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Precooked Jack Bean [*Canavalia ensiformis* (L.) DC] Sprout: Generation of Dipeptidyl Peptidase-IV Inhibitory Peptides during Simulated Digestion

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ABSTRACT: Bioactive peptides generated from jack bean sprouts are reported to function as dipeptidyl peptidase IV (DPP-IV) inhibitors. However, no studies have investigated the effect of precooking followed by simulated digestion using pepsin-pancreatin to increase DPP-IV inhibitory peptide generation in jack bean sprouts. Therefore, the present study aimed to explore the generation of DPP-IV inhibitory peptides from precooked jack bean [*Canavalia ensiformis* (L.) DC] sprouts during simulated digestion with pepsin-pancreatin. The results showed that peptide fractions of the sample hydrolysate with molecular weight <1 kDa exhibited the strongest DPP-IV inhibitory activity (84.77%±0.49%) after simulated digestion. This activity was slightly greater than that (74.12%±0.85%) observed prior to simulated digestion. These findings demonstrate that the DPP-IV inhibitory activity of precooked jack bean sprouts can be retained following simulated digestion. Moreover, our investigation revealed the sequences of two novel peptides following simulated digestion with critical amino acids. The presence of alanine and glycine at the penultimate N-terminus of AAGPKP and LGDLLK confirmed the presence of DPP-IV inhibitors. Both peptide sequences are nontoxic and interact with the catalytic sites of enzymes through hydrogen bonds.

Keywords: dipeptidyl-peptidase IV inhibitors, peptide fraction, peptide sequence, precooked jack bean sprout, simulated digestion

INTRODUCTION

Dipeptidyl peptidase IV (DPP-IV) inhibitory peptides can prevent the degradation of DPP-IV from degrading incretin hormones (Kristin, 2016). According to Kehinde and Sharma (2020), this inhibition can enhance the efficacy of incretins by triggering insulin production and reducing blood glucose levels. Several studies have identified peptides that inhibit DPP-IV from bean sprouts, including soybeans (González-Montoya et al., 2018), pigeon peas (*Cajanus cajan*) (Ohanenye et al., 2021), and common bean (*Phaseolus vulgaris*) (de Souza Rocha et al., 2015). Jack bean sprouts are a source of vegetable protein that contains hydrophobic amino acids (e.g., phenylalanine, proline, valine, leucine, isoleucine, methionine, and alanine) and DPP-IV inhibitory peptides with high inhibitory activity (Agustia et al., 2023a). It is important to retain the stability of bioactive peptides during food processing. Previous studies reported that the heat produced during food processing can affect the generation of DPP-IV inhibitory peptides (Mojica et al., 2015; Bunsroem et al., 2022). Therefore, thermal treatments might affect the application of jack bean sprouts as potential sources of DPP-IV inhibitory peptides. Zhao et al. (2024) found that heating promotes modifications in the protein's tertiary and quaternary structures. As a result, peptide bonds become reoriented, and the cutting sites become more visible. These changes make it easier for enzymes to attack proteins and increase peptide release.

In the gastrointestinal tract, peptides are degraded by digestive enzymes, including pepsin, pancreatin, thermolysin, and alcalase (Wang et al., 2019; Xu et al., 2019). The resistance of peptides to digestive enzyme activity is a factor that determines their bioavailability (Indrati, 2021). However, previous research reported that hydrolysis with digestive enzymes through simulated digestion might increase the activity of DPP-IV inhibitors. These inhibitory activities increased during enzymatic hydrolysis, followed by the fractionation processes of jack bean, pigeon pea, and soybean sprouts (González-Montoya et al., 2018; Ohanenye et al., 2021; Agustia et al., 2024). In particular, peptide fractions with a molecular weight (MW) less than

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1 kDa that function as DPP-IV inhibitors can be produced using the fractionation process of a dialysis membrane (Agustia et al., 2023a). According to Kehinde and Sharma (2020), the MW of DPP-IV inhibitors derived from legumes is typically <1 kDa. Because the active site of DPP-IV is extremely deep and narrow in the enzyme structure, the size of peptides significantly affects the inhibitory affinity of DPP-IV (Ding et al., 2022).

In addition, the amino acid sequence (Nongonierma et al., 2018b), hydrophobicity, and MW (Silveira et al., 2013; Ohanenye et al., 2021) can affect the activity of DPP-IV inhibitory peptides. Peptides that effectively inhibit DPP-IV usually contain polar group aromatic residues, including tryptophan, at N-terminus locations. Moreover, they contain additional branched-chain amino acids, including isoleucine, leucine, or valine (Nongonierma and Fitzgerald, 2014; Nongonierma et al., 2018a). Studies using molecular docking to identify DPP-IV inhibitory peptides and predict their bonds with DPP-IV are increasingly being conducted. You et al. (2022) reported that peptides IPI and IPV from pepsin-trypsin-hydrolyzed quinoa sprouts could bind to the His740, Asn710, and Ser630 residues of DPP-IV via hydrophobic interactions, hydrogen bonds, and electrostatic bonds.

