

Identifikasi dan Uji Potensi Peptida dari Hidrolisat Tripsin Protein Susu Kambing (*Capra hircus*) secara *In Vitro* dan *In Silico*

Dian Riana Ningsih
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INTISARI

Antimicrobial peptides (AMPs) adalah senyawa alami yang diproduksi oleh semua organisme sebagai sistem kekebalan bawaan terhadap patogen. AMPs menunjukkan aktivitas biologis spektrum luas seperti antibakteri dan antijamur. Salah satu sumber AMPs yaitu protein susu kambing. Hidrolisis protein susu kambing menggunakan tripsin akan menghasilkan fragmen peptida bioaktif yang memiliki pengaruh positif terhadap kesehatan. Tujuan penelitian ini adalah mengisolasi dan mengidentifikasi peptida antijamur dan antibakteri dari protein susu kambing, aplikasi fraksi peptida aktif sebagai pengawet roti, sintesis peptida aktif dan menentukan mekanisme aksinya. Tahapan penelitian meliputi: hidrolisis protein susu kambing menggunakan tripsin, mengidentifikasi fraksi peptida aktif terhadap jamur *Aspergillus* sp, *Escherichia coli* dan *Staphylococcus aureus* menggunakan HRMS, uji fraksi peptida aktif sebagai pengawet roti, sintesis peptida aktif dan uji aktivitas terhadap jamur *Aspergillus* sp, *E. coli* dan *S. aureus* serta menentukan mekanisme aksi peptida aktif secara *in silico*.

Fraksi peptida kasein susu kambing pH 5 (K3), pH 6 (K4), pH 7 (K5) dan pH 8 (K6) mampu menghambat *Aspergillus* sp., *Staphylococcus aureus* dan *Escherichia coli* dengan zona hambat terbesar berturut-turut sebesar 26,95; 8,00 dan 2,00 mm. Aktivitas zona hambat masuk kategori sangat kuat, sedang dan lemah. Hasil identifikasi fraksi peptida kasein susu kambing K4 dengan HRMS diperoleh peptida YNVPQLEIVPK (P1), KENINELSK (P2), GLSPEVPNENLLR (P3), dan YLGYLEQLLK (P4). Hasil SEM menunjukkan bahwa fraksi peptida K3, K4, K5 dan K6 mengubah morfologi miselia pada jamur *Aspergillus* sp. Fraksi peptida K4 memberikan pengaruh yang signifikan terhadap morfologi jamur *Aspergillus* sp. Fraksi peptida K3, K4, K5 dan K6 digunakan sebagai pengawet roti tawar. Roti tawar tanpa perlakuan fraksi peptida mulai ditumbuhi jamur pada hari ke 4 dengan jumlah koloni jamur 24×10^7 spora/mL. Roti tawar dengan penambahan fraksi peptida K3, K4, K5 dan K6 mulai ditumbuhi jamur pada hari ke 8. Perlakuan fraksi peptida K4 pada hari ke 8 ditumbuhi jamur yang paling sedikit yaitu 7×10^7 spora/mL. Hasil prediksi struktur 3D CaATPase dengan menggunakan Robetta diperoleh model enzim yang memiliki kualitas struktur yang baik. Hal ini didasarkan pada residu pada *favoured rotamers* 99,53%, residu dengan Nilai rata-rata 3D-1D $\geq 0,2$ memiliki persentase 81,05% dan nilai Nilai molprobability 1,10. Peptida P1, P2, P3, P4 memiliki potensi dalam menghambat CaATPase melalui metode penambatan molekul dengan energi afinitas ikatan berturut-turut sebesar -6,7; -8,2; -7,7; -5,6 kkal/mol. Semua peptida sintetik, P1, P2, P3, dan P4

menunjukkan aktivitas antibakteri terhadap *S. aureus* dan *E. coli*. P4 menunjukkan sifat fisikokimia yang baik dengan struktur amfipatiknya. Interaksi yang kuat juga diamati antara P4 dan enzim MurC dengan afinitas pengikatan $-6,6$ kkal/mol dan Nilai HADDOCK $-42,2 \pm 10,8$. Peptida protein kompleks menunjukkan interaksi ikatan hidrogen pada residu Asp91, Asn70, dan Arg72. Hasil simulasi dinamika molekul, peptida P2, P3 dan P4 mampu berinteraksi dengan *head groups* membran lipid bakteri Gram positif dan Gram negatif. Peptida P4 menunjukkan stabilitas kompleks dan kekompakan yang lebih besar dibandingkan P2 dan P3. Fraksi peptida kasein susu kambing K3, K4, K5 dan K6 berpotensi sebagai pengawet alami pada roti tawar dan peptida aktif P1, P2, P3 dan P4 berpotensi dikembangkan sebagai antibiotik alami.

