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Hydrolyzation of duck meat protein using *Bacillus cereus* TD5B protease, pepsin, trypsin and their potency as an angiotensin converting enzyme inhibitor

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ABSTRAK

Tujuan penelitian untuk mengeksplorasi potensi protein bioaktif daging itik yang dihidrolisis enzim protease-*Bacillus cereus* TD5B, Pepsin, dan Trypsin sebagai agen antihipertensi melalui penghambatan *angiotensin-converting enzyme* (ACE). Sampel yang digunakan: daging itik jantan Mojosari dan Magelang umur 10 bulan, enzim protease-*Bacillus cereus* TD5B, Pepsin, dan Trypsin yang dilakukan secara individual dengan konsentrasi enzim sebesar 0,1% (w/w). Parameter yang diamati: konsentrasi protein, berat molekul protein, aktivitas inhibitor ACE, dan nilai IC_{50} dari ACE. Data konsentrasi protein dianalisis menggunakan t-test, sedangkan data SDS-PAGE dan aktivitas penghambat ACE dianalisis secara deskriptif. Hasil penelitian menunjukkan konsentrasi protein yang larut dari daging itik meningkat karena proses hidrolisis, dari 0,826±0,108 mg/mL menjadi 1,050±0,197 mg/mL (protease-mikrobia); 2,122±0,141 mg/mL (pepsin); 1,641±0,071 mg/mL (trypsin) untuk itik Mojosari dan 0,642±0,038 mg/mL menjadi 1,171±0,534 mg/mL (protease-mikrobia); 2,100±0,376 mg/mL (pepsin); 1,725±0,092 mg/mL (trypsin) untuk itik Magelang. Hasil SDS-PAGE menunjukkan penurunan berat molekul karena hidrolisis, dari 196,53 sampai 43,88 kDa menjadi 71,35 sampai 10,12 kDa. Hidrolisat protein daging itik memiliki aktivitas penghambat ACE 71,7% (Mojosari-Protease-Microbia) IC_{50} 54 µg/mL, 57% (Mojosari-Pepsin) IC_{50} 151µg/mL, 75,8% (Mojosari-Trypsin) IC_{50} 51µg/mL dan 52,8% (Magelang-Protease-mikrobia) IC_{50} 83µg/mL, 78,5% (Magelang-Pepsin) IC_{50} 85µg/mL, 83,9% (Magelang-Trypsin) IC_{50} 22µg/mL. Kesimpulannya, daging itik Magelang yang dihidrolisis dengan Trypsin memiliki potensi yang lebih baik sebagai penghambat ACE.

Kata kunci: Protein Daging Itik; Hidrolisis, Enzim Protease Mikrobia; Penghambatan ACE

ABSTRACT

This study was aimed to explore their potency of protein-bioactive of native ducks-meat after enzymatic hydrolysis by *Bacillus-cereus* TD5B-protease, Pepsin, and Trypsin as an angiotensin-converting enzyme (ACE) inhibitor. The samples: ducks-meats from 10 months age of male Mojosari and Magelang-Duck. The experiments: individually hydrolysis of meat-protein using protease-enzyme (0.1 % w/w) from *Bacillus-cereus* TD5B, Pepsin, or Trypsin. The observed parameters: protein concentration, protein molecular weight, ACE-inhibitor activity, and IC-value (IC_{50}). Data of protein concentration were statistically analyzed using T-Test, while data of SDS-PAGE and ACE-inhibiting activity were analyzed descriptively. The results showed that soluble protein concentration increased due

to the hydrolysis process, from 0.826±0.108 mg/mL to 1.050±0.197 mg/mL (Microbial-protease), 2.122±0.141 mg/mL (pepsin), 1.641±0.071 mg/mL (trypsin) for Mojosari-duck and 0.642±0.038 mg/mL to 1.171±0.534 mg/mL(Microbial-protease), 2.100±0.376 mg/mL(pepsin), 1.725±0.092 mg/mL(trypsin) for Magelang-duck. The SDS-PAGE pattern showed that there was a decrease of molecular weight of duck-meats due to the hydrolysis process, from the range of 196.53-43.88 kDa to the range of 71.35-10.12 kDa. Duck-meat protein hydrolysate had ACE-inhibiting activity 71.7%(Mojosari-Microbial-Protease) IC_{50} 54μg/mL, 57%(Mojosari-Pepsin) IC_{50} 151μg/mL, 75.8%(Mojosari-Trypsin) IC_{50} 51μg/mL and 52.8%(Magelang-Microbial-Protease) IC_{50} 83μg/mL, 78,5%(Magelang-Pepsin) IC_{50} 85μg/mL, 83.9%(Magelang-Trypsin) IC_{50} 22μg/mL. In conclusion, hydrolysate of Magelang duck-meat used Trypsin had better potency as an ACE-inhibitor.

