Angiotensin converting enzyme (ACE) inhibitory and antihypertensive activities of protein hydrolysate from meat of Kacang goat (Capra aegagrus hircus)

by Irdha Mirdhayati

Submission date: 04-May-2023 10:35AM (UTC+0700)
Submission ID: 2083702754
File name: hibitory_peptide_from_Kacang_goat_meat_hydrolysate_published.pdf (419.05K)
Word count: 6550
Character count: 34790

Research Article

Received: 10 March 2015 Revised: 30 October 2015 Accepted article published: 23 November 2015 Published online in Wiley Online Library: 4 January 2016

(wileyonlinelibrary.com) DOI 10.1002/jsfa.7538

Angiotensin converting enzyme (ACE) inhibitory and antihypertensive activities of protein hydrolysate from meat of *Kacang* goat (*Capra aegagrus hircus*)

Irdha Mirdhayati,[a,b] Joko Hermanianto,[a*] Christofora H Wijaya,[a] Dondin Sajuthi[c] and Keizo Arihara[d]

Abstract

BACKGROUND: The meat of *Kacang* goat has potential for production of a protein hydrolysate. Functional ingredients from protein hydrolysate of *Kacang* goat meat were determined by the consistency of angiotensin-converting enzyme (ACE) inhibitory activity and antihypertensive effect. This study examined the potency of *Kacang* goat protein hydrolysate in ACE inhibition and antihypertensive activity.

RESULT: Protein hydrolysates of *Kacang* goat meat were prepared using sequential digestion of *endo*-proteinase and protease complex at several concentrations and hydrolysis times. The highest ACE inhibitory activity resulted from a hydrolysate that was digested for 4 h with 5 g kg^{-1} of both enzymes. An ACE inhibitory peptide was purified and a novel peptide found with a sequence of Phe-Gln-Pro-Ser (IC_{50} value of 27.0 μmol L^{-1}). Both protein hydrolysates and a synthesised peptide (Phe-Gln-Pro-Ser) demonstrated potent antihypertensive activities in spontaneously hypertensive rats.

CONCLUSION: Protein hydrolysate of *Kacang* goat meat produced by sequential digestion with *endo*-proteinase and protease complex has great potential as a functional ingredient, particularly as an antihypertensive agent.

Keywords: protein hydrolysate of *Kacang* goat meat; angiotensin converting enzyme inhibitor; antihypertensive; bioactive peptide

INTRODUCTION

Hypertension is a non-communicable disease known as the silent killer. Individuals with hypertension are at elevated risk of stroke, heart disease and kidney failure.[1] Hypertension affects one billion people worldwide and one-third of adults in the world.[2] Hypertension is responsible for at least 45% of the deaths due to heart disease and 51% of the deaths due to stroke.[3] The prevalence of hypertension in Indonesia is 26.5% as indicated by measurement and medical history.[4] Jenie and Adi[5] examined the acute effects of consumption of goat and beef meat upon blood pressure. The results showed that consumption of goat meat could increase systolic blood pressure in normotensive young males; however, beef did not exhibit this effect. The negative issue facing Indonesian people is that consumption of goat meat can lead to hypertension.[6]

Natural angiotensin converting enzyme (ACE) inhibitory peptides from food used as an alternative antihypertensive agent has received more attention by researchers. Natural ACE inhibitory peptides have several advantages, including the lack of side-effects associated with synthetic drugs, lower inhibition to ACE than synthetic drugs. Consequently, there is potential for safer levels of bradykinin in the body.[7] Much research on ACE inhibitory peptide in food proteins has been done.[8] The ACE inhibitory peptide can be classified according to the animal protein from which it was derived and the type of proteases that were used to generate these bioactive peptides. The animal proteins used as sources of ACE inhibitory peptide were dairy products (milk and fermented milk),[9,10] red meat,[11,12] poultry,[13] fish,[14] seafood,[15] and eggs.[16] The proteases can be single or paired combinations of proteases. Compared to single proteases, paired combinations of *endo*-proteinase and *exo*-peptidase are often preferred due to their ability to produce more hydrolysable material and a greater degree of hydrolysis.[17]

* *Correspondence to: Joko Hermanianto, Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Jl. Lingkar Akademik No 1, Bogor Agricultural University, IPB Campus Darmaga, Bogor 16680, Indonesia. E-mail: jhermanianto01@gmail.com*

a *Department of Food Science and Technology, Researcher at the SEAFAST Centre, Bogor Agricultural University, Bogor 16680, Indonesia*

b *Faculty of Agricultural and Animal Science, Sultan Syarif Kasim Riau State Islamic University, Pekanbaru 28293, Indonesia*

c *Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor 16680, Indonesia*

d *Faculty of Animal Science, School of Veterinary Medicine, Kitasato University, Towada-shi 0348628, Japan*

Meat protein hydrolysate is a potential functional ingredient when ACE inhibitory peptides exhibit antihypertensive activity in spontaneous hypertensive rats (SHR). The high activity of *in vitro* ACE inhibition does not always correspond with their antihypertensive effects.[13,18] Thermolysin digest of chicken muscle has a potent ACE inhibitory activity; however, these purified ACE inhibitory peptides did not show antihypertensive activity in spontaneous hypertensive rats.[13] This inconsistency between *in vitro* ACE inhibitory activity and *in vivo* antihypertensive activity of the active peptides resulted from degradation by gastrointestinal enzymes into inactive peptide fragments.[8] Furthermore, the correlation between *in vitro* ACE inhibitory and *in vivo* antihypertensive activities has not been well investigated.[18]

