COVALENT IMMOBILIZATION OF THIOL PROTEINASES ON CHITOSAN

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The aim of this work was to determine the optimal concentration of a crosslinking agent in the covalent immobilization of ficin, papain and bromelain on a chitosan matrix.

Ficin, papain, bromelain (Sigma) were chosen as objects of study. Azocasein (Sigma-Aldrich) was used as a substrate for hydrolysis. Highmolecular weight chitosan (350 kDa, Bioprogress CJSC) was used as a carrier for immobilization.

The largest amount of protein in immobilized samples (in mg per g of carrier) was observed during covalent immobilization of ficin and papain on a chitosan matrix using glutaraldehyde with a 25% concentration, while binding bromelain – at a concentration of 5%, 10%, 25% (Figure 1).

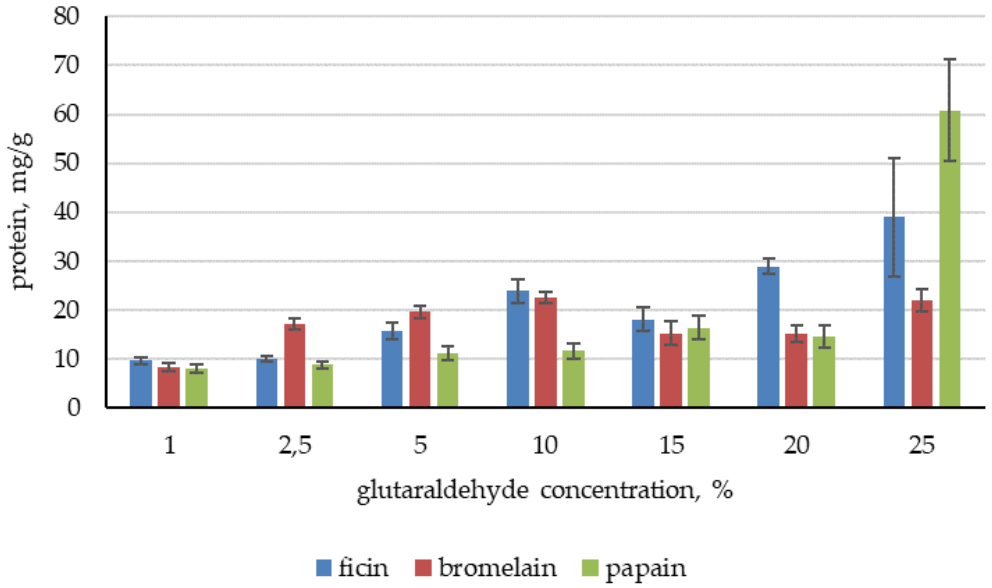

Figure 1. Protein content in immobilized enzymes (in mg per g of carrier)

High values of the total activity (in units per ml of solution) of ficin were observed during its immobilization on chitosan using glutaraldehyde with a 10% concentration. When creating immobilized enzymes based on papain and chitosan, the highest activity was detected applying 20% glutaraldehyde. High activity of bromelain immobilized on a chitosan matrix was detected when using glutaraldehyde with 5%, 10%, 20% concentration (Figure 2).

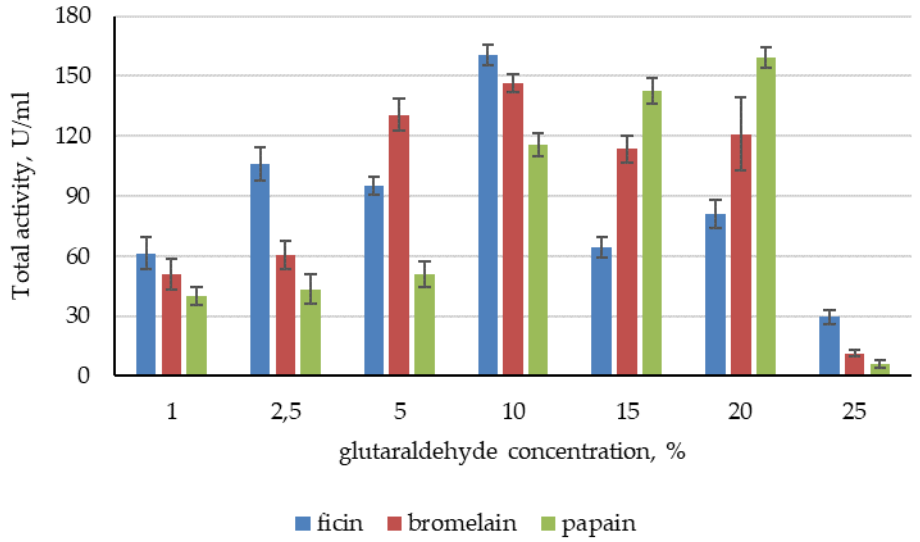

Figure 2. Total activity of immobilized enzymes (in units per ml of solution)

The highest specific activity was shown by ficin and bromelain, immobilized by covalent binding on a chitosan matrix, using glutaraldehyde with a 10% concentration. When developing biocatalysts based on papain and chitosan, the highest specific activity was observed when 20% glutaraldehyde was applied (Figure 3).

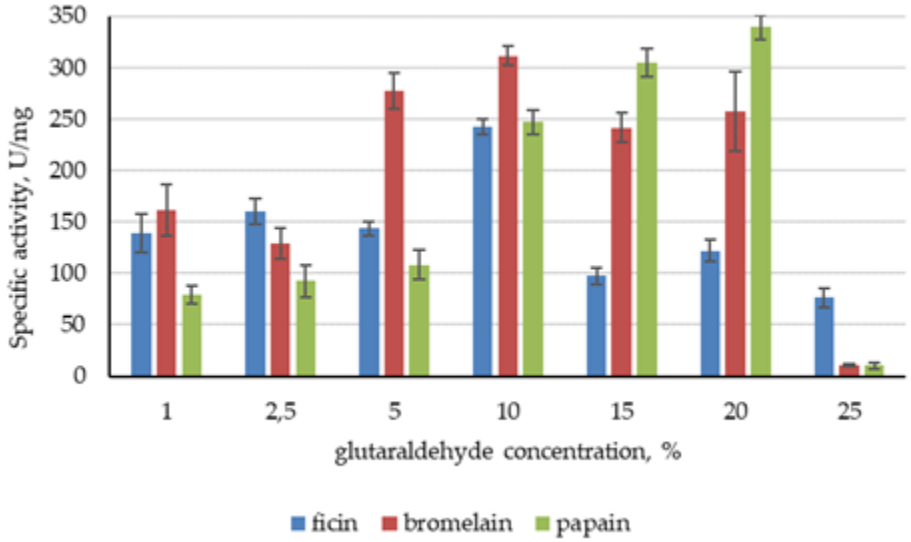

Figure 3. Specific activity of immobilized enzymes (in units per mg of protein)

Conclusion: It was shown that for covalent immobilization of ficin and bromelain on a chitosan matrix, it is most promising to use 10 % glutaraldehyde. For immobilization of papain on chitosan by covalent means, the concentration of glutaraldehyde equal to 20 % is optimal.

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