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Chemical characterization and angiotensin-I converting enzyme inhibitory activity of Indonesian stinky beans

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Abstract

The chemical composition of four Indonesian stinky beans has been explored. Those bean are Petai (*Parkia speciosa*), Lamtoro Gung (*Leucaena leucochepala* ssp. Glabrata (Rose) S. Zarate), Jengkol (*Pithecellobium jiringa*) and Kabau (*Archidendron bubalinum*). This exploration belongs to the local food utilization research theme of functional food sources. Those beans have a unique flavour and usually lead to foul stools, sweat, urine or mouth odour. Their intense flavour may be due to the content of amino acids. High hydrophobic and negative charge hydrophilic amino acids are crucial in preventing hypertension through inhibition of angiotensin I-converting enzyme (ACE) activity. This study evaluated the chemical properties of beans and its angiotensin I-converting enzyme inhibitory (ACE-I) peptides. The amino acid profile was determined as preliminary data to ensure the content of hydrophobic and negative charge hydrophilic amino acids. Their ability to inhibit ACE was studied to represent an antihypertensive effect. *In vitro* gastrointestinal digestion was applied to obtain more small molecular peptides and evaluate their ACE-I activity after digestion. Petai has the highest glutamic acid (20.90 g/100 g protein d.b.), lysine (13.31 g/100 g protein d.b.), arginine (11.76 g/100 g protein d.b.), leucine (10.98 g/100 g protein d.b.), and aspartic acid (10.72 g/100 g protein d.b.). Fresh Jengkol has the highest ACE-I activity (67.59%). Subsequential digestion by pepsin and pancreatin improves the ACE-I activity, and Kabau had the highest (79.87%). Based on the results, Indonesian stinky beans can prevent hypertension by inhibiting ACE activity and being used as a protein source alternative.

1. Introduction

Legumes belong to the Leguminosae family. In tropical countries, legumes are the vital food crops after cereals. Legumes have been essential for food security due to their agricultural and nutritional properties (Calles and Xipsiti, 2019; Edwards *et al.,* 2020). They also have been a critical part of the human diet, considering that their regular intake is associated with degenerative disease prevention (Zhang *et al.,* 2010; Siow and Gan, 2013; Agrawal *et al.,* 2016; Edwards *et al.,* 2020). Legumes have a low-fat content and a high amount of bioactive compounds to contribute to the reduced risk of diet-related diseases (Calles and Xipsiti, 2019; Edwards *et al.,* 2020). They are also high in nutritional compounds, which can play a role in combatting malnutrition and undernourishment (Calles and Xipsiti, 2019; James *et al.,* 2020).

Petai (*Parkia speciosa*), Lamtoro Gung (*Leucaena leucochepala* ssp. Glabrata (Rose) S. Zarate), Jengkol (*Pithecellobium jiringa*), and Kabau (*Archidendron bubalinum*) belong to Leguminosae family. These are Indonesian legumes but are primarily found in other tropical regions, such as Singapore, Malaysia, Thailand and the Philippines (Bhattacharyya and Babu, 2006; Orwa *et al*., 2009; Maxiselly *et al.,* 2017). Petai, Jengkol, and Kabau are often seasonal. However, Lamtoro Gung can be found daily. Petai and Kabau are often consumed in a fresh form. These legumes can be consumed without cooking, only fresh beans that are freshly harvested then cleaned and washed, peeled and eaten as rice

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complement. In Indonesia, consumed fresh vegetables-like dish was known as lalapan. However, Jengkol and Lamtoro Gung are often processed before being consumed.

The amino acid profile of stinky beans needs to be evaluated to know how much hydrophobic and negative charge amino acids are. The presence of those amino acids is essential for the antihypertensive effect. This effect is through its ability to inhibit the activity of ACE (angiotensin-I converting enzyme). Hydrophobic amino acids built hydrophobic interaction between the peptides and the amino acids residue at ACE's active site. This hydrophobic interaction disturbs the active site conformation (Fan *et al.,* 2018). Meanwhile, negatively charged hydrophilic amino acids, such as Asp and Glu, support ACE-I activity through the electrostatic interaction with the Zn^{2+} at ACE's active site (Natesh *et al.,* 2003; Aluko, 2015).

