Immobilized enzymatic preparation of antioxidant peptides from silk fibroin protein and development of edible products

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Cocoon silk is a kind of natural fiber, of which silk protein is a high-quality raw material for new functional protein food, with the help of protease-catalyzed silk protein, which can promote the protein in the enzyme cleavage site enzymatic hydrolysis to obtain silk peptide. However, traditional enzymatic methods cannot separate and recycle the enzyme, which limits its practical application. The immobilization of protease in a certain region allows the enzyme to be catalyzed, which has the advantages of easy separation and recovery, high stability, and reusability. In this paper, we develop the key technology of immobilized protease to hydrolyze silk protein to prepare silk peptide, and add it into food to prepare edible products, and characterize the silk peptide before and after the enzyme digestion, which provides the basis for the broadening of the application field of silk peptide.

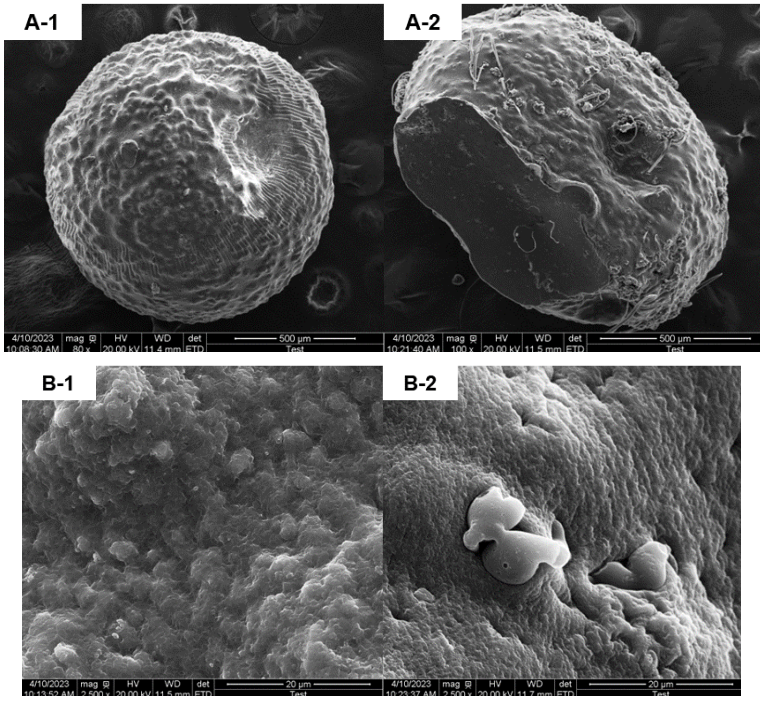

Figure 1 Scanning electron microscopic observation of microstructural features of calcium alginate immobilizing enzyme before (1) and after (2) the enzymatic reaction at magnification of 100x (A) and magnification of 2500x (B)

The dried silkworm cocoons were degummed by Na_2CO_3 boiling water bath method to obtain the silk protein, and the dried silk protein was utilized to dissolve in the ternary system (molar mass 1:2:8 = $CaCl_2$: ethanol: water) to obtain a mixed solution, which was dialyzed out of Ca^{2+} by a dialysis bag of 8 kDa and lyophilized. Six proteases, flavored protease, trypsin, alkaline protease, pineapple protease, neutral protease, and papain, were selected to determine their enzyme activities using the forintol method, and proteases with the same enzyme activities were used to hydrolyze filipin proteins. The optimal protease was screened based on the size

of hydrolysis degree of sericin protein hydrolyzed by the proteases. The results showed that the ability of flavored proteases to hydrolyze filipin proteins was significantly higher than that of other proteases, with a degree of hydrolysis of 55.84±1.21% compared with other proteases. Therefore, flavored protease has the advantage of catalytic hydrolysis of filipin protein.

Based on the enzyme activity after immobilization, the effects of sodium alginate concentration, calcium chloride concentration and enzyme addition on the immobilized enzyme process were investigated, and the preparation process of immobilized flavor protease was optimized by Box-Behnken central composite design to determine the optimal process parameters of immobilized enzyme. The results showed that the immobilized flavor protease reached the maximum enzyme activity of 0.2025 U/g when the concentration of sodium alginate was 3.4%, the concentration of calcium chloride was 5.82%, and the added amount of enzyme was 21.75 U/mL, and the degree of influence was as follows: the added amount of enzyme > the concentration of calcium chloride > the concentration of sodium alginate. Therefore, it is feasible to use sodium alginate to immobilize flavor protease.

Scanning electron microscopy was used to characterize the immobilized enzyme (Figure 1), and Fourier transform infrared spectroscopy and particle size distribution were used to characterize the structure of filipin peptides, and to analyze the correlation between the secondary structure of filipin peptides and their properties. The results showed that there were significant differences between the surfaces before and after the immobilized enzyme reaction, and the secondary structure of the filipin peptide reflected that the filipin protein would appear a broad peak with high peak intensity between 3200 and 3600 cm^{-1} after hydrolysis, and the filipin peptide obtained by using immobilized flavored proteases had an average particle size of 1186.6±161.81 nm, which was similar to the average particle size of the filipin peptide hydrolyzed by immobilized papain enzyme (1518.38±176.72 nm) was not significantly different (p>0.05). The immobilized enzyme maintained more than 80% of its original enzyme activity after 7 cycles of recycling. Therefore, the key technology of sodium alginate immobilized enzyme hydrolysis of filipin protein for the preparation of peptides was developed. Thus, silk peptides have good antioxidant properties when used as food additives. In summary, in this paper, flavor protease was screened as the optimal protease for hydrolysis of silk gum protein, and the process parameters of immobilized enzyme were optimized by response surface design, so as to efficiently prepare silk gum protein peptide with antioxidant activity, broaden the application field of silk gum protein peptide processing, and provide a theoretical basis for further extending the sericulture industry chain.

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