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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Research Article
Increased Inhibition of Angiotensin Converting Enzyme (ACE) Obtained from Indonesian Buffalo Meat Protein Using SEP-PAK Plus C18

[1]Rio Olympias Sujarwanta, [1]Jamhari, [1]Edi Suryanto, [1]Rusman, [1]Setiyono, [1]Endy Triyannanto, [1]Yuny Erwanto and [2]Ragil Yuliatmo

[1]Department of Animal Product Technology, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Department of Leather Processing Technology, Politeknik ATK, Bantul, Yogyakarta, Indonesia

Abstract

Objective: This study aimed to investigate angiotensin converting enzyme (ACE) inhibitory activity derived from Indonesian native buffalo meat and its purified protein using SEP-PAK Plus C18. **Methodology:** Buffalo meat was hydrolyzed with pepsin, trypsin and a combination of pepsin and trypsin. These hydrolysates were assessed for dissolved protein, the molecular weight of proteins and ACE inhibitory activity. The hydrolysate with the highest ACE inhibitory activity was subsequently purified using SEP-PAK Plus C18. The molecular weight of the proteins was analyzed using descriptive statistics and the dissolved protein content and ACE inhibitory activity were evaluated in a complete randomized design. **Results:** Buffalo meat contained protein (22.66±0.2%) and dissolved protein (3.67±0.59 mg mL^{-1}). Enzymatic hydrolysis significantly elevated the level of dissolved protein and decreased the molecular weight of the proteins. The dissolved protein content following hydrolysis by pepsin, trypsin and a combination of pepsin and trypsin was 10.91±0.4, 8.09±0.8 and 10.90±0.4 mg mL^{-1}, respectively. The molecular weight of proteins in the buffalo meat homogenate ranged from 40-70 kDa, whereas, the hydrolysates were generally less than 40 kDa. The ACE inhibitory activity of the purified hydrolysate (IC_{50} = 75.8±20 µg mL^{-1}) was approximately twice that of the hydrolysate (IC_{50} = 133±18 µg mL^{-1}). Purification using SEP-PAK Plus C18 increased ACE inhibitory activity. **Conclusion:** Buffalo meat hydrolyzed by pepsin had the highest ACE inhibitory activity compared with other hydrolysates. Purification using SEP-PAK Plus C18 almost doubled the ACE inhibitory activity.

Key words: Buffalo meat, ACE-I, hydrolysis, enzymes, SEP-PAK plus C18

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Corresponding Author: Jamhari, Department of Animal Product Technology, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hypertension is a major health problem and is a major risk factor for the development of arteriosclerosis, cardiovascular disease, stroke and myocardial infarction[1]. According to Forouzanfar *et al.*[2], the number of adults in the world with hypertension is ~3.5 billion. In 2015, China, India, Russia, Indonesia and the United States accounted for more than half of the global Disability-Adjusted Life-Years (DALYs) related to a systolic blood pressure (SBP) of at least 110-115 mm Hg[2]. Stressful, fiercely competitive lifestyles and food habits have compounded this problem[3].

The treatment of hypertension involves chronic control of blood pressure under normal conditions. Angiotensin-I converting enzyme (ACE) inhibitors are one of several classes of pharmacologic agents that have been widely used in the treatment of hypertension. Natural peptides with ACE inhibitory activity were first found in snake venom[4] and that discovery encouraged the development of synthetic ACE inhibitors. A strong ACE inhibitor was developed in the 1980s and is currently in use[5]. The ACE inhibitors are effective in lowering the mean, systolic and diastolic pressures in hypertensive patients as well as in salt-depleted normotensive subjects[3].

Recently, many bioactive peptides derived from food proteins, including ACE inhibitors, have attracted attention due to their ability to prevent hypertension[6]. Some peptides from food, including porcine skeletal muscle proteins[7-11], salmon[12], fermented egg albumen[13], chicken legs[14] and goat milk casein[15], have been shown to have antihypertensive effects. The ACE inhibitory peptides, such as those reported in the studies above[16], are obtained from enzyme-hydrolyzed proteins that are produced using enzymes, such as trypsin or pepsin. Thus, peptides can be produced by the hydrolysis of food proteins during processing and digestion in the gastrointestinal tract[17]. To purify hydrolysates, Jamhari *et al.*[18] and Katayama *et al.*[6] used SEP-PAK Plus C18 and Yuliatmo *et al.*[14] also reported that using this technique led to an increase in ACE inhibitory activity.

