

# A Study on Enzymatic Hydrolysis and Antioxidant Properties of Porcine Hemoglobin

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## ABSTRACT

The porcine hemoglobin was used as materials to investigate the antioxidant properties before and after enzymatic hydrolysis using Alcalase and Flavourzyme. The antioxidant properties, including reducing power, ferrous ion chelating ability and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were evaluated and compared with those of butylated hydroxyanisole (BHA), alpha-tocopherol or EDTA. As for the results of the antioxidant properties of porcine hemoglobin, it showed that porcine hemoglobin had relatively low reducing power, however, it exhibited increasing chelating ability as the concentration was increased, and the chelating ability was at 74.46 % when the concentration was at 5.0 mg/mL. The DPPH radical scavenging activity of the hemoglobin was at 21.53 % when the concentration was at 2.0 mg/mL. The results for the enzymatic hydrolysis of porcine hemoglobin, it showed that the degree of hydrolysis (DH) for 10-hour Alcalase hydrolysis was 9.84 %, and the DH for 10-hour Flavourzyme hydrolysis was 18.75 %, which was 1.91 times higher than that for Alcalase. The data from two-stage hydrolysis using Alcalase and Flavourzyme showed that the DHs for 4-hour 2.0 % Alcalase hydrolysis followed by 2-, 4-, and 6-hour Flavourzyme (0.5, 1.0 and 2.0 %) hydrolysis were much higher than that for 10-hour 2 % Alcalase hydrolysis. As for the results of the antioxidant activities of porcine hemoglobin hydrolysates, it showed the hydrolysates exhibited low reducing power, however, the ferrous ion chelating ability of the hydrolysates had an increasing chelating ability as the concentration was increased. The porcine hemoglobin hydrolysate, obtained through the hydrolysis using 2 % Alcalase for 4 hours and followed by using 1 % Flavourzyme for 6 hours, had the strongest ferrous ion chelating ability, which was 63.54 % at a concentration of 5.0 mg sample/mL solvent. The result of DPPH radical scavenging activity showed that the hemoglobin hydrolysate obtained through the hydrolysis using 2.0 % Alcalase for 4 hours had relatively higher scavenging activity, which was 41.94 % at a concentration 5.0 mg sample/mL solvent. The peaks of the gel filtration chromatogram for the porcine hemoglobin hydrolysates were divided into three fractions. The results of the relationship between the contents of the protein fractions and the antioxidant indices of the porcine hemoglobin hydrolysates showed that the content of protein fraction I was significantly and positively correlated to the reducing power and the ferrous ion chelating ability, and the protein fraction III was significantly and negatively correlated to the reducing power and the ferrous ion chelating ability. In summary, the results of this study showed that the porcine hemoglobin and its hydrolysates all had strong ferrous ion chelating ability, and some of the porcine hemoglobin hydrolysates exhibited comparable DPPH radical scavenging activity. It can be used as references for the development of natural antioxidants.

Keywords : Porcine hemoglobin ; Antioxidant properties ; Reducing power ; Ferrous ion chelating activity ; DPPH radical scavenging activity ; Enzymatic hydrolysis

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