chitosan (Mr = 350 kDa) enzymes of plant origin (ficin, papain and bromelain) on a matrix.

2. Methods

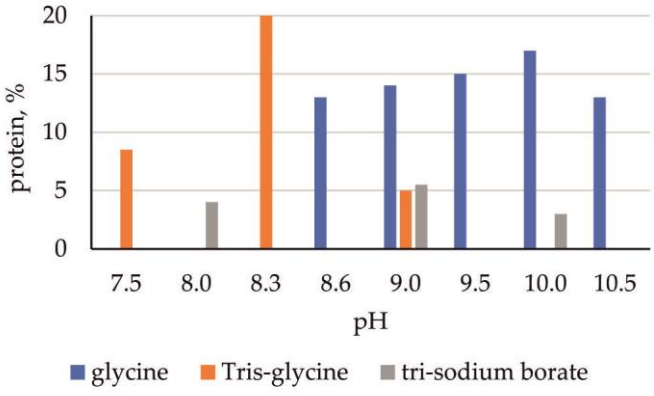
Ficin (EC 3.4.22.3), papain (EC 3.4.22.2), bromelain (EC 3.4.22.4) (Sigma) were chosen as objects of study, azocasein (Sigma) was used as a substrate for hydrolysis, and an acid-soluble high molecular weight chitosan (350 kDa) was used as an immobilization matrix synthesized by Bioprogress CJSC.

Immobilization of ficin, papain, and bromelain on a chitosan matrix was carried out by the adsorption method. To 50 mg of chitosan was added 1 mL of a buffer solution of the enzyme (ficin, papain, bromelain), incubated for 5 h with periodic stirring. After the end of the incubation, the formed precipitate was washed with 50 mM Tris-HCl buffer (pH 7.5) until there was no protein in the washings (control was carried out on a spectrophotometer at λ = 280 nm).

Protein content in immobilized enzymes was determined by the Lowry method [5].

3. Results and Discussion

During immobilization on a chitosan matrix, the largest amount of ficin is sorbed when using Tris-glycine buffer (pH 8.5), glycine buffer (pH 8.6–10.5), borate buffer with the addition of KCl (pH 9.0), bromelain and papain—when using borate buffer with the addition of KCl (pH 8.0–10.0), tris-glycine buffer (pH 8.5–9.0), glycine buffer (pH 8.6–10.5) (Figure 1).



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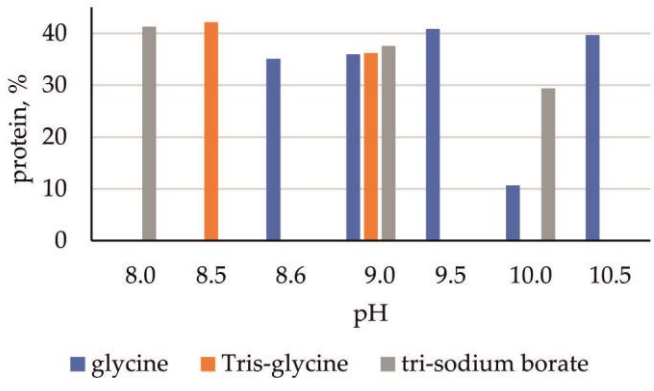

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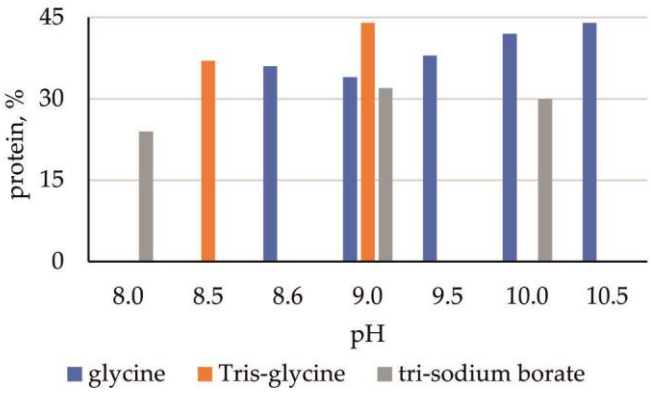
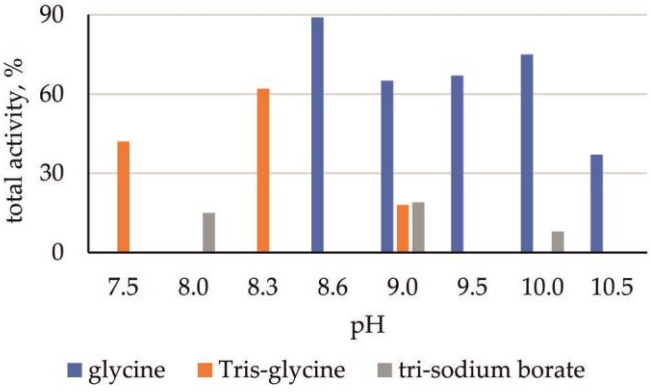


(**c**)

Figure 1. Protein content in immobilized enzymes (in % of native biocatalyst): (**a**) ficin; (**b**) papain, (**c**) bromelain.

High total activity was demonstrated by preparations of immobilized ficin using glycine buffer with pH 8.6, tris-glycine buffer with pH 7.5, 8.5. When immobilized on chitosan, the total activity of papain was found to be higher when using a borate buffer supplemented with KCl at pH 8.0–10.0, glycine buffer at pH 8.6–10.5, and Tris-glycine buffer at pH 8.5–9.5. Bromelain sorbed on chitosan was the most active under immobilization conditions in tris-glycine buffer with pH 8.5 (Figure 2).