Angiotensin I-converting enzyme (ACE) is essential in blood pressure regulation. ACE convert passive angiotensin I to active angiotensin II. Angiotensin II activates vasoconstrictors and increases blood pressure (Bünning and Riordan, 1983). ACE-I peptides prevent increasing blood pressure by inhibiting ACE activity to hydrolyze angiotensin I to angiotensin II (Li *et al.,* 2018). ACE-I peptides can be obtained from protein sources. Legumes (Margatan *et al.,* 2013; Li *et al.,* 2018; Pertiwi *et al.,* 2019; Hermanto *et al.,* 2019; Puspitojati *et al.,* 2019b, 2019a; Putra *et al.,* 2020) are the most commodities had been studied. The others are animal products (Terashima *et al.,* 2010; Escudero *et al.,* 2012), marine products (Alemán *et al.,* 2011; Lee *et al.,* 2011; Wijesekara *et al.,* 2011; Zhao *et al.,* 2019), and dairy products (Saito *et al.,* 2000). The inhibition activity depends on the molecular weight and amino acid composition. Most of them are small peptides called bioactive peptides (de Castro and Sato, 2015; Fan *et al.,* 2018). Enzymatic hydrolysis is frequently used to achieve small peptides. During digestion, there are many proteolytic enzymes, such as pepsin, trypsin, and pancreatin. They degrade high molecular weight peptides to produce the small ones (Puspitojati *et al.,* 2019b) and increase the ACE-I activity (Ratnayani *et al.,* 2019a).

The ACE inhibitory activity of Kabau, Jengkol, and Lamtoro Gung had not been found, but Petai had been studied (Siow and Gan, 2013). However, the effect of in vitro gastrointestinal simulation of all legumes had not been found. This study aimed to explore the chemical composition of Indonesian stinky beans and utilize it as a source of the antihypertensive peptide. Therefore, this article focused on the potential of the stinky bean as a source of ACE based on amino acids' character. *In vitro* gastrointestinal simulation was applied to evaluate their ACE-I activity after digestion.

2. Materials and methods

2.1 Sample preparation

Fresh materials were obtained from the traditional yard. Petai and Jengkol are from Magelang, Lamtoro Gung from Sleman, and Kabau from Bengkulu, Indonesia. The outer skin of those materials was removed, and the peas were washed with tap water and then drained. The fresh peas were immediately chopped and frozen, then dried with a freeze dryer (-40°C, 48 hrs, 0.001 bar). The dried materials were then powdered and sieved at 60 mesh.

2.2 Materials

The solvents and chemical reagents used for the extraction and component analysis were analytical grades, such as petroleum ether, HCL, H_2SO_4, 4% boric acid, NaOH, O-Phthalaldehyde (Merck 111452), ACE (Sigma A6778), Hip-His-Leu (Sigma 859052), HEPES sodium salt (sigma H7006), pepsin (Sigma 77161), pancreatin (Sigma P1750). Solvents and reagents used for amino acid profile analysis were HPLC grade, and they were purified through a Millipore filter (0.45 μm). The amino acid standard was used to quantify the amino acid profile as an external standard.

2.3 In vitro gastrointestinal digestion

Simulation gastrointestinal digestion followed Almeida *et al*. (2015) with slight modification. A total of 250 mg of each sample and 250 μL deionized water (for the blank) were dissolved in 15 mL 0.1 mol/L HCl containing 1.5 mg/mL pepsin (pH 2). The solution was incubated in a water bath shaker (2 hrs, 37°C, 80 rpm). 7.5 mL of 0.5 mol/L NaOH was added to neutralize the solution. Pancreatic digestion started with the addition of 10 mL of 0.2 mol/L phosphate buffer (pH 8) containing 10 mg of pancreatin and was incubated with a water bath shaker (2 hrs, 37°C, 80 rpm). The reaction terminated with the addition of 10% (w/v) trichloroacetic acid. The solution was then centrifugated at 4000×*g* for 20 mins at 4°C. The supernatant was collected for the ACE-I activity assay.