Keywords:Duck Meat Protein; Hydrolysis, Microbial Protease Enzyme; ACE Inhibition

INTRODUCTION

Angiotensin-converting enzyme (ACE) or kininase II was dipeptidyl carboxypeptidase (EC 3.4.15.1) found in various tissues in the body and represents an integral part of setting blood pressure and normal heart function (Shalaby *et al.*, 2006). ACE also reported being responsible for elevating blood pressure (Nakamura *et al*., 1995). Some ACE inhibitors such as captopril, enalapril, lisinopril, and temocapril are clinically used for the treatment of antihypertensives. All of these drugs still produce side effects, so it was necessary to search for a natural ACE inhibitor to reduce a side effect of the drug. Antihypertensive can be reserved by some bioactive peptide through ACE inhibition mechanism and the natural bioactive peptide are resourced from many kinds of natural protein such as pork (Katayama *et al*., 2007), beef (Jang and Lee 2005), goat meat (Jamhari *et al.*, 2013), chicken legs (Yuliatmo *et al*., 2017), collagen from chicken bone (Cheng *et al.*, 2008), chicken breast (Sangsawad *et al*., 2017), milk (Otte *et al*., 2007), *Saccharomyces cerevisiae* (Mirzaei *et al.*, 2015), edible mushroom *Agaricus bisporus* (Lau *et al.*, 2014) and fermented fish (bekasam) (Wikandari *et al*., 2012).

Bioactive protein usually will be active in the metabolism pathway after degraded into oligopeptide and one of their functional prospective character is an antihypertensive agent. The activity of a bioactive peptide from protein as an antihypertensive agent was indicated by IC_{50} value, which was the need for an inhibitor to inhibit 50% of ACE activity (Cheng *et al.*, 2008). The enzymes generally used to bioactive peptide hydrolysis are pepsin (Sangsawad *et al*., 2017), trypsin (Ferreira *et al.*, 2007), and chymotrypsin (Mirzaei *et al.*, 2015). The microbial enzymes have a specific activity in cutting different peptide bonds with other protease enzymes, but microbial enzymes are still not widely used for peptide hydrolysis. Our previous study isolated *Bacillus cereus* TD5B a microbe from the soil around odorous farm area which has protease activity and its was successfully obtained and confirmed to produce extracellular protease enzymes (Fitriyanto *et al.*, 2014).

The ACE inhibition usually tend to low activity in origin protein form and increase by the hydrolysis using proteolysis enzyme. Some researchers applied some proteolytic enzyme such as pepsin, trypsin and khemotripsin to hydrolyse the protein. Whoever due to the many kinds the protein polymer structure, consequently the protein was not well degraded. The optimise protein hydrolysis methods to produce ACE inhibition bioactive peptide are still in progress. Yuliatmo *et. al*. (2017) reported that the protein degradation level was various and depend to the complexities of the origin protein and the ability in ACE inhibition also shown various range. In addition bacterial protease has high enzyme activity and application in the meat protein degradation, and this increases the degree of hydrolysis and increase the bioactivity. Therefore, studies on the application of bacterial enzymes to the hydrolysis of duck meat proteins as antihypertensive agents have never been done. Therefore, it was necessary to investigate and develop the potential of duck meat protein bioactive as an antihypertensive agent by enzymatic hydrolysis by protease enzyme from *Bacillus cereus* bacteria.

MATERIALS AND METHODS

Preparation of Duck Meat Protein Mojosari and Magelang Ducks

Preparation of Magelang and Mojosari duck meat was done by taking 50 g thigh meat from

Duck Male, aged at 10 months old and slaughtered in traditional slaughter method. Samples were added 100 mL of aquadest, then mixed with blender for 5 minutes. The mixture was heated at 70°C for 30 minutes then cooled (Jamhari *et al*., 2013).