Meat protein of *Kacang* goat has a high content of glutamic acid, aspartic acid, lysine, leucine, arginine, and glycine.[19] Its protein hydrolysate has a soluble protein content of approximately 32.0 mg mL^{-1}.[20] An *in vivo* study using SHR showed that meat hydrolysates also decreased the effect of angiotensin II and may prevent conversion of ACE to angiotensin-II through a competitive peptide. A previous study, administering meat hydrolysates, showed blood pressure lowering effect.[21]

In the current study, paired combinations of Protamex® and Flavourzyme® using three different hydrolysis times and enzyme concentrations were used to generate an ACE inhibitory peptide. We prepare a protein hydrolysate of *Kacang* goat meat as a functional ingredient, selecting of the type of proteases to be used and considered food-grade aspects[22] to generate less bitter peptides. Bitterness is an important limitation in utilising protein hydrolysates for many food applications, particularly in hydrolysate-based beverages.[23] Adler-Nissen[24] indicated that *exo*-peptidases combined with *endo-protease* can improve the flavor profile of peptides. Protamex® has been reported to produce non-bitter hydrolysates.[25] Flavourzyme® is a fungal protease complex which contains both *endo*-protease and *exo*-peptidase activities with non-specific peptide cleavage activities.[17,26,27] Flavourzyme® can minimise the bitterness in hydrolysate products[27] and generate ACE inhibitory activity in protein hydrolysates of tilapia fillets.[26]

The *Kacang* goat is indigenous to Indonesia and Malaysia, and is common throughout in Southeast Asia.[28] In Indonesia, the *Kacang* goat has the highest population among goats and its meat is widely consumed.[29] It contains approximately 230 g kg^{-1} w/w protein on a wet weight basis.[19] *Kacang* goats have small bodies and are prolific.[29] From this point of view, the meat of *Kacang* goat has a great potency as a raw material for production of protein hydrolysates.

In a recent study, protein hydrolysates from the loin meat of *Kacang* goat were sequentially digested using pepsin, trypsin and chymotrypsin and showed high ACE inhibitory activity.[20] Furthermore, the sequence of an ACE inhibitory peptide was found.[30] However, there is no information on the antihypertensive activity *in vivo* of protein hydrolysate of *Kacang* goat meat. The purpose of this research was to examine the potency of protein hydrolysates derived from *Kacang* goat in their inhibition of angiotensin converting enzyme (ACE) and antihypertensive activities.

MATERIALS AND METHODS

Materials

The male *Kacang* goats (*Capra aegagrus hircus*) aged 8–24 months from a local farm in East Java were brought to Bogor by Mitra Tani Farm, West Java, Indonesia. Commercial proteinases, Flavourzyme® (*Aspergillus oryzae*, EC.3.4.11.1) and Protamex® were purchased from Novozyme A/S (Bagsvaerd, Denmark). Angiotensin converting enzyme (from rabbit lung) and substrate peptide hippuryl-L-histidyl-leucine (HHL) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade.

Preparation of *Kacang* goat meat

A total of 10 male *Kacang* goats were slaughtered according to Islamic practice at a local slaughterhouse. In Indonesia, generally male goats aged 8–24 months are used for meat. The body part used for this research was the carcass leg. Each of a pair of legs was separated from carcasses prior to manual deboning. Then, the leg meat was cut into small pieces (approximately $2 \times 2 \times 2$ cm^3), packed in plastic pouches laminated with aluminium foil, vacuum sealed and stored at −23 °C. Before grinding, the frozen meats were slowly thawed to 5 °C for 15 h prior to being finely ground five times in a meat grinder. Samples were weighed and prepared for hydrolysis. All the leg meat was pooled to produce protein hydrolysates.

Preparation of protein hydrolysate from *Kacang* goat meat

Hydrolysis was performed according to Chinq-Mars and Li-Chan,[31] with slight modifications. A total of 100 g of minced goat meat and 300 mL of distilled water were homogenised in a blender (National, Tangerang, Indonesia) for 30 s and the homogenate was decanted into a 500 mL glass Szeged beaker. In the next stage, the homogenate was heated to 85 °C for 30 min. After cooling to room temperature (27 °C), the pH of homogenate was adjusted to pH 7 (Milwawkee MW 801; Milwaukee Electronics Kft, Szeged, Romania, Hungary) with 1 mol L^{-1} of NaOH. The temperature of hydrolysis was set to 50 °C in an incubating shaker (Infors HT CH-4103; Infors AG, Bottmingen, Switzerland). The first hydrolysis was conducted by adding Protamex® to the homogenate at several concentrations (5, 10, 15 g kg^{-1}, w/w of the total substrate) and hydrolysed for 60 min. The next hydrolysis was conducted by adding Flavourzyme® at several concentrations (5, 10, 15 g kg^{-1}, w/w of the total substrate) to the first hydrolysate. Digestion proceeded for 1, 3 and 5 h. The pH of homogenate was kept constant by addition of 6 mol L^{-1} NaOH and was monitored every 30 min during hydrolysis. When the hydrolysis time was reached, both proteases were inactivated by heating to 85 °C for 30 min followed by cooling to room temperature. Then, hydrolysates were centrifuged at 11 900 × *g*, at 4 °C for 20 min. The supernatants were filtered using filter paper and pH of supernatants were neutralised. The supernatants were freeze dried (Labconco/LYPH LOCK-18; Labconco corp, Kansas City, MO, USA). The hydrolysate powders were packed into aluminium foil laminated plastic pouches and stored at −20 °C.

Purification and identification ACE inhibitory peptide

Purification and identification of ACE inhibitory peptides from protein hydrolysates of *Kacang* goat meat were performed according to Jang and Lee.[32] The purification steps are discussed below.

