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EFFECT OF DIFFERENT ENZYMES ON PHYSICOCHEMICAL PROPERTIES, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF JACK BEAN (*Canavalia ensiformis*) HYDROLYSATE

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The global meat demand surged by 58% over the past two decades, driven by population growth and economic expansion. However, concerns regarding meat production inefficiencies and health risks have prompted interest in alternative protein sources such as jack bean (*Canavalia ensiformis*), an underutilized high-protein legume. This study investigated the physicochemical properties, antioxidant, antimicrobial activity, and amino acid composition of jack bean protein hydrolysate (JBPH) produced using Alcalase®, bromelain, papain, and Flavourzyme® enzymes. JBPH's crude protein content and molecular weight of protein were determined via the Kjeldahl method and SDS-PAGE, respectively. Additionally, water holding capacity (WHC), oil holding capacity (OHC), degree of hydrolysis (DH), foaming stability (FS), and foaming capacity (FC) were determined. Antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays, while antimicrobial activity was tested via the well diffusion method. Amino acid presence and sequence were identified using HPLC and LC-MS analysis. Five JBPH protein sequences were selected as potential precursors for bioactive peptides using the BIOPEP database via *in silico* methods. It was found that papain-extracted JBPH exhibited the highest protein content (97.16 ± 0.50%), DH (59.16 ± 0.17%), and OHC (12.52 ± 0.30g/g). Flavourzyme®-extracted

JBPH showed the highest WHC (3.52 ± 0.10g/g) and FC (140.13 ± 0.20%), while Alcalase®-extracted JBPH gave the highest FS (100.0 ± 0.00%). JBPH's molecular weight ranged from 29.30 to < 10.0 kDa, with a collapsed building-like structure observed under microscope. Amide A and Amide I peaks were prominent in FTIR analysis. Papain-extracted JBPH exhibited the highest DPPH (35.10 ± 0.01%) and ABTS (94.35 ± 0.01%) inhibition, while bromelain-extracted JBPH had the highest FRAP value (11.21 ± 0.01μM). JBPH also demonstrated inhibition against *Escherichia coli* and *Pseudomonas aeruginosa*. Papain-extracted JBPH contained the most amino acids. Moreover, *in silico* study revealed that chitinase from papain's JBPH showed the most potent antioxidant properties. Overall, JBPH presents a promising alternative protein source with functional food ingredient potential, given its high protein content and beneficial properties.

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KESAN ENZIM YANG BERBEZA TERHADAP SIFAT-SIFAT FIZIKOKIMIA, AKTIVITI ANTIOKSIDAN DAN ANTIMIKROB HIDROLISAT KACANG KORO (*Canavalia ensiformis*)

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Permintaan daging global melonjak sebanyak 58% sepanjang dua dekad yang lalu, didorong oleh pertumbuhan penduduk dan pengembangan ekonomi. Walau bagaimanapun, kebimbangan mengenai ketidakcekapan pengeluaran daging dan risiko kesihatan telah mendorong minat terhadap sumber protein alternatif seperti Kacang Koro (*Canavalia ensiformis*), kekacang protein tinggi yang kurang digunakan. Kajian ini menyiasat sifat fizikokimia, antioksidan, aktiviti antimikrob, dan komposisi asid amino hidrolisat protein Kacang Koro (KKPH) yang dihasilkan menggunakan enzim Alkalase®, bromelain, papain, dan Flavourzyme®. Kandungan protein kasar JBPH dan berat molekul ditentukan melalui kaedah Kjeldahl dan SDS-PAGE, masing-masing. Selain itu, kapasiti pegangan air (WHC), kapasiti pegangan minyak (OHC), tahap hidrolisis (DH), kestabilan buih (FS), dan kapasiti berbuih (FC) telah ditentukan. Aktiviti antioksidan dinilai menggunakan ujian DPPH, ABTS, dan FRAP, manakala aktiviti antimikrob diuji melalui kaedah “well diffusion”. Kehadiran dan jujukan asid amino telah dikenal pasti menggunakan analisis HPLC dan LC-MS. Lima jujukan protein KKPH telah dipilih sebagai prekursor yang berpotensi untuk peptida bioaktif menggunakan pangkalan data BIOPEP melalui kaedah *in siliko*. Didapati KKPH yang diekstrak dengan papain menunjukkan kandungan protein (97.16 ± 0.50%), DH (59.16 ± 0.17%), dan OHC (12.52 ± 0.30g/g) tertinggi. KKPH yang diekstrak dengan Flavourzyme® menunjukkan WHC (3.52 ± 0.10g/g) dan FC (140.13 ± 0.20%)

tertinggi, manakala KKPH yang diekstrak dengan Alcalase® memberikan FS tertinggi (100.0 ± 0.00%). Berat molekul KKPH adalah antara 29.30 hingga < 10.0 kDa, dengan struktur seperti bangunan runtuh diperhatikan di bawah mikroskop. Puncak amida A dan amide I menonjol dalam analisis FTIR. KKPH yang diekstrak dengan papain menunjukkan perencatan DPPH (35.10 ± 0.01%) dan ABTS (94.35 ± 0.01%) tertinggi, manakala KKPH yang diekstrak dengan bromelain mempunyai nilai FRAP tertinggi (11.21 ± 0.01µM). KKPH juga menunjukkan perencatan terhadap *Escherichia coli* dan *Pseudomonas aeruginosa*. KKPH yang diekstrak dengan papain mengandungi paling banyak asid amino. Selain itu, kajian *in siliko* mendedahkan bahawa chitinase daripada KKPH yang dihidrolisis dengan papain menunjukkan ciri antioksida yang paling berpotensi. Secara keseluruhannya, KKPH menunjukkan sumber protein alternatif yang berpotensi sebagai ramuan makanan fungsian, memandangkan kandungan proteinnya yang tinggi dan mempunyai ciri-ciri yang berfaedah.