ABSTRAK

Kolagen merupakan protein dominan pada kulit ikan tuna sirip kuning (*Thunnus albacares*). Proses hidrolisis kolagen berperan penting dalam menghasilkan hidrolisat kolagen dengan aktivitas antioksidan. Penelitian ini terdiri dari 3 tahapan utama, yaitu (1) evaluasi aktivitas antioksidan kolagen kulit tuna sirip kuning secara *in silico* dengan mensimulasikan proteolisis kolagen tuna dengan enzim papain, tripsin, pepsin dan thermolysin. Tahap (2) hidrolisis kolagen kulit tuna sirip kuning dengan enzim papain. Proses hidrolisis *in vitro* menggunakan enzim papain untuk menghasilkan hidrolisat kolagen terbaik dengan variabel konsentrasi enzim dan lama waktu hidrolisis. Parameter pengujian meliputi derajat hidrolisis dan aktivitas antioksidan. Tahap (3) yaitu hidrolisat dengan aktivitas antioksidan tertinggi dianalisis asam amino, gugus fungsi (FTIR), berat molekul dan sifat fungsional protein meliputi kelarutan berbagai pH, kapasitas pengikatan air (WHC), kapasitas pengikatan minyak (OHC), aktivitas buih (FA), stabilitas buih (FS), aktivitas emulsi (EAI) dan stabilitas emulsi (ESI).

Berdasarkan evaluasi *in silico*, prediksi asam amino dengan Protparam Tool menunjukkan bahwa asam amino dominan pada kolagen rantai α1 dan α2 yaitu glisin dan prolin. Hasil proteolysis *in silico* prekursor protein kolagen tuna α1 dan α2 dengan enzim papain mendapatkan nilai derajat hidrolisis teoritis tertinggi sebesar 44,28% dan 42,60%. Hasil prediksi sekuens peptida yang diperoleh dari simulasi proteolisis dengan aktivitas antioksidan pada rantai α1 yaitu HL, AH, EL, PHG, PWG, IR sedangkan pada rantai α2 yaitu AH, EL, PHG, IR.

Evaluasi hidrolisis kolagen tuna sirip kuning dengan enzim papain menunjukkan rendemen berkisar 41,29-46,18%. Variabel lama waktu hidrolisis dan konsentrasi enzim berpengaruh signifikan (p<0,05) sedangkan interaksi antar kedua faktor tersebut tidak berpengaruh (p>0,05) terhadap nilai derajat hidrolisis dan aktivitas antioksidan hidrolisat kulit tuna. Hidrolisat kolagen kulit ikan tuna yang dihidrolisis selama 6 jam dengan konsentrasi enzim 3% mendapatkan rerata persentase derajat hidrolisis 24,03±0,65%, serta rerata aktivitas antioksidan DPPH 56,74±2,51%, daya reduksi dengan nilai absorbansi 0,230±0,01 pada panjang gelombang 700 nm dan ABTS 70,93±1,56%. Hasil analisis asam amino total menunjukkan dominasi glisin dan prolin sebagai penciri kolagen. Pada sampel kontrol, glisin berjumlah 89,6 mg/ g dan prolin 40,7 mg/g, sedangkan pada sampel hidrolisat, glisin berjumlah 174 mg/g dan prolin 74,2 mg/g. Analisis FTIR menunjukkan amida A, I, II dan III pada kontrol dan hidrolisat. Analisis berat molekul SDS-PAGE menunjukkan bahwa proses hidrolisis kolagen dengan enzim papain dapat menurunkan berat molekul kolagen dari 152,56 dan 124,47 kDa menjadi 25,71 kDa. Evaluasi sifat fungsional, kelarutan di berbagai pH sampel kontrol dan hidrolisat kolagen menunjukkan titik isoelektris pada pH 8. Nilai WHC dan OHC sampel kontrol lebih tinggi dibandingkan hidrolisat, yaitu 2,98±1,10 dan 6,95±1,40 mg/g protein (p<0,05). Rerata FA dan FS sampel kontrol yaitu 48,07±6,06% dan 41,20±6,87% lebih tinggi dibandingkan sampel hidrolisat (p<0,05). EAI dan ESI hidrolisat kolagen yaitu 24,63±0,96 m^2/g dan stabilitas emulsi selama 26,26±0,79 menit, rerata tersebut lebih tinggi dibandingkan sampel kontrol (p<0,05).