2.4 Determination of chemical composition

The proximate components of peas were determined by AOAC procedures (Maharani *et al.,* 2022; Yovani *et al.,* 2022). Moisture, protein, lipid, ash, and carbohydrate contents were determined based on oven drying methods, micro-Kjeldahl, soxhlet extraction procedure, dry ashing method, and carbohydrate by difference (Bender, 2009). The crude fibre was analyzed by acid hydrolysis based

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on Madhu *et al*. (2017). According to Merrill and Watt (1973), the total energy was calculated based on the Atwater Bryant factor.

2.5 Crude fibre analysis

Approximately 2 g of sample powder were poured into the Erlenmeyer flask. A total of 0.5 g of boiling stone and three drops of antifoam agent were added. Then, 200 mL of H_2SO_4 was added. The flask was closed with a condenser and boiled for 30 mins. The remaining suspension was filtered through filter paper. The residue was washed with boiled water until the washing was no longer acidic.

The residue was removed, boiled NaOH was added, closed with a condenser, and boiled for 30 mins. The suspension was filtered through filter paper and washed with 10% K_2SO_4 solution. The residue was rewashed with boiled water and 95% alcohol, and then it was removed and transferred to an ashing dish (preweighed dish). The residue was dried for 2 hrs at 100°C until the constant weight was achieved. The crude fibre was calculated according to Equation 1.

$$\%\text{crude fibre} = \frac{(W2-W1)-(W3-W1)}{\text{weight of the sample}} \times 100$$

Where W1: pre-weight dish, W2: Initial dish and samples (g) and W3: Final dish and samples (g)

2.6 Amino acid profile determination

2.6.1 Sample preparation

According to Dai *et al.* (2014) and Fitriani *et al.* (2021), the amino acid was determined with slight modification. One gram samples were hydrolyzed with 20 mL 6 M HCl in an autoclave (110°C for 12 hrs). Then, the solution was neutralized with 20 mL 6 M NaOH and adjusted until 50 mL. The solution was diluted 50 times. Finally, it was filtered using 0.22 µM filter paper, and a 5 µL solution was injected into LC-MS/MS.

2.6.2 Mobile phase preparation

Mobile phase A was 0.1% pentadecafluorooctanoic acid (PDFOA), which consists of water/CH_3CN (99.5%/0.5%) with 0.1% formic acid. Mobile phase B was 0.1% (PDFOA), which consists of water/CH_3CN (10%/90%) with 0.1% formic acid.

2.6.3 HPLC conditioning

Amino acid determination was used HPLC system equipped with SCL-10 A VP, LC-10 AD VP controller system pump, and MS/MS detector, with excitation and emission wavelengths of 340 nm at 455 nm, respectively (Shimadzu, Kyoto, Japan). The column was a euro sphere C18 (4.6×250 mm, 5 µm). Amino acids were separated with a gradient separation system at a 1.1 mL/min flow rate. The gradient elution scheme can be seen in Table 1. Amino acid quantification using amino acid standard.

Table 1. Gradient elution scheme for amino acid profile determination.

Time (mins)	Solvent A (%)	Solvent B (%)
0	90	10
5	50	50
5.2	90	10
6	90	10

2.7 ACE-I activity assay

2.7.1 Sample preparation

Peptide from each stinky bean was extracted by maceration methods (Fitriani *et al.,* 2022). Distilled water was used as the solvent. 1 g powdered sample was dissolved in 10 mL distilled water. The solutions were incubated on a water bath shaker (30°C; 1 hr) and centrifuged. The remaining supernatant was the peptide extract. Captopril was used as a positive control. The concentration of peptide extract and Captopril must be the same.