Homogenisation and ultra-filtration

A total of 2 g of hydrolysate was dissolved in 20 mL of distilled water and stirred for 30 min. Then, the hydrolysate solution was poured into a centrifugal filter unit which contained an ultra-filtration membrane [molecular weight cut-off (MWCO)

3000 Da] (Amicon®; Millipore Corp., Bedford, MA, USA) and was centrifuged at 9391 × *g* for 30 min at 4 °C. The supernatants were re-filtered through a 0.45 µm Advantec syringe filter (Toyo Roshi Kaisha, Tokyo, Japan) and were analysed for ACE inhibitory activity. The supernatant volumes were adjusted with 10 mL of distilled water and subjected to fractionation using gel permeation chromatography.

Gel permeation chromatography

A total of 2.5 mL solution from ultra-filtration was applied to an open column (size 27 × 4 cm) filled with Sephadex™ G-10 (GE Healthcare Bioscience, Uppsala, Sweden) resin. The mobile phase was distilled water at flow rate of 4.2–4.3 mL min^{-1} at room temperature (26–27 °C). The absorbance of the eluents was measured using a spectrophotometer set to 215 nm. Fractionation was repeated four times. Eluents from each fraction were collected and analysed for ACE inhibitory activity. The active fraction was collected and freeze dried (Eyela, Tokyo Rikakikai Co., Tokyo, Japan).

Reversed-phase high-performance liquid chromatography

The active fraction from gel permeation was dissolved in 1.5 mL of distilled water and was re-fractionated using high-performance liquid chromatography in a reverse-phase mode, HPLC-RP (column CAPCELL PAK C18, MG II 4.6 mm ID × 150 mm; Shiseido, Tokyo, Japan). Elution was performed using a linear gradient system from solvent A [0.05% formic acid in distilled water (Millipore)] to solvent B (0.05% formic acid in acetonitrile) at a flow rate of 1 mL min^{-1}. Absorbance was measured at 215 nm. Each fraction was analysed for ACE inhibitory activity (first HPLC run). The active fraction further was purified using HPLC-RP with the same system and analysed for ACE inhibitory activity (second HPLC run). The active fraction from the second HPLC run was dissolved with distilled water and was re-fractionated using HPLC-RP (loaded on column X Bridge™ BEH 130, C18, 3.5 µm, size 2.1 ID × 100 mm; Waters, Dublin, Ireland). Elution was performed with linear gradient [solvent A: 0.08% NH_4HCO_3 in distilled water (Millipore), solvent B: 0.08% NH_4HCO_3 in acetonitrile)] at a flow rate of 1 mL min^{-1} (third HPLC run). The active fraction was further purified using the same HPLC-RP system (fourth HPLC run) and was analysed for ACE inhibitory activity. The molecular weight of the most active fraction (from fourth HPLC run) was confirmed by LCMS-QP8000 (Shimadzu-Biotech, Kyoto, Japan). The most active fraction was concentrated and was applied to a protein sequencer for identification of its amino acid sequence.

Identification of amino acids sequences of the purified peptide

The molecular formula of the most active fraction (from fourth HPLC run) was confirmed using LCMS-QP8000 (Shimadzu-Biotech). Then, it was sequenced using the Edman degradation method with an automated protein sequencer (model PPSQ-31A; Shimadzu-Biotech) equipped with an online detection system for PTH-amino-acids. The sample was dissolved in a 40% acetonitrile solution and then it was transferred onto an activated polyvinylidene fluoride membrane. After fixing with polybrene, the amino acid sequence of the peptide was determined.

Measurement of ACE inhibitory activity

ACE inhibitory activity was measured according to the method of Cushman and Cheung[33] with the modification of Arihara *et al.*[11] A sample solution (15 µL) was mixed with 125 µL of 100 mmol L^{-1} sodium borate buffer (pH 8.3) containing 7.6 mmol L^{-1} of Hip-His-Leu and 608 mmol L^{-1} of NaCl. Then it was pre-incubated for 5 min at 37 °C. The reaction was initiated by addition of 50 µL of ACE dissolved in distilled water. The mixture was incubated for 30 min at 37 °C. For the control, 50 µL of distilled water was used. The reaction was stopped by addition of 125 µL of 1 mol L^{-1} HCl. Hippuric acid liberated by ACE was extracted by addition of 750 µL ethyl acetate to the mixture with vigorous shaking. After centrifugation at 13 760 × *g* for 10 min, 500 µL of the upper layer was collected and then dried at 90 °C for 30 min. Hippuric acid was dissolved in 1 mL of distilled water and its concentration photometrically determined at 228 nm. ACE inhibitory activity was calculated using the equation:

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

where *A* is the absorbance of sample reaction, *B* is the absorbance of the blank, and *C* is the absorbance of the control (distilled water).

Antihypertensive activity

Animal preparation

All procedures involving animals were performed in accordance with 'the animal handling protocol approval' which is issued by the local animal care committee of the School of Veterinary Medicine and Animal Science Kitasato University, Japan. Six-week-old male SHR were purchased from Charles River Japan, Inc. (Yokoyama, Japan). The SHR were housed in cages on a cycle of 12 h of light and 12 h of darkness. The temperature and humidity in the cages were controlled at 24 °C and 50–60%, respectively. The SHR were fed a standard laboratory diet (CE-2; Clea Japan, Inc., Tokyo, Japan), and tap water was available *ad libitum*.

Sample preparation

Protein hydrolysates of *Kacang* goat meat (5 g) was suspended in 100 mL of 100% ethyl alcohol. A precipitate was collected via centrifugation. The precipitate was suspended in 100 mL of 50% ethyl alcohol. After centrifugation, the supernatant (50% ethyl alcohol soluble fraction) was collected and evaporated using a rotary evaporator. The sample remaining after evaporation was dissolved in small amount of distilled water and freeze dried. The resulting powder was used for oral administration to SHR.