Hydrolysis of Meat Proteins with Microbial Protease, Pepsin and Trypsin

The duck meat protein was added with bacterial enzyme *Bacillus cereus* strain TD5B, pepsin and trypsin with the ratio of protein enzyme and protein content of sample 0.1%. The sample was then adjusted to pH 7 by adding NaOH and incubated at 50 °C for 3 hours to hydrolyze protein of duck meat used microbial protease enzyme. The sample was then adjusted to pH 2 by adding HCl and incubated at 37 °C for 3 hours to hydrolyze protein of duck meat used pepsin. The sample was then adjusted to pH 7 by adding NaOH and incubated at 37 °C for 3 hours to hydrolyze protein of duck meat used trypsin. The reaction was stopped by the heating sample at 95 °C for 10 minutes. The sample was then put in ice water until cool. The hydrolysate was filtered using Whatman filter paper No 1. The hydrolysate was then used for dissolved protein test, confirmation with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and ACE inhibitor test (Yuliatmo *et al.*, 2017).

Determination of Protein Content of Duck Meat

This study determined protein content before and after hydrolisis by *B. cereus* enzyme strain TD5B by using Lowry method. The sample was taken at 0.6 mL and added 3 mL reagent C then incubated at room temperature for 10 min. The sample was then added to 0.3 mL reagent E and waited for 30 min. Absorbance was read at 750 nm wavelength using a spectrophotometer. The absorbance of the duck protein hydrolyzate sample was compared with the absorption of bovine serum albumin (BSA) as the standard (Gornal *et al.*, 1949).

Protein Molecular Weight Determination with SDS-PAGE

The electrophoresis process used AE-6530 mPAGE apparatus, ATTO. The process began by making running buffers, sample buffers, commercially blue gel (BRBB) gel dyes, distillation solutions, 12% gel separation, and 3% stacking gel. Then, 7.0 mL of separating gel solution was added 58.5 µL APS 10% and 5.85 µL TEMED. The Solution then was poured on a plate and coated with 1 mL of butanol. After solid, the butanol layer was removed, and the gel stacking solution was added to the separating gel. The stacking gel 2.0 mL was added with 58.5 µL APS 10% and 58.5 µL TEMED, then stirred and pored on the plate. A well-forming comb was inserted between the plate and allowed to stand for 10 minutes later after the solid comb was taken. The solid gel was ready to be assembled in electrophoresis and filled with running buffer. Samples were inserted into columns with volumes 5 to 10 µL / well. The electrophoresis device was connected to a 120 V electric conductor for 3 to 4 hours. Electrophoresis process was stopped until the blue color touches the bottom of the gel. The gel was then stained with CBB dye and shook at 60 rpm for 24 hours. The solution was removed, and gel was washed with a distillation solution for 30 minutes, and finally with 10% acetic acid solution. The gel was then stored in 10% acetic acid solution and could be calculated by molecular weight. Calculation of molecular weight expressed by kilo Dalton (kDa) was done by the descriptive approach. Determination of molecular weight by comparing the result of electrophoresis of protein fraction with protein marker. Determination of molecular weight was done by calculating the value of retardation factor (Rf) of each band with the formula (Wyckoff *et al.*, 1977):

Rf = (Distance of protein movement from start place) / (Distance of color movement from start place)

Then the Rf value was included in the equation linear regression with the formula (Mahasri *et al.,* 2010):

$$Y = aX \pm b$$

where Y= molecular weight, and X = Rf value.

Calculation of the molecular weight of the protein bands contained in the gel was done by comparing the molecular weight of the marker with Rf. The standard curve with Rf value as x-axis and molecular weight logarithm value as y-axis and lined. The molecular weight of the protein fraction was determined by interpolation on the standard curve (Laemmli, 1970).