2.7.2 ACE-I activity determination

With slight modification, ACE-I activity was determined following Fitriani *et al*. (2022). Eight mM substrate (Hip-His-Leu) was prepared with dissolved 0.0034 g HHL on 1 mL 50 mM HEPES buffer containing 300 mM NaCl pH 8.3. The 50 µL sample and 50 µL substrate were poured into a 2 mL Eppendorf tube and incubated at a water bath shaker (37°C; 10 mins). In this step, for the blank and control solution, use distilled water. The HCl was added first in the blank one. 50 µL (92.5 mU/mL) ACE was added and incubated (37°C; 30 mins). The reaction was terminated with the addition of 200 µL 1 M HCl. As the enzymatic reaction product, Hippuric acid was extracted with an additional 1.5 mL of ethyl acetate. It is extracted by the vortex shaking with the highest speed (the hippuric acid extraction needs intense collision) for 2 mins. It was centrifugated using Eppendorf centrifugation (4000 rpm; 15 mins). One millimetre of supernatant was removed from the reaction tube. The solvent was evaporated with boiling water until dried. Approximately 3 mL of bidistilled water was added and homogenized. Finally, the absorbance was read at 228 nm with a spectrophotometer UV-Vis. The inhibitory activity was calculated according to Equation 2.

$$\%\text{ACE} - \text{I activity} = \frac{(A-B)}{(A-C)} \times 100$$

Where A: absorbance of control solution, B:

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absorbance of sample solution and C: absorbance of blank solution

2.8 Data analysis

This experiment has four units of the variance of samples. There were two experimental replications and three analytical replications. Furthermore, there were six raw data for each unit experiment. Data were tested with Analysis of Variance (ANOVA), and if the treatment had a significant effect, post hoc testing was carried out with Duncan's Multiple Range Test (DMRT).

3. Results and discussion

3.1 Chemical composition of beans

Table 2 shows the chemical composition of each bean. Moisture content is one of the most fundamental parameters of a food product (Nielsen, 2010). Jengkol had the significantly highest moisture content, up to 9.48%, and Kabau had the lowest, 4.62%. The drying process causes the low moisture content to result in the powder form. The high moisture content in the beans indicated that a lot of water was bound tightly to the material, which could not be evaporated during the drying process. The moisture content of four stinky beans agrees with James (James *et al.,* 2020), who reported that some selected lesser-known legumes from Nigeria ranged from 9.00% to 12.75%. The moisture content in this study also agrees with Edwards *et al*. (2020), who stated the moisture content of some pulse flour ranged from 3.6 to 13.8%.

Ash content indicates the presence of mineral components in a food matrix. It refers to the inorganic residue after the complete ignition of foodstuffs (Nielsen, 2010). Lamtoro Gung had the highest ash content (4.18%), followed by Petai (3.93%). Jengkol and Kabau had the same value of ash content; each one was 1.81%. The Jengkol ash content of this study is high compared with the Jengkol ash content (0.5%) reported by Sridaran *et al*. (2012). However, low compared with *Acacia nilotica* seeds (39.7%), as reported by Siddhwaju *et al*. (1996).

Petai has the highest crude lipid (22.73%), followed by Lamtoro Gung (2.53%), Jengkol (0.36%), and Kabau (0.38%), which were not significantly different in crude lipids. Petai and Lamtoro Gung crude lipids in this study are high compared with lentils (0.7%), peas (1.0%), and beans (1.8%), which were reported by Piecyk *et al*. (2012). However, the previous research was low compared with Jengkol and Kabau crude lipids in this study. Petai had the same crude lipid of soybean (Chunsuwuhao cultivar) value based on the analysis by Kan *et al.* (2018). The lipid content of Lamtoro Gung is in line with the findings of Ermetice *et al*. (2006), who analyzed the lipid content of legumes. That study reported that the lipid content of peas, common beans, and lentils are 2.34%, 2.52% and 2.36%, respectively.

All stinky beans had high amounts of crude protein. Lamtoro Gung (28.65%) had the significantly highest protein content, followed by Kabau (24.11%), Petai (17.77%), and Jengkol (15.22%). The high amount of protein indicates that they have potential as an alternative protein source (James *et al.,* 2020). Jengkol crude protein was more elevated than Sridaran *et al*. (2012), who reported crude protein of Jengkol in their study was 14.19%. Overall, the protein content in this research ranged from 15.22 to 28.65%. It is in agreement with the finding of Ermetice *et al*. (2006), who reported some legumes with a protein content range of 18.5-23.44%. However, groundnut (29.34%) had a higher crude protein than Lamtoro Gung, the highest crude protein content in this study.