Two synthetic peptides, Phe-Gln-Pro and Phe-Gln-Pro-Ser, used in this study were provided by Thermo Fisher Scientific GmbH (Schwerte, Germany). The tri-peptide Phe-Gln-Pro was used as a standard ACE inhibitory peptide. Both peptides were synthesised using a solid-phase method with a peptide synthesiser. The synthesised peptides were purified using HPLC on a reverse-phased column. The purities of the synthesised peptides were determined using analytical LC-MS.

Dosing of the animals

The SHR were 14–16 weeks old [265–305 g body weight (BW)]. Oral administration of the protein hydrolysate was performed as follows. The protein hydrolysate from *Kacang* goat meat was dissolved in distilled water and assayed for *in vivo* antihypertensive activity at the doses of 0.01 g kg^{-1} and 0.1 g kg^{-1} BW. All the volumes of oral administration were at a dose of 1 mL. The mean of systolic blood pressure (SBP) of the SHR was 206.2 mmHg before administration.

Solutions of synthesised peptides were dissolved in distilled water and assayed for *in vivo* antihypertensive activity at a dose

5 µmol L^{-1} kg^{-1} BW (i.e. 0.00195 g kg^{-1} BW of Phe-Gln-Pro and 0.00239 g kg^{-1} BW of Phe-Gln-Pro-Ser). Distilled water was used as a control. All the volumes of oral doses of peptides were 1 mL. The mean SBP of the SHR was 215.8 mmHg before administration.

Measurement of blood pressure

Rats were each given a sample solution by gastric intubation with a metal tube (Natsume Seisakusho Co., Tokyo, Japan) and were put in a thermostatic box at 40 °C for 15 min. The SBP of each SHR was measured by the tail cuff method with a programmed electrosphygmomanometer (BP-98; Softron Co., Tokyo, Japan). The antihypertensive activity of sample and control were observed by measuring the SBP at 0, 2, 4, 6, 8 and 24 h after a single administration. Results were expressed as the mean value of SBP on nine SHRs at each time. Significant differences from the control were evaluated. The measurements were made in triplicate and average values are reported.

Statistical analysis

Changes in SBP were determined by the difference between SBPs before and after administration. Data are expressed as means value and standard error of the mean (SEM). Data was analysed statistically by two-sample *t*-tests to compare the mean of each dose group with that of the control group. The probability level used to determined statistical significance was $P < 0.05$.

RESULTS AND DISCUSSION

ACE inhibitory activity of protein hydrolysate from *Kacang* goat meat and its purified peptide

In this study, food-grade proteinases in paired combinations of Protamex® and Flavourzyme® were used to produce ACE inhibitory peptide of protein hydrolysates from *Kacang* goat meat with a less bitter taste. Additionally, these paired combinations also produced hydrolysates with various percentages of hydrolysable material and degree of hydrolysis ranging from 49.8% to 76.0%, and 10.4% to 26.3%, respectively.

Initial screening was conducted to find the protein hydrolysate with the highest ACE inhibitory activity. The result showed that all of water soluble fractions of protein hydrolysates from *Kacang* goat meat had ACE inhibitory activity. The highest ACE inhibitory activity resulted from a hydrolysate digested for 4 h with 5 g kg^{-1} of both enzymes (as shown in Fig. 1). This hydrolysate was used in the next purification step and *in vivo* analysis.

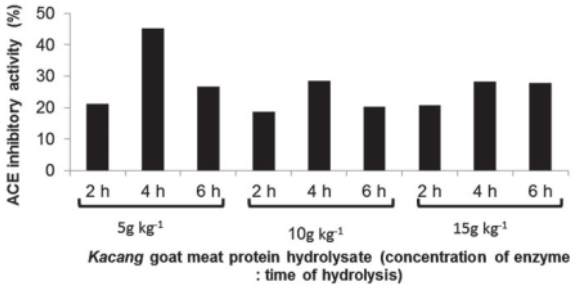

Figure 1. ACE inhibitory activity of protein hydrolysates from *Kacang* goat meat. Analysis was done in two replicates. Concentration of the hydrolysate was 0.5 mg mL^{-1}

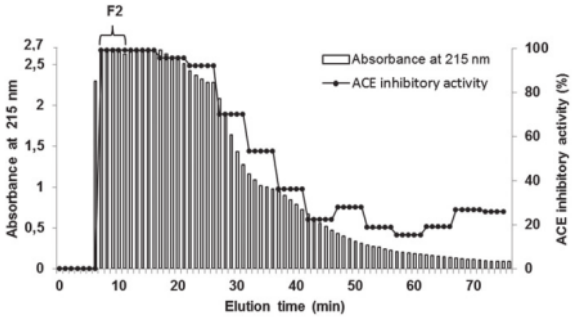

Figure 2. Fractionation of protein hydrolysates of *Kacang* goat meat on a Sephadex G-10 gel filtration column and ACE inhibitory activity of each fraction. F2 (fraction 2), elution time 8–12 min, was the most active fraction and was further analysed using high-performance liquid chromatography with a reversed-phase mode (HPLC-RP).

