

Selection of the optimal medium for adsorption of plant proteases

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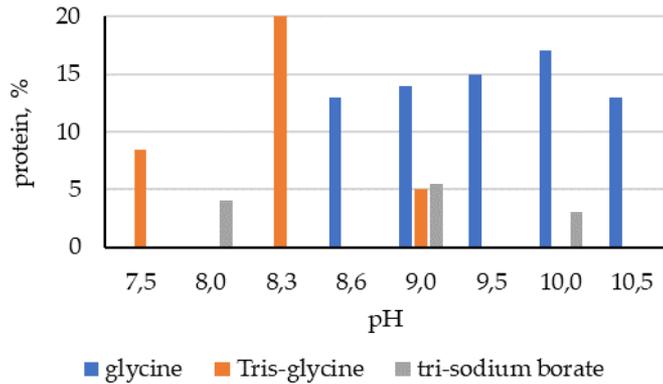
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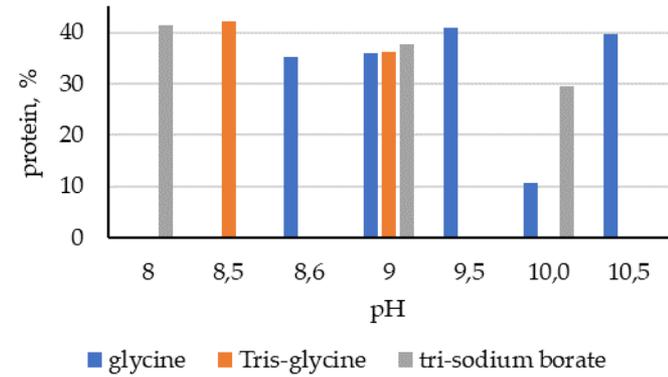
The aim of the work was the selection of optimal conditions for adsorption immobilization of acid-soluble chitosan ($M_r = 350$ kDa) enzymes of plant origin (ficin, papain and bromelain) on a matrix.

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.

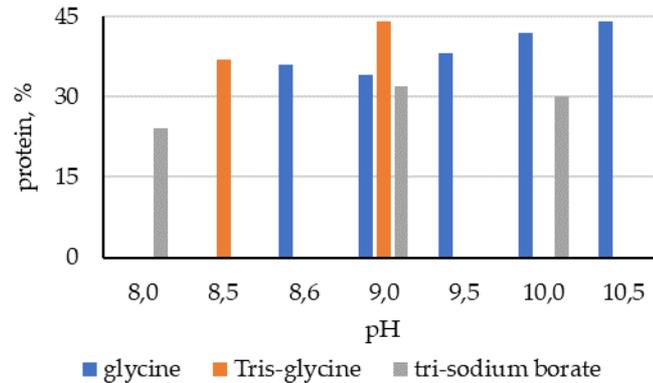
During immobilization on a chitosan matrix, the largest amount of ficin is sorbed when using Tris-glycine buffer (pH 8.5), glycine buffer (pH 8.6-10.5), borate buffer with the addition of KCl (pH 9.0), bromelain and papain - when using borate buffer with the addition of KCl (pH 8.0-10.0), tris-glycine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



(a)



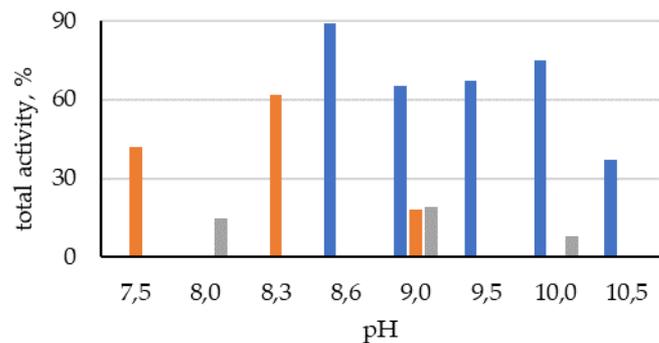
(b)



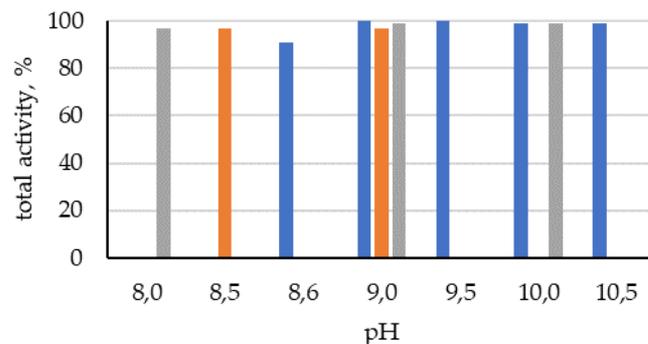
(c)