Buffalo meat has several properties that differentiate it from beef, such as less intermuscular fat and cholesterol, fewer calories, more essential amino acids and higher iron content, it also has significant potential for the production of bioactive peptides[19]. In Indonesia, buffalo meat has become an important source of protein[20] because it contains as much protein as beef. However, no studies have been conducted on the peptides that can be derived from buffalo meat. This study aimed to investigate the potential of buffalo meat as a source of bioactive peptides that increase the angiotensin-converting enzyme inhibitory activity. Additionally, given the findings of Yuliatmo *et al.*[14], the use of SEP-PAK Plus C18 to purify protein was investigated to determine whether this technique increases bioactivity.

MATERIALS AND METHODS

Sample collection and preparation: Buffalo meat was obtained from a buffalo loin (*Longissimus dorsi*) from the Central Java area in Indonesia. Pepsin (porcine stomach mucosa) and trypsin were purchased from the Wako Pure Chemical Industry Ltd., Tokyo, Japan. Angiotensin-converting enzyme (ACE) from rabbit lung was obtained from the Sigma Chemical Co., St. Louis, USA and hippuryl-L-histidyl-leucine (HHL) free base was purchased from Nacalai Tesque, Kyoto, Japan.

The meat was homogenized following the method of Jamhari *et al.*[18]. Water (100 mL) was added to a 50 g sample of meat and blended using a Philips HR 2116 food processor (Philips, Indonesia) for 5 min. The meat extract was then homogenized using a Silverson L4R homogenizer (Silverson, England) for 10 min. The meat homogenates were incubated in a Memmert water bath (Memmert, Schwabach, Germany) for 30 min at 70°C and then cooled with ice. The homogenates were made and used immediately prior to analysis.

Chemical composition analysis: The moisture content, crude fat and crude protein of the buffalo meat were determined using standard methods of the Association of Official Analytical Chemists (AOAC)[21].

Preparation of hydrolysates: Buffalo meat homogenates were separated into three treatments, hydrolyzed by pepsin (BMP), trypsin (BMT) or a combination of pepsin and trypsin (BMPT). The hydrolyzation by pepsin was adjusted to pH 2, the hydrolyzation by trypsin was adjusted to pH 7.0 and the third treatment was hydrolyzed first by pepsin then hydrolysis was continued with trypsin. Prior to incubation, 0.01 g of enzyme was added to the homogenate. After 2 h of incubation at 37°C, the pH of the hydrolysate was adjusted to 7.0 and hydrolysis was terminated by heating to 95°C for 10 min followed by cooling in ice. The hydrolysates obtained were assayed to determine the concentration of dissolved protein, the molecular weight of the proteins and the ACE inhibitory activity.

Determination of dissolved protein: The concentration of dissolved protein in the hydrolysates was determined with the Biuret method using a spectrophotometer (Shimadzu, Tokyo, Japan) at a wavelength of 750 nm[22]. The protein concentration was determined by comparing the absorbance of the sample with the absorbance of a bovine serum albumin (Sigma, St. Louis, USA) standard.

Molecular weight of proteins using SDS PAGE: To determine the molecular weight of the proteins, the extracts were subjected to SDS PAGE according to the method of Laemmli[23] using 12% resolving gel and 5% stacking gel at a constant voltage of 140 V for 3 h. Protein bands were visualized by staining with Coomassie Brilliant Blue R-250 (Sigma, St. Louis, USA). The molecular weights of the proteins were estimated by comparison with molecular weight standards.