Determination of ACE Inhibitor Activity

Determination method of ACE inhibitor activity was based on the (Cushman *et al.*, 1981). Protein hydrolyzate sample 6 µL was mixed with 50 µL 7.6 mM of HHL substrate and dissolved in

100 mM borate buffer (pH 8.3) and 608 mm NaCl. Before reacting with ACE, the sample was preincubated for 5 minutes in a water bath at 37°C. The reaction began with the addition of 20 µl 60 mU/mL ACE dissolved in borate buffer (pH 8.3). Incubation was done in the water bath for 30 minutes at 37°C. The reaction was discontinued by the addition of 554 µL 0.1 N HCl, except on blanks having added 554 µL 0.1 N HCl prior to incubation. The product of the reaction (hippuric acid) was extracted with the addition of 1.5 ml ethyl acetate and was shaken for 2 minutes. The mixture was then centrifuged at 2,500 rpm (117 x 10 g) for 15 minutes. One milliliter of supernatant was taken and transferred to another test tube, then dried at 100 °C for 10 minutes. The reaction tube was then cooled to room temperature for 30 seconds. Hippuric acid released by ACE was determined by a spectrophotometer with a wavelength of 228 nm. The concentration of ACE inhibitors to inhibit 50% of ACE activity was called IC value (IC_{50}). IC_{50} was obtained by making a resistor activity regression equation of 4 series dilution samples. The percentage of ACE-inhibiting activity was calculated by the formula:

Inhibitor = (Ec-Es) / (Ec-Eb) x 100%

Ec = absorbance control; Es = absorbance of sample; Eb = absorbance blank

RESULTS AND DISCUSSION

Degree of Hydrolysis

Determination of protein contents of Mojosari and Magelang duck meat before and after hydrolysis were determined using Lowry method could be seen in Table 1. The results showed that the Mojosari duck and Magelang ducks showed different soluble protein concentrations both before hydrolysis and after hydrolysis with the bacterial enzyme *Bacillus cereus* strain TD5B, pepsin and trypsin. Quantitatively, the protein concentrations after hydrolysis showed higher level than before hydrolysis. The hydrolysis process would (cleavage) the binding of the Mojosari duck and Magelang duck peptides so that the protein concentration would be higher because of the unbreakable protein into dissolved protein (Nurhayati *et al*., 2007). the both of samples, the highest value from the results of hydrolysis was by used pepsin ie 2.122±0.141 mg/mL (Mojosari duck meat) and 2.100±0.376 mg/mL (Magelang duck meat).

Protein enzymatic hydrolysis is a technique used for the isolation of peptides from meat sources, and the most commonly used digestive enzymes are pepsin, trypsin and chymotrypsin (Ryan *et al.*, 2011). Protein hydrolysis is done by cutting peptide bonds to break protein molecules into smaller peptides using acids, bases or enzymes, which are applied to improve the functional properties of proteins. The advantages of enzymatic reactions are non-extreme reaction conditions such as hydrolysis with acids and bases, easy to control reactions, minimal formation of by products and do not reduce the protein nutritional value. Hydrolysis with acids or bases tends to be more difficult to control than enzymatic hydrolysis (Kim *et al.,* 2003). Hydrolyzed food proteins will produce peptides as inhibitors of angiotensin converting enzyme (ACE), an enzyme that plays a role in increasing blood pressure (Nakamura *et al*. 1995).

Protein Molecular Weight

The SDS-PAGE method was used to determined the degree of protein hydrolisis on the basis of molecular weight. Results of SDS-PAGE method on samples of Mojosari and Magelang duck meat before and after hydrolysis could be seen in Figure 1. . The molecular weight readings aided by using standard curves with y was the log

Table 1. The Soluble Protein Concentrations of Mojosari and Magelang Ducks Meat Before and After Hydrolysis (mg/mL)

Type of Duck Meat	Soluble Protein Concentration (mg/mL)			
	Before hydrolysis	*B. cereus* Protease hydrolisis	Pepsin hydrolisis	Trypsin hydrolisis
Mojosari	0.826±0.108	1.050±0.197	2.122 ± 0.141	1.641 ± 0.071
Magelang	0.642±0.038	1.171±0.534	2.100 ± 0.376	1.725 ± 0.092