However, no studies have investigated the effect of precooking followed by pepsin-pancreatin hydrolysis on jack bean sprouts as a possible supplier of DPP-IV inhibitory peptides. Hence, the present study aimed to explore the production of DPP-IV inhibitory peptides generated from precooked jack bean sprouts during simulated digestion using pepsin-pancreatin. Furthermore, the DPP-IV inhibitory peptides generated from simulated digestion were fractionated using dialysis membranes with molecular weight cut-off (MWCO) values of 1.0, 3.5, and 14 kDa and characterized to establish the peptide sequence that functions as a DPP-IV inhibitor and to compute the inhibitory peptide and DPP-IV binding configuration model. The novelty of this study is that it provides comprehensive information concerning the DPP-IV inhibitory peptide profile generated from precooked jack bean sprouts.

MATERIALS AND METHODS

Materials

Jack beans [*Canavalia ensiformis* (L.) DC.] were acquired from Wonogiri, Central Java, Indonesia. The reagents for simulated digestion and analysis included human recombinant DPP-IV enzyme (EC.3.4.14.5), Gly-Pro p-nitroanilide substrate (Sigma-G0513), pepsin from porcine gastric mucosa (EC.3.4.23.1), and pancreatin from porcine pancreas (EC.232-468-9) obtained from Sigma-Aldrich Co.. Sitagliptin (Januvia™) as a commercial inhibitor was obtained from MSD Pharmaceuticals, and MWCO dialysis membranes [Spectrum™ Spectra/Por™ RC (regenerated cellulose) Dialysis Membrane] with standard grade size of 1 kDa (Cat No. 08-670-12C), 3.5 kDa (Cat No. 08-670-5B), and 14 kDa (Cat No. 08-66-7D) were obtained from Fisher Scientific.

Preparation of precooked jack bean sprouts

Based on the methods of Agustia et al. (2023b), jack bean sprouts were obtained by germinating jack beans for 60 min. The word "precooked" in this study refers to sprout samples that underwent various processing methods, including boiling and oven drying. The samples were precooked by boiling them in water at 100°C for 0, 1, 2, 3, 4, and 5 min before being oven-dried at 55°C for 24 h and powdered (60-mesh sieve). Peptide extract was prepared in accordance with the methods of Agustia et al. (2023a). The samples were distilled in water (1:20) w/v at 30°C for 60 min and then centrifuged at 3,200 *g* for 20 min at 4°C (Sorvall™ ST 8 Centrifuge, Thermo Fisher Scientific Inc.). The obtained supernatant was referred to as the peptide extract, and its degree of hydrolysis (DH), peptide concentration, and DPP-IV inhibitory activity were measured.

Simulated digestion

Simulated digestion was performed in accordance with the methods of Agustia et al. (2024) with slight modifications. Precooked jack bean sprout was dissolved in distilled water at a ratio of 1:10 w/v. Thereafter, it was blended for 3 min (Stirrer Ultra Turrax Fluko FM30D, Fluko), incubated at 30°C for 60 min (Memmert Waterbath WNB 29, Memmert), and centrifuged at 3,200 *g* for 15 min (Sorvall™ ST 8 Centrifuge). About 2 mL of the supernatant, which contained a protein concentration of 5 mg/mL, was acidified to pH 3.0 by adding 1 M HCl. Subsequently, 0.32 mL of pepsin solution (2,000 U/mL) was combined with the samples and allowed to react for 2 h at 37°C. The hydrolysis process was monitored by taking samples every 30 – 120 min. The samples were immersed in hot water (95°C) for 15 min and then cooled in an ice bath to stop the reaction. The pH of samples was adjusted to 7 by adding 2 M NaOH. Then, the samples were centrifuged at 3,200 *g* for 10 min at 4°C and stored at −18°C until evaluated. The pH of samples with an unstopped pepsin solution was adjusted to 7.5 by adding 2 M NaOH. Hydrolysis with pancreatin was started by adding 5 mL of pancreatin (100 U/mL), followed by incubation at 37°C for 2 h. To observe the development of hydrolysis, samples were taken at 30-min intervals, starting at 150 min and continuing until 240 min. The hydrolysis reaction was stopped using the previous method, and 1 M HCl was added to set the pH to 7. The samples were centrifuged at 3,200 *g* for 10 min at 4°C and stored at −18°C until evaluated.

Analysis methods

***Protein solubility*:** The Lowry method determine the protein solubility. One milliliter was used to of protein extract and 0.9 mL of Lowry A were combined in a reaction tube. Following shaking incubation at 50°C for 10 min at 80 rpm (Memmert Waterbath WNB 29), the solution was chilled to room temperature and added with 0.1 mL of Lowry B. Afterward, the solution was incubated for 10 min at room temperature and then added with 3 mL of Lowry C. Subsequently, the solution was incubated at 50°C for 10 min at 80 rpm and chilled to 25°C−28°C. Ultraviolet-visible spectrophotometers (GENESYS 10S, Thermo Scientific) were utilized to measure the absorbance at 650 nm. The standard stock solution comprised 100 mg/L of bovine serum albumin.

