Enzyme-assisted extraction and characterization of collagen from Basa fish (*Pangasius bocourti*) skin

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It's generally accepted that type I collagen is a fibrillar structure collagen, which plays an important role as the essential structural composition and mechanical scaffold of several tissues. The objective of this study is to extract type I collagen from Basa fish skin using acetic acid as the extraction solvent with the assistance of pepsin. The small pieces of Basa fish skin were immersed in 0.2 M sodium hydroxide solution under stirring in 24 h to eliminate lipids. After that, the skin was washed to neutral pH with distilled water. In the next step, the skin pieces were dipped and stirred in 0.003 M citric acid in 30 min to remove minerals. The skin pieces were immersed in 1% hydrogen peroxide solution for 3 h for colorant and odorant elimination. The collagen sample was obtained with 0.5 M acetic acid solution in addition to pepsin in 24 h. The extract filtration was performed through filter paper. The protein precipitation was done by adding dropwise sodium chloride solution in 24 h to the extract so that the final sodium chloride concentration was 2.5 M. The precipitate was collected after centrifugation at 4°C and 3000 rpm in 20 min. The extraction process was followed by dialysis in phosphate buffer with pH 7.8. Finally, the sample was lyophilized and stored in sealed containers until use at -20°C. The obtained collagen was characterized by quantitative and qualitative methods. The denaturation temperature of collagen samples was determined based on the change in viscosity of collagen solution as increasing temperature. The purity of collagen proteins was examined by ultraviolet-visible spectroscopy (UV-Vis). The molecular weight (Mw) of collagen subunits was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The amino acids composition was analysed by high performance liquid chromatography (HPLC). The surface morphology of collagen was observed by scanning electron microscope (SEM) images. The chemical groups were determined by fourier transform infrared spectroscopy (FTIR). The results showed that the collagen extraction yield was 520.5 mg/g of fresh fish skin on the basis of lyophilized dry weight. The denaturation temperature (T_d) was 34.8°C by measuring viscosity. UV-Vis spectrum with one peak at the wavelength of 230 nm confirmed the purity of the

collagen. Based on SDS-PAGE, Mw of collagen α_1, α_2, and β subunits were approximately 130, 118, and above 200 kDa, respectively. By HPLC, 17 proteinogenic amino acids were found in the collagen sample, in which the hydroxyproline content was 68.3 mg/g. SEM images confirmed the fibril structure of collagen. FTIR spectrum indicated characteristic bands according to the presence of amide A, B, I, II, and III bonds in collagen chemical structure. It is concluded that the preparation of collagen from Basa fish skin (*Pangasius bocourti*) was done. Therefore, the purified collagen obtained from this study can be further used in various fields of application.

Keywords: Basa fish skin, type I collagen, enzymatic extraction, proteinogenic amino acids, hydroxyproline