Previous research by Jamhari *et al.*[20] found that protein hydrolysate from loin meat of *Kacang* goat sequentially hydrolysed with digestive enzymes had ACE inhibitory activity of approximately 80.6% with an IC_{50} of 316 µg mL^{-1}. Differences in types of proteases and muscle parts used in the study caused differences in ACE inhibitory activity. ACE inhibitory activity was affected by peptide composition arising from hydrolysis and its binding ability with the active site of ACE.[34]

Raghavan and Kristinsson[26] reported that tilapia protein hydrolysate digested using Flavourzyme® showed potent ACE inhibitory activity with a 25% degree of hydrolysis. Sequentially digestion using a combination commercial protease and Flavourzyme® to produce protein hydrolysate of red meat has not yet been reported.

Fractionation of protein hydrolysate of *Kacang* goat meat was started by concentrating the selected hydrolysate using a centrifugal ultra-filtration membrane (MWCO, 3000 Da). This ultra-filtration aimed to eliminate all proteins in the hydrolysate with molecular weights over 3000 Da. The ultra-filtered extract had a two-fold higher ACE inhibitory activity over the original extract (45% increased to 94%). Furthermore, peptides with molecular weights lower than 3 kDa had the highest ACE inhibitory activity. This fraction was loaded onto a Sephadex G-10 column. ACE inhibitory activities of each of the fractions are shown in Fig. 2. The results showed that fraction 2 (elution time 8–12 min) had the greatest ACE inhibitory activity.

Eluent of fraction 2 from the Sephadex G-10 column was subjected to a reversed-phase high-performance liquid chromatography analysis (HPLC-RP). The chromatogram is shown in Fig. 3. Two active fractions were selected from the first HPLC run. They are fraction 3 (elution time 5–7.5 min) and fraction 5 (elution time 10–12.5 min) with ACE inhibitory activities of 74.4% and 64.3%, respectively. Both of these fractions were further purified using a second HPLC run. An active sub-fraction from fraction 5 was obtained with an ACE inhibitory activity of approximately 30.7%.

The most active fraction was further purified using a different column and procedure (third HPLC run, Fig. 4). Another active fraction (A) was obtained corresponding to an elution time of 4–5 min. Then, fraction A was further purified using a fourth HPLC run with similar conditions as the previous run producing a single peptide. This single peptide had a molecular weight of 478 Da determined using the *m/z* of mass spectra from LC-MS analysis. This single peptide was then collected, concentrated and subjected to amino acid

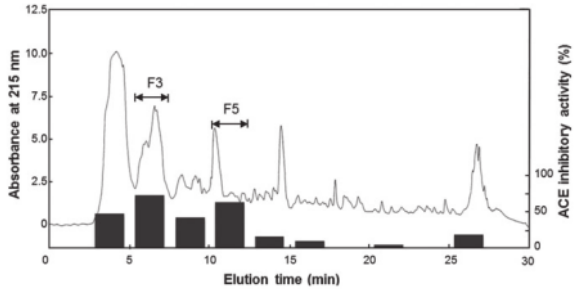

Figure 3. Fractionation of protein hydrolysates from *Kacang* goat meat using reversed-phase high performance liquid chromatography (RP-HPLC) (first HPLC run). Fractions were collected and assayed for ACE-inhibitory activity. Fraction 3 had an elution time of 5–7.5 min and fraction 5 corresponded to an elution time of 10–12.5 min.

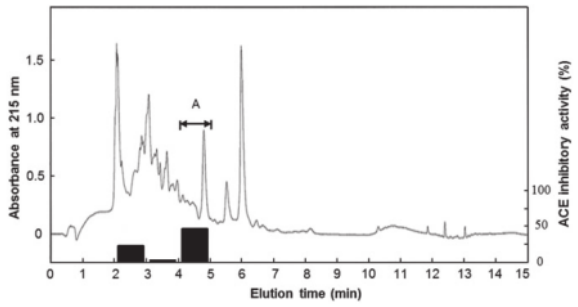

Figure 4. Fractionation of protein hydrolysates from *Kacang* goat meat using reversed-phase high performance liquid chromatography (RP-HPLC) (third HPLC run). Fractions were collected and assayed for ACE-inhibitory activity. The most active fraction (A) corresponded to an elution time of 4–5 min.

sequencing using the Edman degradation method. The amino acid sequence was identified as a *tetra*-peptide, Phe-Gln-Pro-Ser. This sequence of peptide has not yet been determined to be an ACE inhibitory peptide derived other protein hydrolysates.[21,34,35]

The *tetra*-peptide Phe-Gln-Pro-Ser was found to be an ACE inhibitory peptide with an IC_{50} of 27.0 μmol L^{-1}. Phe-Gln-Pro-Ser also was found in the actin sequence of muscle skeletal material of *Sus scrofa* (wild swine), *Oncorhynchus keta* (chum salmon), *Bombyx mori* (silkworm) and *Crassostrea gigas* (pacific oyster) based on multiple alignment of homology analysis in a protein database.[35] The *tri*-peptide Phe-Gln-Pro was found to be an ACE inhibitory peptide derived the thermolysin digest of dried bonito with an IC_{50} of 12.0 μmol L^{-1}.[36] Jamhari *et al.*[30] found the sequence of an ACE inhibitory peptide derived the protein hydrolysate from loin meat of *Kacang* goat. The sequence was Leu-Thr-Glu-Ala-Pro-Leu-Asn-Pro-Lys-Ala-Arg-Asn-Glu-Lys with a molecular weight of 1581 Da and had an IC_{50} value equal to 190 μg mL^{-1} (120 μmol L^{-1}). Compared to the results obtained by Jamhari *et al.*,[30] the ACE inhibitory peptide found in this study had a shorter sequence and IC_{50} value 4.4-fold lower than that of protein hydrolysate from loin meat of *Kacang* goat. Norris and Fitzgerald[7] postulated that the potent ACE inhibitory peptides commonly have short sequences, i.e. two to 12 amino acids in length. Some structural features of potent ACE inhibitory peptides are characterised by the presence hydrophobic residues at the C-terminus and N-terminus, existence of aromatic or branched