***Peptide concentration and degree of hydrolysis (DH)*:** The analysis of DH and peptide concentrations was performed in accordance with the methods of Agustia et al. (2023a). The DH was estimated using the groups of free amino acids generated during simulated digestion, whereas peptide concentrations were measured from hydrolyzed samples at specific times.

***Dialysis using molecular weight cut-off (MWCO)*:** The peptides were fractionated using a dialysis membrane based on the MW, in accordance with the methods of Agustia et al. (2023a). Three dialysis membranes with MWCO values of 1, 3.5, and 14 kDa were used to gradually dialyze the hydrolysates with the most potent inhibitory activity. First, the dialysis membrane (1 kDa) was filled with 10 mL of hydrolysate, and membrane clips were used to fasten both edges of the membrane. This membrane was then placed in 90 mL of distilled water and shaken at 4°C for 12 h. The same procedures were applied to transfer the residual peptide solution to the 3.5 kDa membrane. Meanwhile, other solutions were filtered using the 14 kDa membrane. The identified peptide fractions had a MW of <1.0, 1.0−3.5, 3.5−14, and >14 kDa. The distribution of peptide fractions and DPP-IV inhibitory activities were examined in all fractions.

***Dipeptidyl peptidase IV (DPP-IV) inhibitory activity*:** The DPP-IV inhibitory activity was assessed using the protocol of Nongonierma et al. (2018b) with slight modifications. First, 25 μL of hydrolysate was added with Tris-HCl buffer (100 mM, pH 8.0) and placed in a 96-well microplate (Biologix 07-6096 Sterile Cell Culture Plate, Biologix). Then, 50 μL of Gly-Pro p-nitroanilide substrate (0.2 mM) and 50 μL of 0.0025 U/mL DPP-IV were added to the microplate. The 96-well microplate was placed in a shaking water bath (WB-4MS, Biosan) and incubated at 37°C for 60 min to activate the enzymatic reaction. The absorbance of each well was detected using a spectrophotometer at 405 nm (ELISA Reader 270, Biomerieux). The DPP-IV inhibitory activity was calculated using the following equation:

$$\text{DPP-IV Inhibitory Activity (\%)} = 1 - \frac{(T-B)}{(P-N)} \times 100$$

where T is the absorbance of the test sample (sample+enzyme+substrate), B is the absorbance of the blank sample (sample+buffer+substrate), P is the absorbance of the positive control (enzyme+substrate+buffer), and N is the absorbance of the negative control (substrate+buffer).

***Peptide sequence identification*:** Peptide sequences were identified based on the method of Fitriani et al. (2022). Liquid chromatography-mass spectrometry (LC-MS; Nano LC Ultimate 3000 series system tandem Q Exactive Plus Orbitrap HRMS, Thermo Scientific) was used to characterize and identify the peptide fraction from simulated digestion that exhibited the strongest DPP-IV inhibitory effect. The LC-MS system was outfitted with a trap column (Thermo Fisher Scientific 164649, 5 mm in length and 30 μm in diameter) and a capillary column (PepMap RSLC C18, 100 pore size and 3 μm particle size, 75 μm× 15 cm, part number ES 800). The fractionated peptide extract was dissolved using a water-acetonitrile solution (ratio of 8:2 by volume). Next, 50 μL of solution was inserted into the LC-MS system by injection. The mobile phase consisted of two solvents: solvent A, which was a solution of water and 0.1% formic acid, and solvent B, which was a solution of acetonitrile and 0.1% formic acid. The flow rate was adjusted to 300 nL per min, and the mass range was set between 200 mass-to-charge ratio units and 2,000 mass-to-charge ratio units. The eluents in the gradient consisted of 2% B from 0 min to 2 min, a linear increase from 2% to 99% B from 3 min to 40 min, 99% B from 40 min to 55 min, and 2% B from 55 to 60 min. Then, the peptide sequence was identified using Proteome Discoverer 2.2 (Thermo Fisher Scientific Inc.).

***Evaluation of peptide sequence bioactivity*:** BIOPEP-UWM was used to evaluate the potential bioactivity of the fractionated peptide in accordance with the methods of Fitriani et al. (2022) (https://biochemia.uwm.edu.pl/biopep-uwm, accessed April 24, 2024). The bioactivity of peptide sequences was determined based on the bioactive fragment frequency and the protein fragment bioactivity from the database. Afterward, the toxicity of peptide sequences was examined using the ToxinPred database (https://webs.iiitd.edu.in/raghava/toxinpred/, accessed April 25, 2024).