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

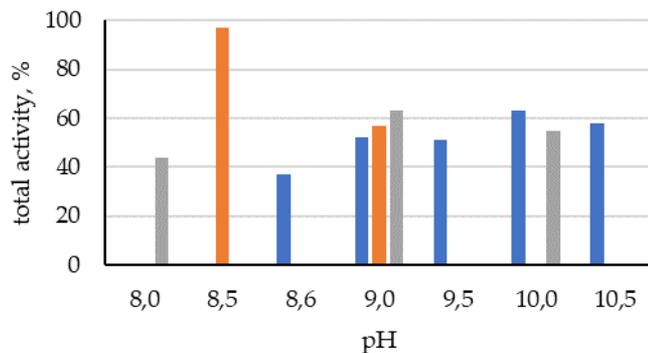
High total activity was demonstrated by preparations of immobilized ficin using glycine buffer with pH 8.6, tris-glycine buffer with pH 7.5, 8.5. When immobilized on chitosan, the total activity of papain was found to be higher when using a borate buffer supplemented with KCl at pH 8.0-10.0, glycine 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).



(a)



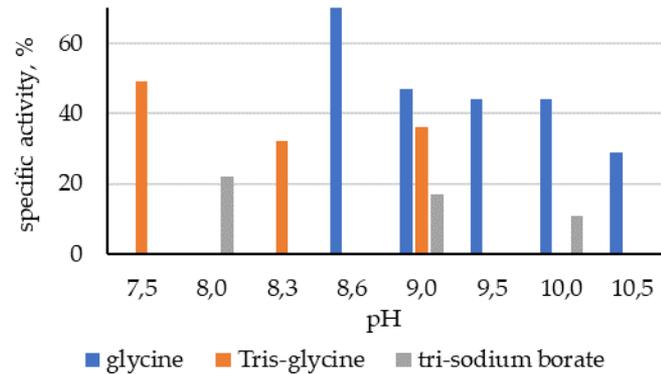
(b)



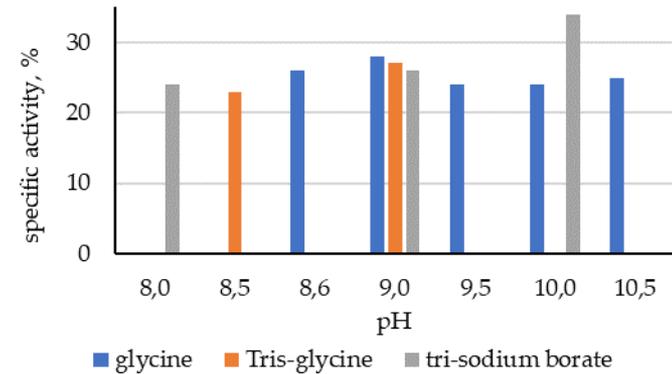
(c)

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

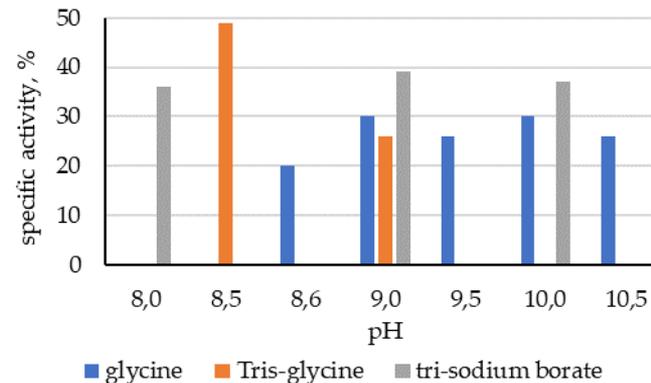
The highest specific activity during the immobilization of ficin on chitosan was revealed when using a glycine buffer with pH 8.6, during the sorption of papain - using glycine with pH 9.5-10.5, tris-glycine 8.5-9.0, borate with the addition of KCl with pH 9.0, and adsorption of bromelain - when using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



(a)



(b)



(c)

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

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.

This work was financially supported in the form of a grant from the President of the Russian Federation for state support to young Russian scientists - doctors of sciences (MD-1982.2020.4. Agreement 075-15-2020-325).