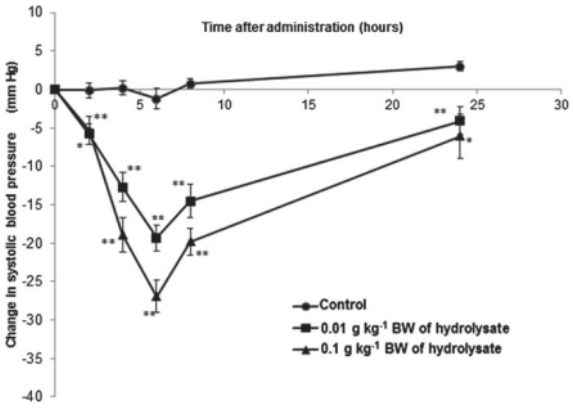

Figure 5. Antihypertensive activities of single oral administration of protein hydrolysate from *Kacang* goat meat at concentrations 0.01 g kg^{-1} BW and 0.1 g kg^{-1} BW. This experiment used 14- to 16-week-old rats. Each point indicates the mean of systolic blood pressure of nine SHRs, and the vertical bars represent the standard error. Distilled water was used as a control. Significant difference from control at each time: $^{*}P < 0.05$, $^{**}P < 0.01$.

side chain amino acids, and proline at one or more positions. In previous studies, ACE inhibitory peptides derived from muscle protein of domestic animals were commonly found in pork, beef and chicken,[11,13,35,37] whereas ACE inhibitory peptides derived goat meat are still considered rare.

Results of the current study emphasise that differences in muscle protein and type of protease used to generate hydrolysate caused differences in ACE inhibitory peptide. This is in agreement with Jang and Lee[32] and Di Bernardini *et al.*,[37] who showed that ACE inhibitory peptides from beef rump sarcoplasmic protein hydrolysate differed from ACE inhibitory peptides derived from bovine brisket sarcoplasmic protein. Arihara *et al.*[11] and Muguruma *et al.*[12] explained that protein hydrolysate of porcine leg meat (*bicep femoris*) hydrolysed with different commercial proteases produced different ACE inhibitory peptides.

Antihypertensive activity of *Kacang* goat meat protein hydrolysate and synthetised peptides

The changes of SBP of SHR after a single oral administration of protein hydrolysate of *Kacang* goat meat at concentrations 0.01 g kg^{-1} BW and 0.1 g kg^{-1} BW are shown in Fig. 5. Distilled water was used as a control. SBP of SHR did not change significantly during 24 h of observation. As can be seen in Fig. 5, SBP of SHR after administering 0.01 g kg^{-1} BW of hydrolysate was lower than of the control group between 2 h to 24 h of observation ($P < 0.01$). The reduction in SBP was 19.3 ± 1.66 mm Hg at 6 h after administration. A similar pattern occurred in SBP of SHR after administering 0.1 g kg^{-1} BW of hydrolysate. The reduction was greater due to higher concentration of hydrolysate given. The highest reduction of SBP was 26.9 ± 2.11 mm Hg, occurred at 6 h after administration when compared that of the control ($P < 0.01$). SBP of SHR was still significantly lower than that of the control group after 24 h ($P < 0.05$).

As the highest reduction of SBP in SHR occurred in 6 h after administration and both concentrations of hydrolysates induced similar effects in lowering blood pressure over 24 h. This indicates that protein hydrolysate from *Kacang* goat meat digested using Protamex® and Flavourzyme® contained ACE inhibitory peptides that presumably included a true inhibitor type and pro-drug type. This is in line with Fujita *et al.*[13] Both inhibitor and pro-drug

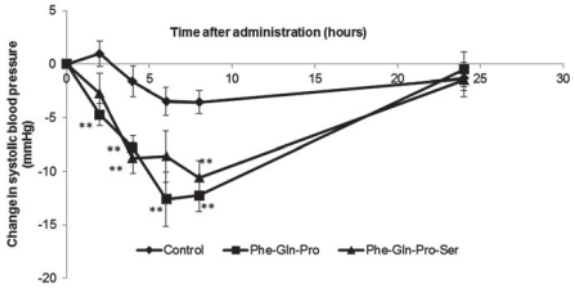

Figure 6. Antihypertensive activities of single oral administration of synthesised peptides at concentrations 0.00195 g kg^{-1} BW of Phe-Gln-Pro and 0.00239 g kg^{-1} BW of Phe-Gln-Pro-Ser. This experiment used 18- to 20-week-old rats. Each point indicates the mean of systolic blood pressure of nine SHRs, and the vertical bars represent the standard error. Distilled water was used as a control. Significant difference from control at each time: $^{**}P < 0.01$.

type peptides are characterised by long-lasting antihypertensive effects after oral administration to SHR.

The trend of reduction in SBP values in this study was similar to that of Nakashima *et al.*[38] SBP of SHR was significantly lowered after 24 h administering thermolysin hydrolysate of water insoluble protein from porcine skeletal muscle at 2.1 g kg^{-1} BW and thermolysin hydrolysate of myosin at 0.03 g kg^{-1} BW. The highest reductions occurred after 8 h (22.9 ± 5.4 mm Hg) and 6 h (24.9 ± 5.1 mm Hg).