Computational modeling for peptide and DPP-IV bond structure

***Preparation of molecules*:** The peptide sequence was inserted into chimera 1.16 to build the peptide three dimensional (3D) structure (Haddad et al., 2020). In this study, sitagliptin was used as a control (commercial) inhibitor, and

the 3D structure was gained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) with CID 4369359. Then, the PyRx software (Autodock Vina) was used to create a 3D structure of peptides and control by lowering energy with an open babel. PDB ID 3VJK was also used to acquire the 3D structure of the DPP-IV enzyme from the Protein Data Bank (https://www.rcsb.org/). Next, extraneous ligands and water were eliminated using BIOVIA Discovery Studio to prepare the enzyme structure.

***Molecular docking*:** A specific docking method was used for the molecular docking process. The grid settings for the Centre were X:62, Y:59, and Z:36, and those for the Dimension (Angstrom) were X:19, Y:15, and Z:19. The catalytic sites of the DPP-IV enzyme, located at His740, Asp708, and Ser630, were assigned a grid box (Ojeda-Montes et al., 2018). Moreover, PyRx and BIOVIA Discovery Studio tools were utilized for molecular docking and visualization (Nafisah et al., 2022).

Statistical analysis

Data were analyzed using the independent sample *t*-test and analysis of variance. Duncan's multiple range test was used to analyze for significant differences ($P<0.05$). IBM SPSS Statistics 26 (IBM Corp.) were used for all analyses.

RESULTS AND DISCUSSION

Protein solubility of precooked jack bean sprouts

The protein solubility of precooked jack bean sprouts is shown in Fig. 1A. The protein solubility of precooked jack bean sprouts significantly decreased ($P<0.05$) from 24.35%±0.05% dry weight at the beginning to 15.37%±0.1% dry weight after boiling for 5 min. This decrease is because using high heat can denature and coagulate proteins. Sashikala et al. (2015) found that heating proteins between 50°C and 80°C will cause denaturation. Proteins that have undergone denaturation may become less soluble in water because of changes in their structure. Moreover, protein denaturation changes or modifies the protein conformation of tertiary and quaternary structures without breaking covalent connections, causing the protein to precipitate (Nikfarjam et al., 2020). In a previous study, legume cultivars were heated to enhance their flavor and minimize anti-nutritional elements, thereby making them safe for ingestion; however, this process can lead to significant compositional changes (Sashikala et al., 2015).

DPP-IV inhibitory activity of precooked jack bean sprout

The DPP-IV inhibitory activity of precooked jack bean sprouts increased from 41.57%±0.34% before boiling to 46.16%±0.29% after boiling for 5 min (Fig. 1B). Protein hydrolysis can occur during boiling, leading to the formation of bioactive peptides that inhibit DPP-IV activity. According to Hernández-Ledesma et al. (2011), bioactive peptides could be formed throughout food processing under heat or alkaline conditions. Before precooking, the jack bean sprouts had high DPP-IV inhibitory activity (41.57%±0.34%) because of the existence of precursor amino acids that dominate the DPP-IV inhibitory peptide, including positively charged amino acids (lysine and arginine), negatively charged amino acids (aspartic acid and glutamic acid), and hydrophobic amino acids (alanine, leucine, phenylalanine, and proline) (Agustia et al., 2023a).

Precooking produces heat, which enhances the DPP-IV inhibitory activity. Meanwhile, denaturation promotes the release of inhibitory peptides from DPP-IV. Similarly, Harnedy-Rothwell et al. (2021) reported that heat treatment of a tomato-based product (soup and juice) for 1 min (90°C) increased the DPP-IV inhibitory activity. Furthermore, the inhibitory activities assessed in the pres-