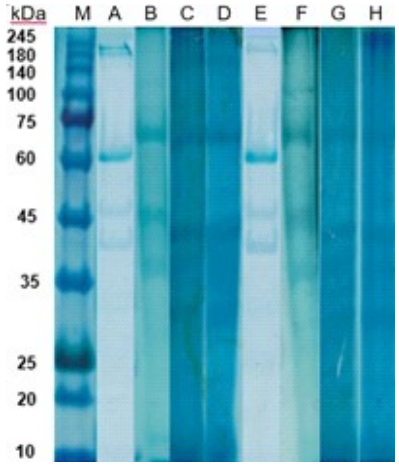

Figure 1. SDS-PAGE of Mojosari and Magelang Duck Meat

M : Protein Marker
A : Mojosari duck meat before hydrolysis
B : Mojosari duck meat after hydrolysis of *B.cereus* TD5B
C : Mojosari duck meat after hydrolysis of Pepsin
D : Mojosari duck meat after hydrolysis of Trypsin
E : Magelang duck meat before hydrolysis
F : Magelang duck meat after hydrolysis of B.cereus TD5B
G : Magelang duck meat after hydrolysis of Pepsin
H : Magelang duck meat after hydrolysis of Trypsin

of molecular weight (MW), and x was the ratio between the migration of the protein band of the sample with the migration of the protein marker band (Rf) at Figure 2. The results of molecular weight readings on protein bands of samples before and after hydrolysis could be seen in Table 2.

The result of SDS-PAGE based on a calculation of molecular weight according to protein standard curve showed that there are some visible proteins. Protein bands before hydrolysis appeared to be identical to the samples of Mojosari duck and Magelang duck, so in outline, the two samples had the same molecular weight value that was between 196.53 kDa to 43.88 kDa. The protein bands in both samples after hydrolysis had the same molecular weight between 71.25 kDa to 10.12 kDa, but there were differences in molecular weight in the second and third band by using *B. cereus* TD5B protease, i.e., in Mojosari duck samples of 44.68 kDa and 38.40 kDa whereas in the Magelang duck 51.27 kDa and 36.73 kDa. The samples those were hydrolyzed by pepsin and trypsin only resulted a different in the third band, i.e.29.41 kDa for Mojosari duck meat and 28.13 kDa for Magelang duck meat.

The sample after hydrolysis has a lower molecular weight; this was because when the process of protein hydrolysis would split into shorter peptides, thereby reducing the molecular

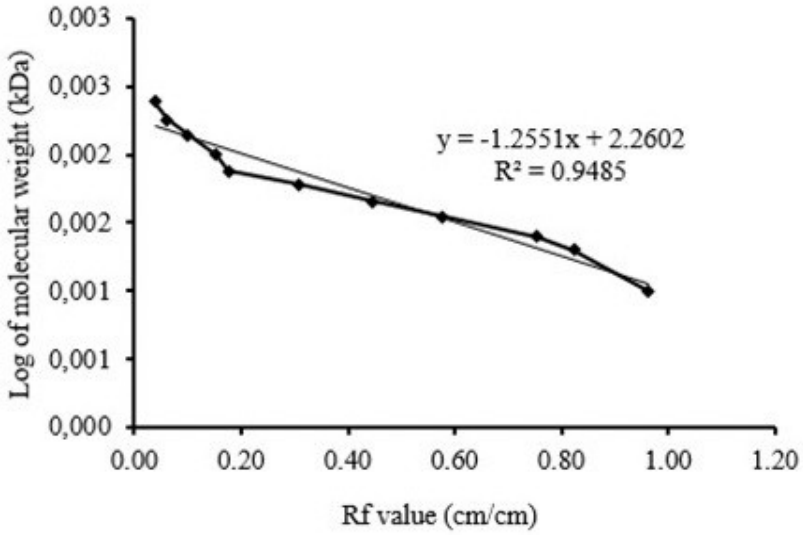

Figure 2. Equation Curve between Log Value and Rf. Symbols (♦) Represent Y. Linear Regression Equation was and $R^2 = 0.9485$

weight (Ryder *et al.*, 2016). The SDS would wrap the chain of proteins that are bonded with the same negative charge forming the SDS-protein complex. SDS-protein complexes have identical charge densities and move on the gel-based solely on protein size; the larger SDS-protein complexes have lower mobility than the smaller SDS-protein complexes (Fatmawati *et al.*, 2009). The basic principle of SDS-PAGE was the process of filtering proteins through a polyacrylamide gel. Molecules that have a smaller or lighter weight would migrate faster and move below, whereas larger or heavier molecules would migrate more slowly so that they remain at the top of the gel. The thick band indicates that the protein content was large or the concentration was large while the thin band indicates that the protein content was small.