The changes of SBP of SHR after single oral administering each of the synthesised peptides, Phe-Gln-Pro and Phe-Gln-Pro-Ser, are shown in Fig. 6. Distilled water was used as a control and SBP of SHR did not change significantly during 24 h of observation. The *tri*-peptide Phe-Gln-Pro showed the highest reduction of SBP, 12.6 ± 2.54 mm Hg, at 6 h after administration. It was significantly different compared to the control ($P < 0.01$). The *tetra*-peptide Phe-Gln-Pro-Ser showed the highest reduction of SBP, 10.6 ± 1.58 mm Hg at 8 h, and was significantly different when compared to the control ($P < 0.01$). The SBP of SHR was not different among the controls at 2, 6 and 24 h. It can be concluded that the antihypertensive activity of the *tri*-peptide Phe-Gln-Pro is more stable than that of the *tetra*-peptide Phe-Gln-Pro-Ser.

Result in this study show that hydrolysate resulting from 4 h incubation with 5 g kg^{-1} of Protamex® and Flavourzyme®, respectively, produced both ACE inhibitory and antihypertensive activities. The synthetic peptide Phe-Gln-Pro-Ser from the protein hydrolysate of *Kacang* goat meat also demonstrated antihypertensive activity. This sequence has not yet been found as an antihypertensive peptide derived from other protein hydrolysates.[21,34] Therefore, Phe-Gln-Pro-Ser can be considered a novel antihypertensive peptide.

ACE inhibitory peptide of gastrointestinal enzyme hydrolysate of *Kacang* goat meat has been reported.[30] In the current study, the result of ACE inhibitory activity and antihypertensive tests indicated that protein hydrolysate from *Kacang* goat meat has great potential as an antihypertensive agent. Results of this study may considerably increase value addition to goat meat.

Use of a paired combination, Protamex® and Flavourzyme®, to generate protein hydrolysate was considered. Improved sensory properties of hydrolysate were achieved since Flavourzyme® is a known de-bittering agent in hydrolysate products.[27] The bioactive peptide in *Kacang* goat meat protein hydrolysate can perhaps be applied as a bioactive compound in development of functional food. Thus, *Kacang* goat meat protein hydrolysate has great potential as functional ingredient, as well as use as a primary or supplement ingredient to enrich foods or drinks.

CONCLUSION

This study demonstrated that a water soluble fraction of a protein hydrolysate from *Kacang* goat meat sequentially digested using Protamex® and Flavourzyme® exhibited ACE inhibitory activity. Also, novel ACE inhibitory peptides from *Kacang* goat meat were found, namely Phe-Gln-Pro-Ser (478 Da), with an IC_{50} of 27.0 µmol L^{-1}. These peptides exhibit an antihypertensive effect. The results of the current study indicate that protein hydrolysate from *Kacang* goat meat has great potential as a functional ingredient and antihypertensive agent in the development of functional foods.

ACKNOWLEDGEMENT

This study was financially supported by the Directorate of Islamic Higher Education of the Ministry of Religious Affairs Republic of Indonesia through the program of PROSALE 2013 and Laboratory of Food Function and Safety, School of Veterinary and Medicine, Kitasato University, Japan.

REFERENCES

1 Vasdev S and Stuckless J, Antihypertensive effects of dietary protein and its mechanism. *Int J Angiol* **19**:e7–e20 (2010).
2 Cohen DL, Townsend RR, Angell SY and DiPette DJ, The World Health Organization recognizes non-communicable diseases and raised blood pressure as global health priority for 2025. *J Clin Hypertens* **16**:624 (2014).
3 WHO, A Global Brief on Hypertension, Silent killer, global public health crisis. Document number: WHO/DCO/WHD/2013. Available: http//:www.who.int [18 September 2014].
4 Ministry of Health Affair, Republic of Indonesia, *Basic Health Research*. Department of Research and Development of Health, Jakarta, pp. 122–124 (2013).
5 Jenie IM and Adi DK, Acute pressor response to the consumption of goat meat. *Mutiara Medika* **8**:35–43 (2008).
6 Sutama IK and Budiarsana IGM, *Panduan Lengkap Kambing Dan Domba, Cetakan 3*. Penebar Swadaya, Jakarta, p. 6 (2011).
7 Norris R and FitzGerald RJ, *Antihypertensive Peptides from Food Proteins, Bioactive Food Peptides in Health and Disease*. Available: www.intechopen.com [30 January 2013].
8 Li G-H, Le G-W, Shi Y-H and Shrestha S, Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutr Res* **24**:469–486 (2004).
9 Espejo-Carpio FJ, De Gobba C, Guadix A, Guadix EM and Otte J, Angiotensin I-converting enzyme inhibitory activity of enzymatic hydrolysates of goat milk protein fractions. *Int Dairy J* **32**:175–183 (2013).
10 Korhonen H, Milk-derived bioactive peptides: From science to applications. *J Funct Foods* **1**:177–187 (2009).
11 Arihara K, Nakashima Y, Mukai T, Ishikawa S and Itoh M, Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Sci* **57**: 319–324 (2001).
12 Muguruma M, Ahhmed AM, Katayama K, Kawahara S, Maruyama M and Nakamura T, Identification of pro-drug type ACE inhibitory peptide sourced from porcine myosin B: evaluation of its antihypertensive effects *in vivo*. *Food Chem* **114**:516–522 (2009).
13 Fujita H, Yokoyama K and Yoshikawa M, Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *J Food Sci* **65**:564–572 (2000).

14 Cho S-S, Lee H-K, Yu C-Y, Kim M-J, Seong E-S, Ghimire BK, *et al.*, Isolation and charaterization of bioactive peptides from Hwangtae (yellowish dried Alaska pollack) protein hydrolysate. *J Food Sci Nutr* **13**:196–203 (2008).