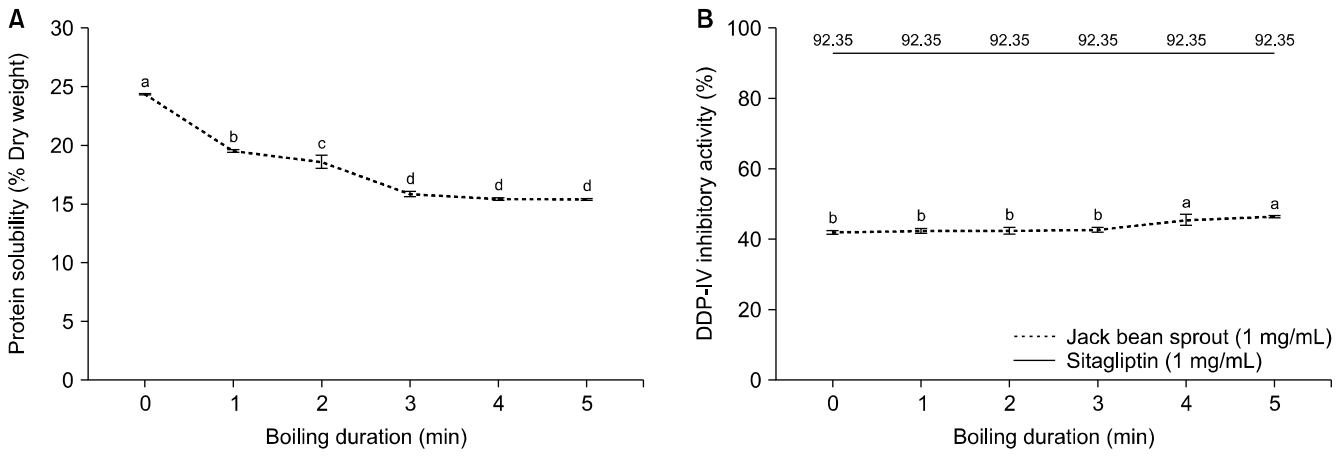

Fig. 1. Protein solubility and DPP-IV inhibitory activity of jack bean sprout during boiling treatment. Mean value±standard deviation of three replications. Different letters show significant differences (Duncan's multiple range test, $P<0.05$). DPP-IV, dipeptidyl peptidase IV.

ent study surpassed those of *Amaranthus hypochondriacus* L. observed by López-Sánchez et al. (2016). However, the strongest DPP-IV inhibitory activity obtained in this study (46.16%±0.29%) was lower than that of the commercial drug sitagliptin (92.35%). Jack bean sprout samples precooked for 5 min were then hydrolyzed with pepsin-pancreatin and assessed for DPP-IV inhibitory activity.

Simulated digestion using pepsin-pancreatin

***DH and peptide concentration*:** The percentage of DH and peptide concentration were investigated to analyze the improvement in DPP-IV inhibitory activity after simulated digestion using pepsin followed by pancreatin for 240 min. Pepsin-pancreatin hydrolysis releases free amino groups and peptides, which are expressed as a percentage (%) by DH. These tiny peptides played a role in the inhibitory activity. The DH significantly increased ($P<0.05$) after pepsin-pancreatin hydrolysis (Fig. 2A). When comparing pepsin hydrolysis to pancreatin hydrolysis, the rise in the DH was not statistically significant. In particular, the DH significantly increased at 150 min of hydrolysis, peaking at 210 min (60.62%±1.12%). After pepsin-pancreatin hydrolysis, the DH was 58.48%±0.92%. This value is slightly greater than that of cooked koro kratok (*Phaseolus lunatus* L.) tempeh (43.23%) and boiled pigeon pea (*Cajanus cajan*) tempeh (54%) obtained in previous studies (Pertiwi et al., 2020; Putra et al., 2021). These findings show that precooking effectively improves protein breakdown through pepsin and pancreatin, which increase the DH. In this study, pepsin hydrolysis samples had lower DH than pancreatin hydrolysis samples. Every enzyme has a distinct selectivity that influences the DH and the released peptide. A high DH indicates that the digestive enzyme pancreatin (chymotrypsin, trypsin, exoprotease such as carboxypeptidase A and B, and elastase) is more selective than pepsin at creating short peptides (Andriamihaja et al., 2013). Putra et al. (2021) discovered that pepsin hydrolysis had lower DH than pancreatin hydrolysis during the simulated digestion of boiled pigeon pea (*Cajanus cajan*) tempeh.

The increased peptide concentrations during pepsin-pancreatin hydrolysis are shown in Fig. 2A. Higher DH values lead to higher peptide concentrations. After pepsin-pancreatin hydrolysis, precooked jack bean sprouts had a peptide concentration of 62.38±1.03 mg/g. These findings were comparable to those of Pertiwi et al. (2020), who found that cooked koro kratok (*Phaseolus lunatus* L.) tempeh produced more peptides and amino acids than raw tempeh. Food processing may affect peptide synthesis during digestion. The heating procedure modifies the tertiary and quaternary structures of proteins. This leads to peptide bond reorientation, which promotes the release of peptides by exposing cleavage sites and increasing the susceptibility of proteins to enzyme activity (Michael et al., 2018).

***DPP-IV inhibitory activity*:** Simulated digestion enhanced the DPP-IV inhibitory activity of precooked jack bean sprouts (Fig. 2B) and showed significant differences ($P<0.05$) between each sample. This improvement is likely because of the degradation of bioactive peptides during simulated digestion. This process produces novel peptides that effectively inhibit DPP-IV (Agustia et al., 2024). In this study, precooked jack bean sprouts had the strongest DPP-IV inhibitory activity (62.72%±0.92%) after simulated digestion with pepsin-pancreatin for 180 min. This result indicates that the 180-min digestion period inhibits DPP-IV more effectively than other periods. This enhancement may be because this period generates more peptides with a low MW and a DPP-IV-specific structure. Moreover, the inhibitory activity of DPP-IV remained constant from 210 to 240 min, even though the peptide concentration and DH data changed as the enzymes broke down proteins. This phenomenon is possible be-