Kata kunci: derajat hidrolisis, konsentrasi enzim, peptida, sifat fungsional, waktu hidrolisis

ABSTRACT

Collagen is the dominant protein of yellowfin tuna (*Thunnus albacares*) skin. The hydrolysis process is essential in producing collagen hydrolysates with antioxidant activity. This study consists of 3 main stages, (1) evaluation of the antioxidant activity of yellowfin tuna skin collagen based on in silico approach, and the evaluation was performed by simulating the proteolysis of tuna collagen with the papain, trypsin, pepsin, and thermolysin enzymes. The second stage (2) is in vitro hydrolysis of yellowfin tuna skin collagen using papain enzyme. In vitro hydrolysis process using the enzyme papain to produce the best collagen hydrolysate with enzyme concentration and hydrolysis time as independent variables. The parameters of analysis include the degree of hydrolysis and antioxidant activity. In stage (3), hydrolysates with the highest antioxidant activity were analyzed amino acids, functional groups (FTIR), molecular weight, and functional properties of proteins, including relative solubility in various pH, *water holding capacity* (WHC), *oil holding capacity* (OHC), *foaming activity* (FA), *foam stability* (FS), *emulsifying activity index* (EAI) dan *emulsion stability index* (ESI)

Based on the in silico evaluation, amino acid prediction using Protparam Tool shows the dominant amino acids in collagen chains are glycine and proline on α1 and α2 chains. The results of proteolysis in silico of tuna collagen as precursor protein α1 and α2 chains, papain enzyme got the highest theoretical hydrolysis degree value of 44.28% and 42.60%. The simulation of proteolysis showed that the sequences with antioxidant activity on the chain α1 were HL, AH, EL, PHG, PWG, and IR, while on the chain α2, were AH, EL, PHG, and IR.

Evaluation of yellowfin tuna collagen hydrolysis using papain enzyme showed yield ranged from 41.29-46.18%. The variable length of hydrolysis time and enzyme concentration showed a significant effect (p<0.05); meanwhile, the interaction between the two factors had no effect (p>0.05) on the degree of hydrolysis and antioxidant activity of collagen hydrolysates. The highest hydrolysis degree and antioxidant activity of tuna skin collagen hydrolysates at 3% enzyme concentration and 6 h hydrolysis time was 24.03%±0.65%, and the average antioxidant activity of DPPH was 56.74±2.52%, reducing power 0.230±0.01 at wavelength 700 nm and ABTS 70,93±1,45%. The amino acid analysis results showed glycine and proline dominance as collagen markers. Glycine and proline of the control sample were 89.6 mg/ g and 40.7 mg/g; meanwhile, glycine and proline of collagen hydrolysates were 174 mg/g and 74.2 mg/g. FTIR analysis showed both Amida A, I, II, and III on control and hydrolysates. After hydrolysis, the collagen's SDS-PAGE protein molecular weight decreased from 152.56 and 124.47 kDa to 25.71 kDa. Evaluation of functional properties, control and collagen hydrolysates on relative solubility at various pH showed an isoelectric point at pH 8. WHC and OHC of control collagen higher than collagen hydrolysates, were 2.98±1.10 and 6.95±1.40 mg/g protein (p<0.05). FA and FS of control were higher than the hydrolysates (p<0.05), were 48.07±6.06% and 41.20±6.87%. EAI and ESI evaluation of collagen hydrolysates had a higher value than the control on the parameters of emulsifying activity of 24.63±0.96 m^2/g and emulsion stability at 26.26±0.79 minutes (p<0.05).

Keywords: degree of hydrolysis, enzyme concentration, peptide, functional properties, hydrolysis time