Table 2 shows the carbohydrate content range between 49.27-73.13%. Jengkol had the significantly highest value, followed by Kabau, Lamtoro Gung and Petai. Those values are within the carbohydrate content range reported by James *et al*. (2020) and Ermetice *et al*. (2006). James *et al*. (2020) reported that the groundnut in their study has the lowest (7.34%) content of carbohydrates, and the Bambara nut has the highest (64.74%) one. Ermetice *et al*. (2006) stated that the

Table 2. The chemical composition of four Indonesian stinky beans.

	Lamtoro Gung	Jengkol	Kabau	Petai
Moisture content (%wb)	8.81±0.26^{b}	9.48±0.01^{a}	4.62±0.16^{d}	6.30±0.08^{c}
Ash content (%db)	4.18±0.26^{a}	1.81±0.22^{c}	1.81±0.00^{c}	3.93±0.03^{b}
Crude lipid (%db)	2.53±0.03^{b}	0.36±0.08^{c}	0.38±0.01^{c}	22.73±0.35^{a}
Crude protein (%db)	28.65±0.18^{a}	15.22±0.02^{d}	24.11±0.21^{b}	17.77±0.03^{c}
Carbohydrate by difference (%db)	55.83±0.29^{c}	73.13±0.32^{a}	69.08±0.04^{b}	49.27±0.22^{d}
Crude fibre (%db)	12.76±0.75^{a}	3.37±0.05^{c}	2.35±0.03^{c}	11.08±0.63^{b}
Energy (kcal)	360±2.28^{c}	356±0.51^{c}	376±0.53^{b}	472±2.21^{a}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different (P≤0.05).

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carbohydrate content of peas, common beans, chickpeas, and lentils are 52.5%, 54.3%, 54.0% and 56.4%, respectively. Crude fibre determined in this study has varying values. Lamtoro Gung has the significantly highest value (12.76%), then Petai (11.08%). Jengkol (3.37%) and Kabau (2.35%) had the lowest crude fibre content, and both of them didn't have significant values. However, Jengkol crude fibres in this study are higher than Sridaran *et al*. (2012), who reported the crude fibre of Jengkol in his study was 1.76%.

3.2 Amino acid profiling

The amino acid profile is shown in Table 3. Glutamic acid was the most dominant amino acid in Lamtoro Gung (10.83 g/100 g protein d.b.), followed by aspartic acid (8.89 g/100 g protein d.b.), and arginine (8.89 g/100 g protein d.b.). Lamtoro Gung has 32.73 and 33.58 g/100 g protein d.b. for total essential and non-essential amino acids. Jengkol was rich in lysine (9.37 g/100 g protein d.b.) and arginine (8.51 g/100 g protein d.b.). Kabau had the lowest content of total amino acids (28.87 g/100 g protein d.b.). Petai amino acid was dominated by glutamic acid (20.90 g/100 g protein d.b.), followed by lysine (13.31 g/100 g protein d.b.), arginine (11.76 g/100 g protein d.b.), leucine (10.98 g/100 g protein d.b.), and aspartic acid (10.72 g/100 g protein d.b.).

It shows that Lamtoro Gung and Petai have a high amount of aspartic acid and glutamic acid. The aspartic and glutamic acid presence indicates that Lamtoro Gung and Petai are good umami ingredient sources (Dermiki *et al.,* 2013). Having a high amount of total essential amino acids, Lamtoro Gung (32.73 g/100 g protein d.b.), Jengkol (39.29 g/100 g protein d.b.), and Petai (60.21 g/100 g protein d.b.) have potential as an essential amino acid alternative to reduce malnutrition (James *et al.,* 2020).

The presence of negative charge hydrophilic amino acids (glutamic and aspartic acid) and hydrophobic amino acids (phenylalanine, isoleucine, leucine, methionine, valine, proline and alanine) have an essential role as an ACE-I agent (Aluko, 2015; Pebrianti, 2019; Puspitojati *et al.,* 2019a). Hydrophobic amino acids can

Table 3. The amino acid profiling of Indonesian stinky bean.