ACE Inhibitor Ativity

Testing of ACE-inhibiting activity was performed with 4 dilution samples. Dilution was done to obtain IC_{50} value. The results of the ACE inhibitory activity of the Mojosari duck protein hydrolysate are presented in Table 3 for hydrolyzes using *Bacillus cereus* TD5B protease, in Table 4 for hydrolyzes using pepsin, in Table 5 for hydrolyzes using trypsin, and the result of the linear regression curve between in assay concentration and the inhibiting activity can be seen in Figure 3. The curve shows the line gradient was more than 0.9 with linear regression obtained value $Y = ax \pm b$. The IC_{50} value obtained from the linear regression equation was created by changing the value of Y to 50, so that the value of IC_{50} are presented in Table 6.

Meat protein hydrolysate of Mojosari and Magelang duck meats had ACE-inhibiting activity

Table 2. Protein Molecular Weight of Mojosari and Magelang Duck Meat Before and After Hydrolysis (kDa)

The protein bands	Mojosari Duck				Magelang Duck			
	Before hydrolysis	*B.cereus* Protease	Pepsin	Trypsin	Before hydrolysis	*B.cereus* Protease	Pepsin	Trypsin
1 (top)	196.53	71.25	71.25	71.25	196.53	71.25	71.25	71.25
2	183.34	44.68	40.18	40.18	183.34	51.27	40.18	40.18
3	62.73	38.40	29.41	29.41	62.73	36.73	28.13	28.13
4	51.27	10.12	10.12	10.12	45.00	10.12	10.12	10.12

Table 3. ACE-inhibiting Activity of Hydrolysate from Mojosari and Magelang Duck Meat After Hydrolysis of *Bacillus cereus* TD5B

Mojosari Duck Meat			Magelang Duck Meat		
Protein Sample (mg/mL)	Protein in Assay (mg/mL) (x)	Inhibiting Activity (%)	Protein sample (mg/mL)	Protein in Assay (mg/mL) (x)	Inhibiting Activity (%)
1.050	0.083	71.7	1.171	0.092	52.8
0.525	0.041	43.1	0.586	0.046	37.1
20.262	0.021	23.8	0.293	0.023	22.8
30.131	0.010	13.6	0.146	0.011	21.4

Table 4. ACE-inhibiting Activity of Hydrolysate from Mojosari and Magelang Duck Meat After Hydrolysis of Pepsin

Mojosari Duck Meat			Magelang Duck Meat		
Protein Sample (mg/mL)	Protein in Assay (mg/mL) (x)	Inhibiting Activity (%)	Protein Sample (mg/mL)	Protein in Assay (mg/mL) (x)	Inhibiting Activity (%)
2.122	0.168	57.0	2.100	0.166	78.5
1.061	0.084	25.2	1.050	0.083	60.0
0.531	0.042	17.8	0.525	0.041	32.6
0.265	0.021	12.6	0.263	0.021	16.3

Table 5. ACE-inhibiting Activity of Hydrolysate from Mojosari and Magelang Duck Meat After Hydrolysis of Trypsin

Mojosari Duck Meat			Magelang Duck Meat		
Protein Sample (mg/mL)	Protein in Assay (mg/mL) (x)	Inhibiting Activity (%)	Protein Sample (mg/mL)	Protein in Assay (mg/mL) (x)	Inhibiting Activity (%)
1.641	0.130	75.8	1.725	0.136	83.9
0.821	0.065	60.1	0.863	0.068	60.4
0.410	0.032	48.0	0.431	0.034	56.4
0.201	0.016	30.5	0.216	0.017	48.0

71.7% (Mojosari duck meat was hydrolysed by *Bacillus cereus* TD5B Protease) with IC_{50} values was 54 µg/mL, 57% (Mojosari duck meat was hydrolysed by Pepsin) with IC_{50} values was 151 µg/mL, 75.8% (Mojosari duck meat was hydrolysed by Trypsin) with IC_{50} values was 51 µg/mL and 52.8% (Magelang duck meat was hydrolysed by *Bacillus cereus* TD5B Protease) with IC_{50} values was 83 µg/mL, 78,5% (Magelang duck meat was hydrolysed by Pepsin) with IC_{50} values was 85 µg/mL, 83.9% (Magelang duck meat was hydrolysed by Trypsin) with IC_{50} values was 22 µg/mL