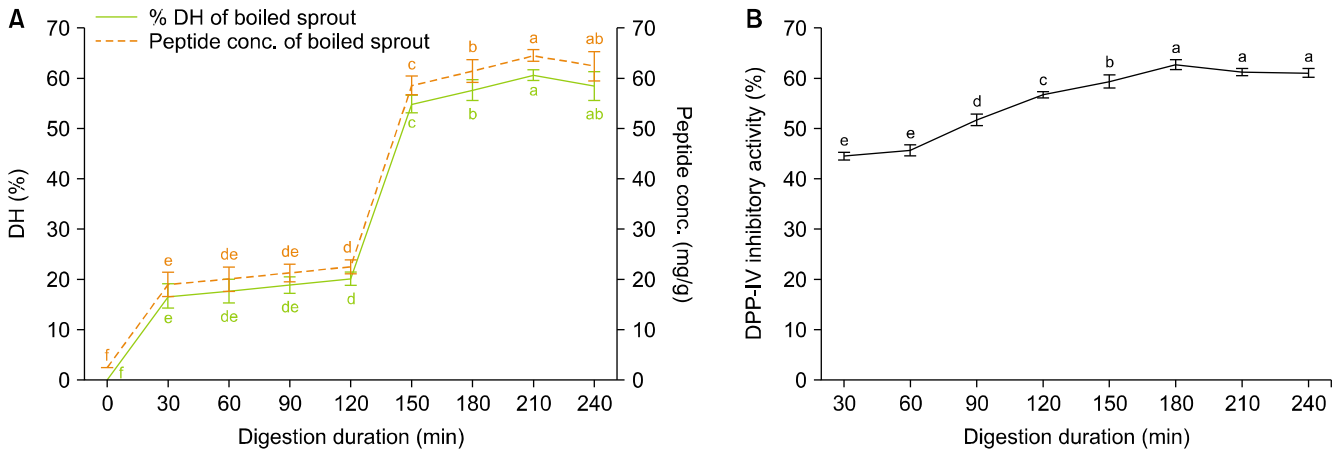

Fig. 2. Peptide concentration (mg/g), DH (%), and DPP-IV inhibitory activity (%) of precooked jack bean sprout during 240 min simulated digestion by pepsin-pancreatin. Mean value±standard deviation of three replications. Different letters show significant differences (Duncan's multiple range test, $P<0.05$). DH, degree of hydrolysis; DPP-IV, dipeptidyl peptidase IV; conc., concentration.

Table 1. Percentage of peptides and DPP-IV inhibitory activity of peptide hydrolysate fractions of precooked jack bean sprout

Fraction (kDa)	Before simulated digestion		After simulated digestion	
	Percentage of peptide (%)	DPP-IV inhibitory activity (%)	Percentage of peptide (%)	DPP-IV inhibitory activity (%)
<1	38.53±0.61^{Ab}	74.12±0.85^{Ab}	72.01±0.79^{Aa}	84.77±0.49^{Aa}
1-3.5	17.94±0.58^{Da}	65.18±0.32^{Bb}	14.85±0.49^{Bb}	72.84±0.32^{Ba}
3.5-14	19.85±0.87^{Ca}	59.53±0.98^{Ca}	11.08±0.95^{Cb}	56.76±0.67^{Db}
>14	23.67±0.58^{Ba}	56.23±0.64^{Db}	2.06±0.61^{Db}	61.77±0.80^{Ca}

Mean value±standard deviation of three replications.
Superscript (a, b) in every fraction in parameter percentage of peptide before and after simulated digestion show significant differences (*t*-test, $P<0.05$).
Superscript (a, b) in every fraction in parameter DPP-IV inhibitory activity before and after simulated digestion show significant differences (*t*-test, $P<0.05$).
DPP-IV, dipeptidyl peptidase IV.

cause peptides are constantly breaking down. The structure and sequences of peptides enable them to bind to the DPP-IV enzyme, which has a strong inhibitory effect.

***Molecular weight (MW) distribution*:** According to the data presented above, an elevation in DH (which leads to shorter peptides) is associated with an elevation in DPP-IV inhibitory activity. Consequently, we used dialysis membranes to fractionate the MW. Statistical data showed significant ($P<0.05$) differences between each sample in a different fraction and also in each fraction in a different sample (before or after simulated digestion). The maximum percentage of peptide is seen in the MW <1 kDa fraction before and after simulated digestion (Table 1). After simulated digestion, the rate of peptides with a MW <1 kDa increased. This improvement was because of the increased production of low-MW peptides during pepsin-pancreatin hydrolysis. The percentage of peptides with higher MW (i.e., 3.5, 3.5 – 14, and >14 kDa) will decrease as the percentage of peptides with MW <1 kDa increases. Wang et al. (2019) found that the structural stability of high-MW peptides during simulated digestion was weaker than that of low-MW peptides. The samples also had the strongest DPP-IV inhibitory activity at <1 kDa before and after simulated digestion (Table 1). There was a correlation between smaller peptide sizes and higher levels of DPP-IV inhibitors. According to You et al. (2022), peptides with a MW <1 kDa showed the strongest DPP-IV inhibitory activity in quinoa (*Chenopodium quinoa* Willd.) sprouts. Moreover, they had higher concentrations of hydrophobic amino acids particularly around the N-terminus. The low MW of peptides enhances the accessibility of the enzyme's active site (Ding et al., 2022).