Inhibition of angiotensin I-converting enzyme activity: The ACE inhibition assays were performed using the method of Cushman and Cheung[24] after modification. Briefly, 30 μL of sample was added to 250 μL of 0.1 M borate buffer (pH 8.5) that included 7.6 mM hippuryl-L-histidyl-L-leucine (HHL) (Nacalai Tesque Inc., Kyoto, Japan) as a substrate and 0.608 M sodium chloride. The reaction was started by the addition of 100 μL of 60 mU mL^{-1} rabbit lung ACE in 0.25 M borate buffer (pH 8.5). To terminate the reaction, 544 μL of 1 M hydrochloric acid (Merck, Darmstadt, Germany) was added. To extract the hippuric acid liberated from the HHL by the ACE, 1.5 mL of ethyl acetate (Merck, Darmstadt, Germany) was added the tubes, which were vigorously shaken and then centrifuged for 10 min at 3,600 rpm. About 1 mL of the ethyl acetate layer was collected into another tube and dried for 10 min at 100°C. The residue obtained was dissolved in 1 mL of 1 M sodium chloride (Merck, Darmstadt, Germany) and the absorbance of the solution was determined at 228 nm. The ACE inhibitory activity was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{C-S}}{\text{C-B}} \times 100$$

where, S is the absorbance of the sample (inhibitor) plus ACE (enzyme) and HHL (substrate), C is absorbance of the water (positive control) plus ACE (enzyme) and HHL (substrate) and B is the absorbance of water (negative control) plus ACE (enzyme), HHL (substrate) and hydrochloric acid (added prior to the reaction). The inhibitory activity was expressed as the IC_{50} (the concentration needed to inhibit 50% of the original ACE activity) of the sample. A decrease in the IC_{50} indicates an increase in ACE inhibitory activity. The inhibitory activity of the hydrolysate fraction was expressed as a percentage of ACE inhibition.

Purification of hydrolyzed peptides using a SEP-PAK Plus C18 cartridge: The hydrolysates with the highest ACE inhibitory activity were purified using a SEP-PAK Plus C18 cartridge (Waters Co., Milford, MA, USA) and eluted with 2% acetonitrile (Merck, Darmstadt, Germany) and 0.1% trifluoroacetic acid (Merck, Darmstadt, Germany) in water followed by 65% acetonitrile (Merck, Darmstadt, Germany) and 0.1% trifluoroacetic acid in water. The purpose of this step was to remove the non-protein components in each hydrolysate. The filtrates were dried using a freeze dryer and were then diluted with water[14].

Statistical analysis: The data on chemical composition and the molecular weight of proteins were analyzed using descriptive statistics, whereas, the data on dissolved protein concentration and ACE inhibitory activities were analyzed using a one-way ANOVA in SPSS v15.0. The results are expressed as the mean±standard deviation. Differences were considered significant at $p<0.05$. When significant differences were detected, the analysis was followed with a Duncan's new multiple range test[25].

RESULTS

Chemical composition of buffalo meat: The average chemical composition of the buffalo loin consisted of 72.16±0.24% water, 2.44±0.03% fat, 1.46±0.18% collagen and 22.67±0.20% protein and is shown in Table 1.

Dissolved protein content of buffalo meat hydrolysate: The dissolved protein of the hydrolysates of buffalo meat using trypsin (8.09±0.8 mg mL^{-1}) showed a significant increase ($p<0.05$) compared with the homogenate without hydrolysis (3.67±0.59 mg mL^{-1}), the highest levels of dissolved protein occurred in samples buffalo meat hydrolyzed by pepsin (BMP) (10.91±0.4 mg mL^{-1}). These results are shown in Table 2.