ACE-inhibiting activity was performed by (Cushman *et al.*, 1981). One of the ingredients used was hippuryl-histidyl-leucine (HHL). ACE inhibitor activity can be determined by HHL by measuring the release of HHL hypersensitivity (Attwood *et al.,* 1984). In the event of termination of 2 amino acids, the activity of ACE inhibitors was low, but if there was no complete removal of two amino acids then ACE inhibitor activity was high (Azizi *et al.*, 2000). The protein hydrolysis with protease enzymes produces simple peptides. The hydrolysis peptide derived from skeletal porcine muscle myosin was detected as octapeptide Val-Lys-Lys-Val-Leu-Gly-Asn-Pro (Katayama *et al.,* 2007). Some of the protein hydrolysis peptides have ACE inhibitory activity. ACE inhibitory activity was shown by IC_{50} (Cheng *et al.*, 2008).

If ACE inhibitory activity increases, IC_{50} becomes lower (Saiga *et al.,* 2008). The extracted porcine protein was crude and mainly included Myosin light chain and troponin have inhibitory activity with an IC_{50} value of 225 µg/mL (Katayama *et al.*, 2007). The peptide purification results showed that the amino acid sequence of the ACE inhibitor peptide from the bean protein hydrolysate protein was leu-thr-glu-ala-proleu-asn-pro-lys-ala-asn-arg-glu-lys. The peptide had a molecular weight (MW) of 1,581 and was in a