Kata kunci: antijamur; antibakteri; *in vitro*; *in silico*; peptida; susu kambing

Identification and Testing of The Potential Peptides From Goat Milk Protein Trypsin Hydrolysate (*Capra hircus*) *In Vitro* and *In Silico*

Dian Riana Ningsih
21/476107/SPA/00780

ABSTRACT

Antimicrobial peptides (AMPs) are natural compounds produced by all organisms as an innate immune system against pathogens. AMPs exhibit broad-spectrum biological activity such as antibacterial and antifungal. One source of AMPs is goat milk. Goat milk is easily digested and does not cause allergies. Hydrolysis of goat milk protein using trypsin produce bioactive peptide fragments that have a positive effect on health. The purpose of this study was to isolate and identify antifungal and antibacterial peptides from goat milk protein, application of active peptide fractions as bread preservatives, synthesis of active peptides and determining their mechanism of action. The stages of the study include: hydrolysis of goat milk protein using trypsin, identifying active peptide fractions against *Aspergillus* sp, *Eschericia coli* and *Staphylococcus aureus* using HRMS, testing active peptide fractions as bread preservatives, synthesis of active peptides and activity testing against *Aspergillus* sp, *S. aureus*, *E. coli* and determining the mechanism of action of active peptides *in silico*.

The goat milk casein peptide fractions pH 5 (K3), pH 6 (K4), pH 7 (K5) and pH 8 (K6) were able to inhibit *Aspergillus* sp., *S. aureus* and *E. coli* with the largest inhibition zones of 26.95; 8.00 and 2.00 mm respectively. The inhibition zone activity was categorized as very strong, moderate and weak. The results of the identification of the goat milk casein peptide fraction K4 with HRMS obtained peptides YNVPQLEIVPK (P1), KENINELSK (P2), GLSPEVPNENLLR (P3), and YLGYLEQLLK (P4). SEM results showed that the peptide fractions K3, K4, K5 and K6 changed the mycelial morphology of *Aspergillus* sp. The K4 peptide fraction had a significant effect on the morphology of *Aspergillus* sp. The K3, K4, K5 and K6 peptide fractions were used as preservatives for white bread. White bread without peptide fraction treatment began to grow mold on day 4 with the number of mold colonies of 24×10^7 spores/mL. White bread with the addition of peptide fractions K3, K4, K5 and K6 began to grow mold on day 8. The treatment of peptide fraction K4 on day 8 was grown by the least mold, namely 7×10^7 spores/mL. The results of the 3D structure prediction of CaATPase using Robetta obtained an enzyme model that has good structural quality. This is based on the residues in the favored rotamers of 99.53%, residues with an average score of $3D-1D \geq 0.2$ have a percentage of 81.05% and a molprobit score of 1.10. Peptides P1, P2, P3, P4 have the potential to inhibit CaATPase through the molecular penambatan method with successive binding affinity energies of -6.7; -8.2; -7.7; -5.6 kcal/mol. All synthetic peptides, P1, P2, P3, and P4 showed antibacterial activity against *S. aureus* and *E. coli*. P4 showed good physicochemical properties

with its amphipathic structure. Strong interaction was also observed between P4 and MurC enzyme with binding affinity of -6.6 kcal/mol and HADDOCK score of -42.2 ± 10.8 . The peptide protein complex showed hydrogen bonding interactions at residues Asp91, Asn70, and Arg72. The results of molecular dynamics simulations, peptides P2, P3 and P4 were able to interact with head groups of lipid membranes of Gram-positive and Gram-negative bacteria. Peptide P4 showed greater complex stability and compactness compared to P2 and P3. The goat milk casein peptide fractions K3, K4, K5 and K6 have the potential as natural preservatives in white bread and the active peptides P1, P2, P3 and P4 have the potential to be developed as natural antibiotics.

Keywords: antifungal; antibacterial; *in vitro*; *in silico*; peptida; goat milk