Amino acid	Amino acid concentration of Kabau (mg/100 g d.b.)			
	Lamtoro Gung	Jengkol	Kabau	Petai
Essential amino acid				
Arginine	8.89±0.03^{b}	8.51±0.36^{b}	2.74±0.03^{c}	11.76±0.096^{a}
Threonine	2.17±0.08^{c}	2.76±0.14^{b}	1.28±0.07^{d}	5.15±0.11^{a}
Methionine	0.07±0.03^{c}	0.22±0.01^{b}	0.06±0.005^{c}	0.37±0.001^{a}
Phenylalanine	3.14±0.07^{c}	4.25±0.04^{b}	2.33±0.006^{d}	6.35±0.16^{a}
Isoleucine	2.63±0.09^{c}	3.36±0.014^{b}	1.25±0.01^{d}	5.81±0.03^{a}
Leucine	5.68±0.13^{a}	7.64±0.03^{b}	2.75±0.06^{d}	10.98±0.07^{a}
Lysine	7.06±0.28^{c}	9.37±0.17^{b}	3.14±0.13^{d}	13.31±0.04^{a}
Valine	3.09±0.16^{b}	3.18±0.006^{b}	1.27±0.02^{c}	6.48±0.22^{a}
Total	32.73	39.29	14.82	60.21
Non-essential amino acid				
Aspartic acid	8.89±0.009^{b}	7.37±0.25^{c}	3.00±0.08^{d}	10.72±0.07^{a}
Glutamic acid	10.84±0.35^{b}	7.90±0.15^{c}	3.34±0.008^{d}	20.90±0.21^{a}
Tyrosine	0.48±0.02^{a}	0.09±0.006^{b}	0.06±0.01^{b}	0.47±0.02^{a}
Glycine	3.03±0.16^{b}	2.95±0.10^{b}	0.88±0.10^{c}	4.49±0.38^{a}
Alanine	2.66±0.01^{b}	2.85±0.25^{b}	1.35±0.01^{c}	5.81±0.03^{a}
Serine	2.71±0.06^{c}	2.94±0.03^{b}	1.20±0.07^{d}	5.23±0.002^{a}
Histidine	3.46±0.78^{b}	4.69±0.27^{ab}	3.51±0.56^{b}	5.28±0.12^{a}
Proline	1.51±0.04^{c}	2.76±0.21^{b}	0.71±0.01^{d}	3.26±0.11^{a}
Total	33.58	31.55	14.05	56.16
Total (essential amino acid and	66.31	70.84	28.87	116.37
Total HLAA	47.54	46.59	19.16	77.31
Total HLAA Negative charge	19.73	15.27	6.34	31.62
Total HBAA	18.79	24.26	9.71	39.06

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different (P≤0.05).

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build some hydrophobic interactions with the enzyme's active sites and decrease their activities (Fan *et al.,* 2018). Negative charge hydrophilic amino acids form an electrostatic interaction with Zn^{2+} ions, which attach to the active site, and then catalytic activity decreases (Natesh *et al.,* 2003; Aluko, 2015). Since the stinky beans contain a high value of negative charge and hydrophobic amino acid, they have the potential for the ACE-I effect.

Petai showed the highest negative charge of hydrophilic amino acids (31.62 g/100 g protein d.b.) and a total of hydrophobic amino acids (39.06 g/100 g protein d.b.). On the other hand, the Jack bean, which has been studied for its ACE-I properties, has 25.63 and 19.97 g/100 g protein d.b. hydrophobic and negative charge hydrophilic amino acids (Puspitojati *et al.,* 2019b). The hydrophobic amino acid of Petai in this study is higher compared with pigeon pea (21.28 g/100 g protein) (Pebrianti *et al.,* 2019) and *Phaseolus lunatus* L. tempeh (4.18 g/100 g protein) (Pertiwi *et al.,* 2019).

3.3 ACE-inhibitory activity

ACE-I activity was studied on the fresh sample and their hydrolysates. Pepsin and pancreatin were used to hydrolyse stinky beans. Those enzymes represent the human body's digestion simulation, so using stinky beans as an antihypertensive can be evaluated well. Figure 1 shows the ACE-I activity of all stinky beans.