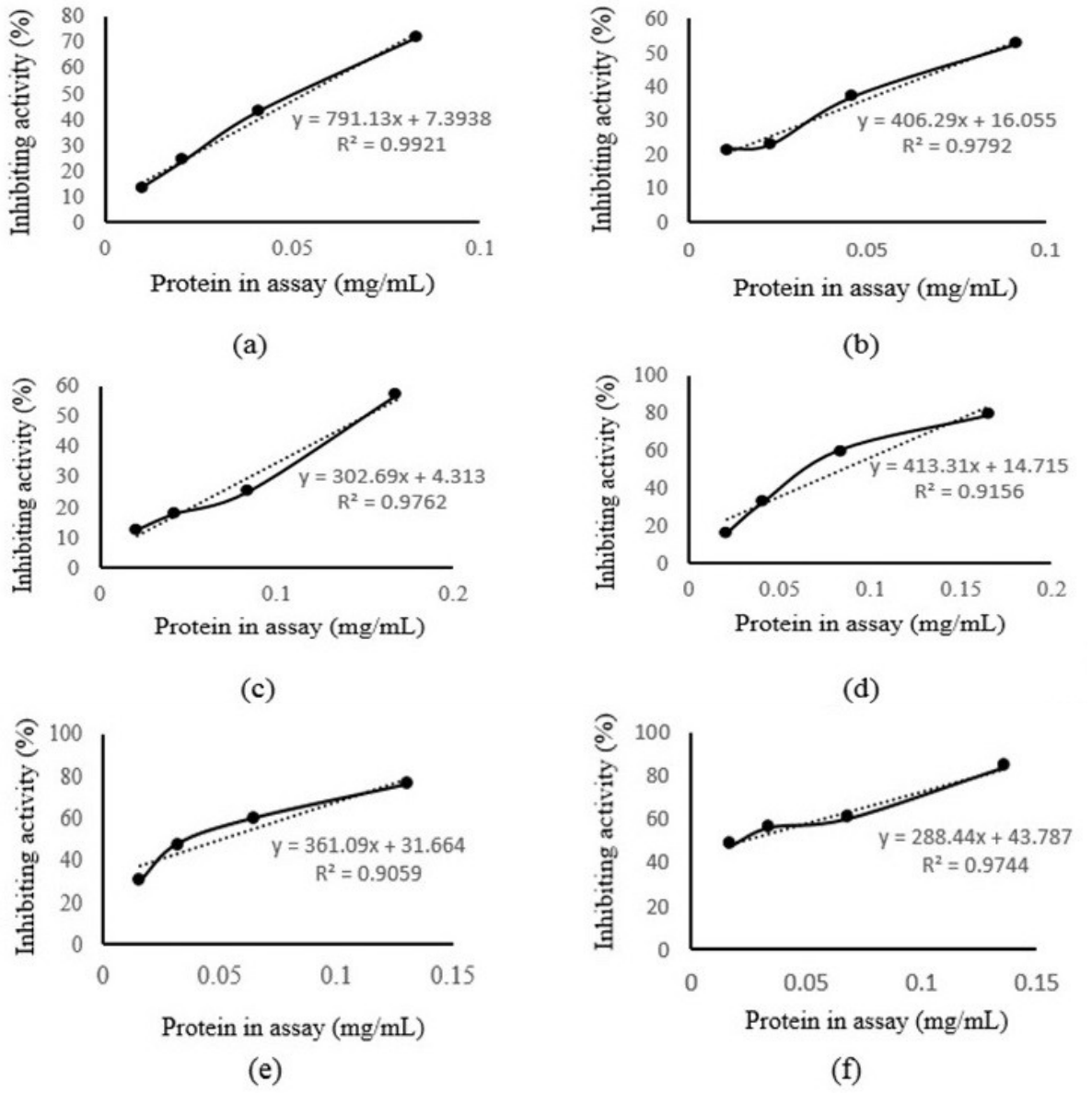

Figure 3. Linear Regression Curve of ACE Hydrolyzed Inhibition Activity of Mojosari and Magelang Duck Meat Protein. Symbols (●) represent point of ACE inhibition, while trendline (----) represent linear regression line from ACE inhibition. Results of linear regression equation were: (a) y = 791.13x + 7.3938 and R^2 = 0.992 for Mojosari duck meat – B.cereus TD5B; (b) y = 406.29x + 16.055 and R^2 = 0.979 for Magelang duck meat – B.cereus TD5B; (c) y = 302.69x + 4.313 and R^2 = 0.976 for Mojosari duck meat – Pepsin; (d) y = 413.31x + 14.715 and R^2 = 0.915 for Magelang duck meat – Pepsin; (e) y = 361.09x + 31.661 and R^2 = 0.906 for Mojosari duck meat – Trypsin; (f) y = 288.44x + 43.787 and R^2 = 0.975 for Magelang duck meat – Trypsin

residual position of 20 to 33 from goat meat b-actin protein; the peptide has ACE inhibitor activity (IC_{50}) of 190 µg/mL or 120 µM (Jamhari *et al*., 2013). Beef hydrolysate IC_{50} has value of 248.99 µg/mL (Jang and Lee 2005). Chicken leg protein hydrolysate showed an IC_{50} of 330 µg/mL (Yuliatmo *et al.*, 2017)]. The IC_{50} value of hydrolysates derived from chicken leg bone protein has value of 945 µg/mL (Cheng *et al*., 2008). Collagen extracted from chicken legs had activity IC_{50} has value of 260 µg/mL (Saiga *et al*., 2008). The IC_{50} values for thermolysin hydrolysates of caseins is 45 µg/mL (Otte *et al*., 2007). Yeast protein hydrolysate IC_{50} value was

Table 6. The IC_{50} Value of Bioactive Peptide from Mojosari and Magelang Ducks Meat (µg/mL)

Type of Duck Meat	IC_{50} after Hydrolysis (µg/mL)		
	B. cereus Protease	Pepsin	Trypsin
Mojosari	54	151	51
Magelang	83	85	22

420 µg/mL (Mirzaei *et al*., 2015). The whey protein concentrate hydrolyzate (WPC) has an IC_{50} value of 52.9 ± 2.9 µg/mL, but its peptide fraction with a molecular weight below 3 kDa shows an IC_{50} value of 23.6 ± 1.1 µg/mL (Tavares *et al*., 2011).

The lower the IC_{50} value, the better the ability of a protein to inhibit ACE activity. Based on the literature it can be seen that the hydrolyzate protein duck Mojosari and Magelang ducks have a higher IC_{50} value compared with milk-based samples, whereas when compared with samples made from meat, the value of IC_{50} hydrolyzate protein duck meat was lower. It was known that the duck protein hydrolyzate has the ability to inhibit ACE activity.

CONCLUSION

Hydrolysis of protein from Mojosari and Magelang duck meat with microbial enzyme *Bacillus cereus* TD5B, pepsin, and trypsin decreased the protein molecular weight. . Protein hydrolysate of Mojosari and Magelang duck meat were using trypsin enzyme have the highest potential as ACE inhibitors with IC_{50} values of Mojosari duck meat was 51 µg/mL and Magelang duck meat was 22 µg/mL.

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