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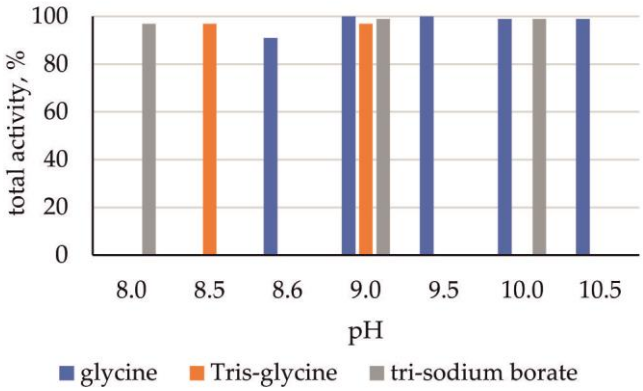

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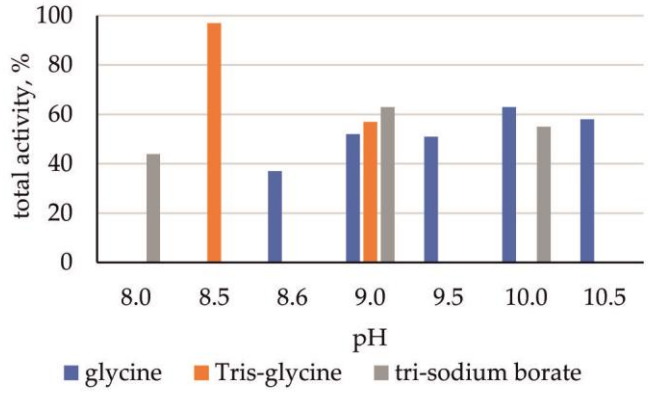
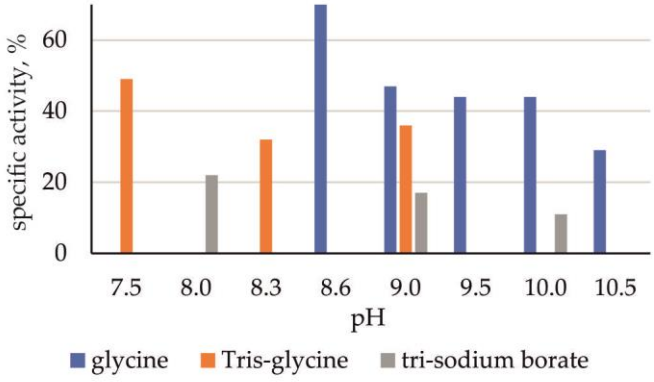


(**c**)

Figure 2. Total activity of immobilized enzymes (in % of native biocatalyst): (**a**) ficin; (**b**) papain, (**c**) bromelain.

The highest specific activity during the immobilization of ficin on chitosan was revealed when using a glycine buffer with pH 8.6, during the sorption of papain—using glycine with pH 9.5–10.5, tris-glycine 8.5–9.0, borate with the addition of KCl with pH 9.0, and adsorption of bromelain—when using tris-glycine buffer with pH 8.5 (Figure 3).



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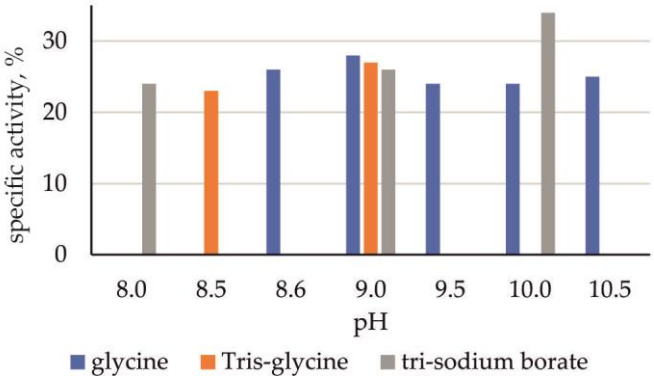

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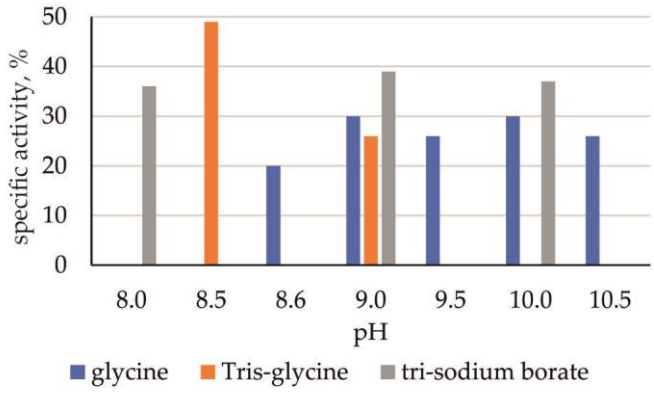

(**c**)

Figure 3. Specific activity of immobilized enzymes (in % of native biocatalyst): (**a**) ficin; (**b**) papain, (**c**) bromelain.

4. Conclusions

The optimal buffer systems were selected for the adsorption immobilization of enzymes on the chitosan matrix, namely, glycine buffer pH 8.6 is promising for the sorption of ficin, glycine buffer pH 8.6–10.5—for the adsorption of papain, and Tris-glycine buffer pH 8.5—for the immobilization of bromelain.

5. Patents

Holyavka, M., Artyukhov, V., Koroleva, V. Method for obtaining heterogeneous preparation of various dispersities based on bromelain and chitosan. RU 2677232 C2. Date of publication: 16.01.2019 Bull. № 2.

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