***Peptide sequence*:** The MW <1 kDa peptide fraction had the strongest inhibitory effect against DPP-IV. Therefore, the peptide sequence of this sample was determined using LC-MS and further analyzed using Proteome Discoverer 2.2. Moreover, the potential bioactivity of the peptide sequence was identified through analysis using the BIOPEP-UWM database. Two peptide sequences with a MW between 540 and 658 Da and length of six amino acids were identified (Table 2). These peptide sequence fragments were dipeptides and tripeptides that exhibit multiple bioactivities, including DPP-IV and angiotensin-converting enzyme (ACE) inhibition. Mojica et al. (2015) also found the LL peptide fragment in precooked common bean (*Phaseolus vulgaris*) peptide sequences, which has potential bioactivity as a DPP-IV and ACE inhibitor. AAGPKP and LGDLLK peptide sequences demonstrated hydrophobicity at the N-terminus. According to González-Montoya et al. (2018), DPP-IV can be inhibited by the hydrophobic amino acids present in the N-terminus of soybean sprout peptide. The hydrophobic DPP-IV binding site has an affinity for hydrophobic amino acids, including alanine (A), isoleucine (I), and leucine (L) (Nongonierma and Fitzgerald, 2014). The active site of DPP-IV may create hydrogen bonds and hydrophobic interactions with peptides that have hydrophobic residues at the N-termi-

Table 2. Peptide sequences of precooked jack bean sprout acquired from simulated digestion (MW<1 kDa peptide fraction)

Number	Peptide sequence	MW (Da)	Toxicity prediction	Activity	Frequency of bioactive fragments	Potential bioactivity of protein fragments	Fragments peptide	Accession number
1	AAGPKP	540.31	Nontoxin	DPP-IV *inhibitor*	0.83	0.00010	GP, KP, AA, AG, PK	A0A0D3D0B2
			Nontoxin	ACE *inhibitor*	0.83	0.00887	GP, AA, AG, KP, AGP	
2	LGDLLK	658.42	Nontoxin	DPP-IV *inhibitor*	0.17	0.0000001	LL	A0A444XD59
			Nontoxin	ACE *inhibitor*	0.50	0.000037	LG, GD, DL	

MW, molecular weight.