15 Wang J, Hu J, Cui J, Bai X, Du Y, Miyaguchi Y, *et al.*, Purification and identification of a ACE inhibitory peptide from oyster proteins hydrolysate and the antihypertensive effect of hydrolysate in spontaneously hypertensive rats. *Food Chem* **111**:302–308 (2008).

16 Majumder K and Wu J, Purification and characterisation of angiotensin I converting enzyme (ACE) inhibitory peptides derived from enzymatic hydrolysate of ovotransferrin. *Food Chem* **126**:1614–1619 (2011).

17 Nchienzia HA, Morawicki RO and Gadang VP, Enzymatic hydrolysis of poultry meal with *endo*- and *exo*peptidases. *Poult Sci* **89**:2273–2280 (2010).

18 Jao C-L, Huang S-L and Hsu K-C, Angiotensin I-converting enzyme inhibitory peptides: Inhibition mode, bioavailability, and antihypertensive effects. *BioMed* **2**:130–136 (2012).

19 Mirdhayati I, Hermanianto J, Wijaya CH and Sajuthi D, Carcass profile and chemical characteristic of male *Kacang* goat (*Capra aegragus hircus*). *Indonesian J Anim Vet Sci* **19**:25–33 (2014).

20 Jamhari J, Yusiati LM, Suryanto E, Cahyanto MN, Erwanto Y and Muguruma M, Comparative study on angiotensin converting enzyme inhibitory activity of hydrolysate of meat protein of Indonesian local livestocks. *J Indonesian Trop Anim Agric* **38**:27–33 (2013).

21 Ahhmed AM and Muguruma M, A review of meat protein hydrolysates and hypertension. *Meat Sci* **86**:110–118 (2010).

22 Ngo D-H, Ryu B and Kim S-K, Active peptides from skate (*Okamejei kenojei*) skin gelatin diminish angiotensin-I converting enzyme activity and intracellular free radical-mediated oxidation. *Food Chem* **143**:246–255 (2014).

23 Kamara MT, Amadou I, Kexue Z, Kelfala Foh MB and Huiming Z, The influence of debittering and desalting defatted foxtail millet (*Setaria italica* L.) protein hydrolysate. *Am J Biochem Mol Biol* **1**:39–53 (2011).

24 Adler-Nissen J, *Enzymic Hydrolysis of Food Proteins*. Elsevier Applied Science Publishers, New York (1986).

25 Liaset B, Julshamn K and Espe M, Chemical composition and theoretical nutritional evaluation of the produced fractions from enzymic hydrolysis of salmon frames with Protamex™. *Process Biochem* **38**:1747–1759 (2003).

26 Raghavan S and Kristinsson HG, ACE-inhibitory activity of tilapia protein hydrolysates. *Food Chem* **117**:582–588 (2009).

27 Nilsang S, Lertsiri S, Suphantharika M and Assavanig A, Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. *J Food Eng* **70**:571–578 (2005).

28 Tsukahara Y, Chomei Y, Oishi K, Kahi AK, Panandam JM, Mukherjee TK, *et al.*, Analysis of growth patterns in purebred *Kambing Katjang* goat and its crosses with the German Fawn. *Small Rumin Res* **80**:8–15 (2008).

29 Ginting S and Mahmilia F, Kambing Boerka: Kambing tipe pedaging hasil persilangan Boer x Kacang. *Wartazoa* **18**:115–126 (2008).

30 Jamhari J, Yusiati LM, Suryanto E, Cahyanto MN, Erwanto Y and Muguruma M, Purification of angiotensin converting enzyme inhibitory peptide derived from *Kacang* goat meat protein hydrolysate. *J Indonesian Trop Anim Agric* **38**:239–246 (2013).

31 Chinq-Mars CD and Li-Chan EC, Optimizing angiotensin I-converting enzyme inhibitory activity of Pacific hake (*Merluccius productus*) fillet hydrolysate using response surface methodology and ultrafiltration. *J Agric Food Chem* **55**:9380–9388 (2007).

32 Jang A and Lee M, Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. *Meat Sci* **69**:653–661 (2005).

33 Cushman DW and Cheung HS, Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem Pharmacol* **20**:1637–1648 (1971).

34 Ryan JT, Ross RP, Bolton D, Fitzgerald GF and Stanton C, Bioactive peptides from muscle sources: Meat and fish. *Nutrients* **3**:765–791 (2011).

35 Vercruysse L, Van Camp J and Smagghe G, ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: A Review. *J Agric Food Chem* **53**:8106–8115 (2005).

36 Yokoyama K, Chiba H and Yoshikawa M, Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried Bonito. *Biosci Biotechnol Biochem* **56**:1541–1545 (1992).

37 Di Bernardini R, Mullen AM, Bolton D, Kerry J, O'Neill E and Hayes M, Assessment of the angiotensin-I-converting enzyme (ACE-I) inhibitory and antioxidant activities of hydrolysates of bovine brisket sarcoplasmic proteins produced by papain and characterisation of associated bioactive peptidic fractions. *Meat Sci* **90**:226–235 (2012).

38 Nakashima Y, Arihara K, Sasaki A, Mio H, Ishikawa S and Itoh M, Antihypertensive activities of peptides derived from porcine skeletal muscle myosin in spontaneously hypertensive rats. *J Food Sci* **67**:434–437 (2002).

Angiotensin converting enzyme (ACE) inhibitory and antihypertensive activities of protein hydrolysate from meat of Kacang goat (Capra aegagrus hircus)

ORIGINALITY REPORT

18%	13%	13%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

1%

www.biorxiv.org

Internet Source

< 1%

/node/53356.full.pdf

< 1%

★ *https://www.bioRxiv.org/content/10.1101/174300v2.full.pdf*

< 1%

/content/10.1101/2020.06.25.170605v1

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		