Tilapia skin peptides restore cyclophosphamide-induced premature ovarian failure via inhibiting oxidative stress and apoptosis in mice

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1. Screening of proteases

Oxidative stress plays a vital role in the development of POF. Here, alcalase, neutrase, papain, pepsin, and trypsin were chosen for enzymatic hydrolysis of tilapia skin based on antioxidant activity (Supplementary Table 1). The enzymolysis process parameters were obtaind according to the previously studies with appropriate modification.[1-4]

1.1 Determination of degree of hydrolysis

When the protein is hydrolyzed by the enzyme, the peptide bond will be broken, and free amino and carboxyl groups will be released simultaneously. Therefore, the degree of hydrolysis of the protein can be determined by measuring the free amino acid nitrogen. The degree of hydrolysis was measured according to the previously protocol with appropriate modification.[1]

Degree of hydrolysis (%) = $(A_1 - A_0) / A \times 100\ \%$

A_1, amino acid nitrogen content after hydrolysis; A_0, amino acid nitrogen content before hydrolysis; A, total nitrogen content of the sample

1.2 DPPH radical scavenging assay

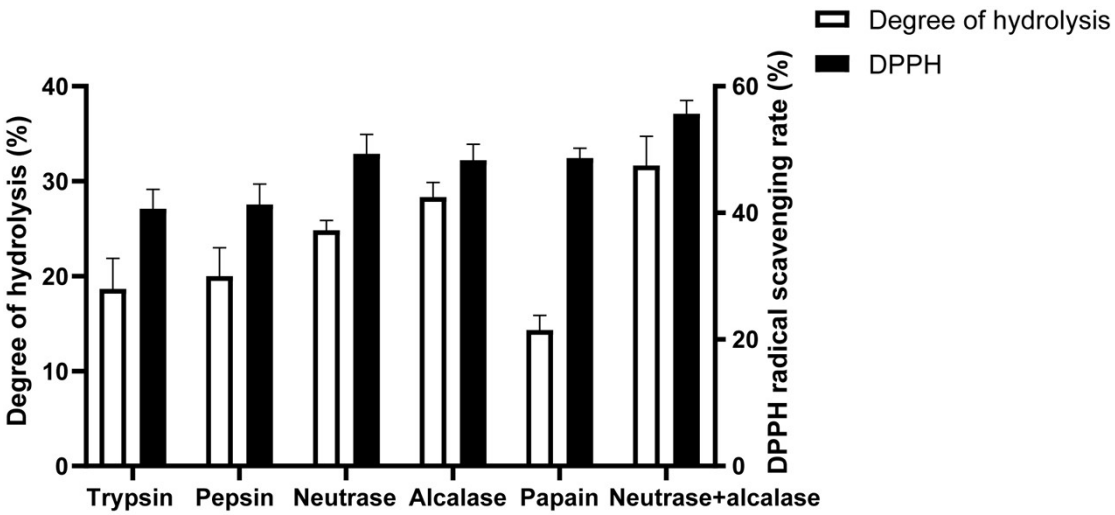
TSP was configured as a 5.0 mg/ml sample solution, the 2.0 ml sample solution was placed in the tube, 2.0 ml DPPH solution (0.1 mmol/L) dissolved in absolute ethanol was added, and the mixture was shaken vigorously for 10 s and protected from light at room temperature for 30 minutes. After the reaction, the absorbance of the reaction mixture was measured at 517 nm. The DPPH radical scavenging was assessed according to the previously method with appropriate modification.[5]

DPPH radical scavenging rate (%) = $(B_{water} - B_{samble} + B_{ethanol}) / B_{water}$

B_{water}, absorbance of blank; B_{samble}, absorbance of samble; $B_{ethanol}$, absorbance of control.

Supplementary Table 1. Enzymolysis process parameters

Protease	Temperature (℃)	pH	The amount of protease (%)	Time (h)	Solid-liquid ratio (V/W)
Trypsin	37	7.8	3	8	5：1
Neutrase	50	7.0	3	6	5：1
Alcalase	55	9.0	3	5	5：1
Pepsin	37	2.0	3	6	5：1
Papain	55	7.0	3	8	5：1



Supplementary Fig. 1 Effects of enzymes on the degree of hydrolysis of tilapia skin and DPPH radical scavenging rate. All data were shown as the mean ± SD (n=3).

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