Generally, the raw form of all stinky beans has ACE-I activity with various intensities. Jengkol has the highest inhibitory activity (67.77%) than other fresh samples. Pepsin hydrolysis does not increase ACE-I activity in all the samples yet. However, pancreatin hydrolysis increases the ACE-I activity of Lamtoro Gung and Kabau. Subsequent hydrolysis with pepsin and pancreatin success increases the ACE-I activity of all samples mostly twice. Kabau had the highest (79.87%) ACE-I activity from hydrolysate products, followed by Jengkol, Lamtoro Gung, and Petai. Kabau Pepsin-Pancreatin hydrolysate had higher ACE-I activity than pigeon pea protein hydrolysate (70.51%) by the pepsin and the combination of trypsin and chymotrypsin (Ratnayani *et al.,* 2019b). However, the present result had lower ACE-I activity than jack bean tempeh (72 h fermentation) hydrolysate by the pepsin and pancreatin. Tempe hydrolysate had 88.2% ACE-I activity (Puspitojati *et al.,* 2019a).

Kabau pepsin-pancreatin hydrolysates result in higher ACE-I activity than others and are close to Captopril. Captopril, as the positive control, has 90.55% inhibitory activity. Hydrolysate products have high ACE -I activity due to the peptide size obtained after hydrolysis. High molecular peptides were hydrolyzed, forming the small peptides (Ratnayani *et al.,* 2019b). The importance of peptide size is related to the active site of ACE because its active site is in a narrow location. Therefore, smaller peptides enter the active site and disturb the activity of ACE. In contrast, a larger peptide size will reduce peptide accessibility to the ACE active site (Natesh *et al.,* 2003).

4. Conclusion

All stinky beans can potentially be used as an antihypertensive agent to prevent hypertension through the capability to inhibit ACE activity. Fresh Jengkol has the highest ACE-I activity than other fresh forms. Stinky beans hydrolyzed by pepsin-pancreatin have high ACE-I activity. Kabau has the most increased ACE-I activity than other hydrolysate products. It was expected the presence of a high smaller peptide size (need to be investigated in further research). Subsequently, hydrolysis by pepsin and pancreatin as gastrointestinal digestion simulation can degrade high molecular peptides to obtain the smaller ones and increase the ACE

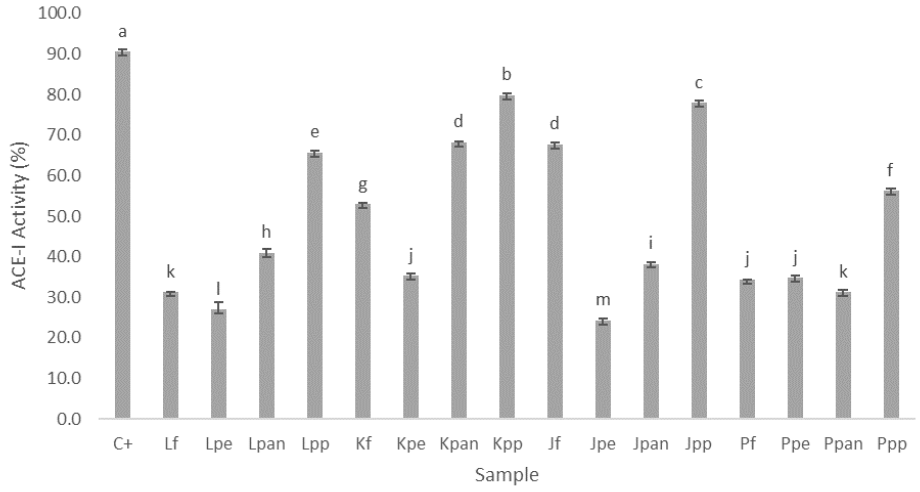

Figure 1. ACE-I activity of stinky bean. Bars with different notations are statistically significantly different (P≤0.05). C+: Positive control, L: Lamtoro Gung, K: Kabau, J: Jengkol, P: Petai, f: fresh, pe: pepsin, pan: pancreatin, pp: pepsin-pancreatin.

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-I activity close to Captopril. The high protein and amino acid value of all the stinky beans make them potentially used as a protein source alternative. In addition, low lipids and high crude fibre content can help reduce the risk of diet-related diseases.

Conflict of interest

The authors declare no conflict of interest.

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