

## ISOLATION OF GARUT SHEEP SKIN PROTEIN AND IDENTIFICATION THEIR BIOACTIVE PEPTIDES AS AN ANGIOTENSIN CONVERTING ENZYME INHIBITOR AGENT

### ABSTRACT

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Hypertension remains one of the leading global health concerns, driving the continuous research for natural antihypertensive derived from sustainable protein sources such as animal by-products. This study aimed to extract protein from Garut sheep skin and investigate the potential of its bioactive peptides as Angiotensin-Converting Enzyme (ACE) inhibitors. The samples used were ten fresh skins from 1.5-year-old purebred male Garut sheep. The research was conducted in two stages. The first stage involved the extraction and characterization of protein from Garut sheep skin using plant and microorganism-derived enzymes including bromelain, neutrase, alcalase, protease from *Aspergillus oryzae* and protease from *Bacillus subtilis*. Among the extracts obtained, bromelain-soluble protein showed the highest yield. The fourier transform infrared spectroscopy (FTIR) analysis of bromelain-soluble sheepskin protein (BSP) showed five amide bands (A, B, I, II, and III) with characteristics typical of collagen structures. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiling indicated that BSP predominantly consisted of collagen and keratin proteins. The second stage of the study involved *in vitro* gastrointestinal digestion (GID) process to produce bromelain-soluble protein hydrolysate (BSPH). Purification of ACE-inhibitory peptides from BSPH was carried out through ultrafiltration using 30, 10, and 3 kDa molecular weight cut-off membranes, followed by fractionation using RP-HPLC. The most potent peptides were identified by tandem mass spectrometry and *in silico* analysis. The SDS-PAGE profiles of BSP and BSPH showing that high molecular weight proteins were hydrolyzed into smaller molecules. The ACE-inhibitory activity of 1 mg/mL of BSPH was 48.99%. Arginine, tyrosine, leucine, and lysine were the predominant amino acid residues found in BSPH. The GID process resulted in protein degradation and the formation of small peptides. ACE-inhibitory activity of BSPH increased compared to BSP in the same size fraction. The ultrafiltration fraction <3 kDa showed the highest inhibitory activity. The reverse phase-high performance liquid chromatography (RP-HPLC) fractionation of the best fraction revealed 25 fraction and four fractions (fractions 2, 3, 12, and 15) have the highest ACE-inhibitory activities. Sequence identification and *in silico* analysis selected two peptide sequences in fraction 12 and 15, GPAGPAGPR and QGPPGPAGPR, as promising peptide candidates for ACE inhibition from Garut sheep skin collagen.

**Keywords:** Garut sheep skin, protease, collagen, bioactive peptides, ACE-inhibition

## ISOLASI PROTEIN KULIT DOMBA GARUT DAN IDENTIFIKASI PEPTIDA BIOAKTIFNYA SEBAGAI AGEN PENGHAMBAT ANGIOTENSIN CONVERTING ENZYME

### INTISARI

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Hipertensi merupakan penyakit yang menjadi salah satu masalah kesehatan global utama yang mendorong peningkatan penelitian terhadap penemuan antihipertensi alami yang berasal dari sumber protein berkelanjutan, terutama hasil samping hewani yang berpotensi mengandung peptida bioaktif. Penelitian ini bertujuan mengekstrak protein dari kulit domba Garut serta mengevaluasi potensi peptida bioaktifnya sebagai penghambat *Angiotensin-Converting Enzyme* (ACE). Sampel berupa sepuluh kulit segar domba Garut galur murni, berjenis kelamin jantan dan berusia 1,5 tahun. Penelitian dilakukan dalam dua tahap. Tahap pertama mencakup ekstraksi dan karakterisasi protein menggunakan enzim tumbuhan dan mikroorganisme antara lain bromelin, alkalase, neutrase, protease dari *Aspergillus oryzae* dan *Bacillus subtilis*. Di antara ekstrak yang diperoleh, protein larut bromelin atau *bromelain-soluble sheepskin protein* (BSP) menunjukkan rendemen yang paling tinggi. Analisis *fourier transform infrared spectroscopy* (FTIR) mengidentifikasi lima pita amida khas kolagen, sementara *sodium dodecyl sulfate- polyacrylamide gel electrophoresis* (SDS-PAGE) dan *tandem mass spectrometry* memperlihatkan bahwa BSP terutama tersusun atas kolagen dan keratin. Tahap kedua adalah *in vitro gastrointestinal digestion* (GID) menggunakan pepsin dan pancreatin pada BSP, menghasilkan *bromelain-soluble sheepskin protein hydrolysate* (BSPH). Pemurnian peptida penghambat ACE dari BSPH dilakukan melalui ultrafiltrasi (30, 10, dan 3 kDa) dan fraksinasi *reverse phase high-performance liquid chromatography* (RP-HPLC). Hasil menunjukkan GID berhasil mendegradasi protein bermassa besar menjadi peptida kecil. Aktivitas penghambatan ACE dari BSPH menunjukkan kenaikan dibandingkan BSP mencapai 48,99% pada konsentrasi 1 mg/mL. Analisis asam amino menunjukkan dominasi residu arginin, tirosin, leusin, dan lisin pada BSPH. Fraksi dari BSPH dengan ukuran berat molekul <3 kDa memiliki aktivitas penghambatan ACE paling baik. Fraksinasi RP-HPLC terhadap fraksi terbaik menghasilkan 25 fraksi, dengan empat fraksi (2, 3, 12, dan 15) memiliki aktivitas penghambatan ACE terkuat. Hasil identifikasi urutan asam amino peptida dan analisis *in silico* berhasil mengidentifikasi dua peptida dengan urutan GPAGPAGPR dan QGPPGPAGPR, dari fraksi 12 dan 15, sebagai kandidat potensial penghambat ACE dari kolagen domba Garut.

**Kata Kunci:** Kulit domba Garut, protease, kolagen, peptida bioaktif, penghambatan ACE