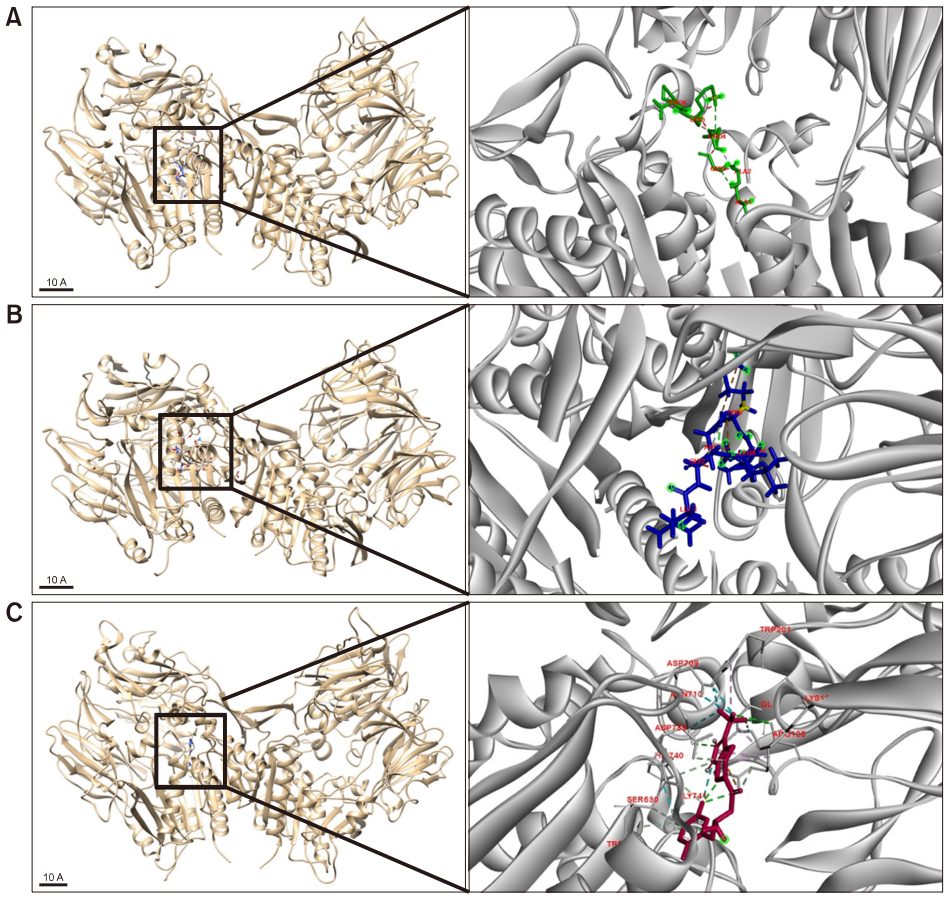

Fig. 3. Interaction of DPP-IV enzyme and (A) AAGPKP, (B) LGDLLK, and (C) sitagliptin. The cream-colored, ribbon-like structure depicts DPP-IV (scale bar: 1×10^{-9} m), and objects in green, dark blue, and red color indicate AAGPKP, LGDLLK, and sitagliptin, respectively. DPP-IV, dipeptidyl peptidase IV.

nus (Ojeda-Montes et al., 2018). AAGPKP and LGDLLK peptide sequences also have alanine and glycine at the penultimate N-terminus. Moreover, the DPP-IV enzyme cleaves alanine, proline, and glycine residues at the penultimate N-terminus (Rivero-Pino et al., 2020). These peptide sequences are believed to function as a DPP-IV inhibitor. Additionally, we used the ToxinPred database to evaluate the toxicity of peptide sequences. The nontoxic properties displayed by both peptide sequences suggest that the peptide obtained from precooked jack bean sprouts is safe to eat.

***Molecular docking*:** Predicting the bond configuration of the peptide inhibitor and the DPP-IV enzyme through computer simulations provides a detailed visualization of the binding structure, binding energy, and dynamic processes associated with the formation of the enzyme-peptide complex. AAGPKP and LGDLLK peptide sequences exhibited an ideal conformation upon binding to DPP-IV (Fig. 3). AAGPKP has a lower binding energy (−4.6 kcal/mol) than LGDLLK (−1.4 kcal/mol) and sitagliptin (−3.7 kcal/mol) (Table 3). When the binding energy is lower, the peptide and DPP-IV exhibit a more stable configuration and a more robust connection (You et al., 2022). The AAGPKP peptide sequence provides the lowest binding energy and has a more powerful interaction than sitagliptin and LGDLLK. According to Nongonierma et al. (2018a), the peptide containing glycine and alanine at its penultimate N-terminus may bind to the DPP-IV active site through a competitive form of inhibition. The enzyme's catalytic triad site, which includes His740, Asp708,

Table 3. Binding energy and hydrogen bond interaction of peptides and DPP-IV

Number	Peptide	Binding energy (kcal/mol)	Amino acid residue	Interaction category
1	AAGPKP	−4.6	Lys122, Asp708, His740, Gly741, Val546, Glu205	Hydrogen bond
2	LGDLLK	−1.4	Lys122, Arg125, Asn710, Asp739, Gln123, Tyr547, Ser630, Asp739, Glt741	Hydrogen bond
3	Sitagliptin	−3.7	Arg125, Asn710, Gly741, Lys122, Trp629, Ser630, His740, Asp739	Hydrogen bond
			Glu205, Asp709, Asn710, His740	Halogen
			Arg125	Electrostatic
			Arg125, Trp201	Hydrophobic

DPP-IV, dipeptidyl peptidase IV.

and Ser630, was the interaction site for both peptide sequences through the interaction of hydrogen bonds. According to You et al. (2022), catalytic residues are involved in the inhibitory activity of peptides.

Precooking followed by pepsin-pancreatin hydrolysis modifies the protein's structures. This process leads to the release of inhibitory peptides from DPP-IV. The jack bean sprout precooked for 5 min and then hydrolyzed with pepsin-pancreatin for 180 min showed the strongest DPP-IV inhibitory effect. Moreover, MW<1 kDa peptide fractions exhibited greater DPP-IV inhibitory activity after undergoing pepsin-pancreatin hydrolysis than before hydrolysis. This finding indicates that peptides with lower MW have a stronger inhibitory effect than those with higher MW. Additionally, the AAGPKP and LGDLLK peptide sequences of the MW<1 kDa peptide fraction contain alanine and glycine at the penultimate N-terminus, confirming the presence of DPP-IV inhibitors. The BIOPEP-UWM database indicated that the precooked jack bean sprout sequences have the ability to inhibit ACE. However, more studies are needed to provide conclusive evidence. Both nontoxic peptide sequences interact with the catalytic sites of enzymes through hydrogen bonds. Precooked jack bean sprouts could serve as a food source for DPP-IV inhibitors. However, more studies are needed to focus on how DPP-IV inhibitory peptides are absorbed in the small intestine.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: FCA. Analysis and interpretation: FCA, DUP. Data collection: FCA, UFR. Writing the article: FCA. Critical revision of the article: DUP, UFR. Final approval of the article: all authors. Statistical analysis: UFR. Obtained funding: FCA, DUP, UFR. Overall responsibility: FCA.

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