Table 1: Chemical composition of buffalo meat

Components	Percentage
Moisture	72.161±0.24
Fat	2.445±0.03
Protein	22.666±0.20

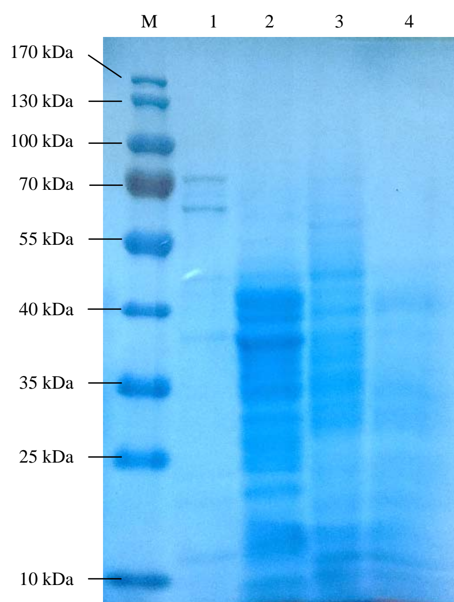

Fig. 1: Results of SDS PAGE

M: Marker, 1: Homogenate, 2: BMP, 3: BMT, 4: BMPT

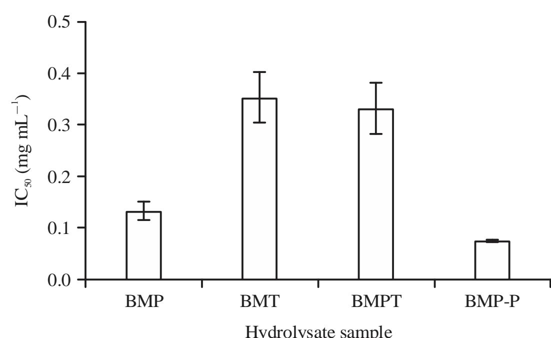

Fig. 2: Determination of the IC_{50} values

BMP: Buffalo meat hydrolyzed by pepsin, BMT: Buffalo meat hydrolyzed by trypsin, BMPT: Buffalo meat hydrolyzed by pepsin+trypsin, BMP-P: Purified protein of BMP

Table 2: Dissolved protein contents after hydrolysis of buffalo meat

Hydrolysate sample	Dissolved protein content (mg mL^{-1})*
Homogenate (BM)	3.67 ± 0.59^a
Hydrolysis with pepsin (BMP)	10.91 ± 0.37^c
Hydrolysis with trypsin (BMT)	8.09 ± 0.85^b
Hydrolysis with pepsin then trypsin (BMPT)	10.90 ± 0.38^c

*Different superscripts in the same column indicate significant differences (p<0.05)

Analysis of molecular weights of proteins using SDS PAGE: The homogenate of the non-hydrolyzed samples produced three bands of molecular weights between 40-70 kDa (Fig. 1), whereas, the hydrolysates produced many bands all with a molecular weight below ~40 kDa.

Inhibition of ACE activity: Buffalo homogenate did not inhibit the ACE. The average IC_{50} of the sample BMP and BMT were 133 ± 18 and 353 ± 48 μg mL^{-1}, respectively, whereas, the sample BMPT was 333 ± 45 μg mL^{-1} (Fig. 2). However, the purified hydrolysates showed higher ACE inhibitory activities ($IC_{50} = 75.8\pm20$ μg mL^{-1}). The IC_{50} of the purified hydrolysates was significantly decreased ($p<0.05$) compared with the non-purified hydrolysates, indicating greater ACE inhibitory activity as a result of this treatment.

DISCUSSION

The protein content of the buffalo meat was high (22.66%) and the concentration of the protein differed slightly from the value reported by Kandeepan *et al.*[26], who found that frozen buffalo meat contained 20.41% protein. The result suggested that its protein content was higher than other meats such as beef[27] (18.48%) and pork[28] (20.57%). This high nutritional content makes food products from buffalo meat functionally important for purposes such as antihypertension.

Hydrolysis significantly increased the content of soluble proteins ($p<0.05$). This increase was due to the hydrolytic process, which is able to break peptide bonds and convert complex peptides to simpler peptides. As a result, the number of peptides increased and the concentration of dissolved proteins also increased. As a result, the hydrolysates from protein hydrolyzed by digestive enzymes showed activities up to 3 times higher compared with the treatment without the enzyme.

The results of the SDS PAGE showed differences among the samples. Homogenized protein had bands at the top of the lane (approximately 70 kDa) and typically had only 3 bands. One of these bands corresponded to homogenate protein as described by Darewicz *et al.*[12], also called the myosin heavy chain (MHC) fragment. These types of peptides are commonly found in muscle or meat. According to Wells *et al.*[29], MHC is a motor protein composed of thick muscle filaments. Most organisms produce many MHC isoforms with regular temporal and spatial expression patterns.

Protein samples that were hydrolyzed by pepsin and trypsin, such as BMP, BMT and BMPT, had more bands of lower molecular weight: Approximately 40 kDa and below. The decrease in the molecular weight of these bands may be due to the process of enzymatic hydrolysis. The hydrolytic process results in changes in peptide structure producing shorter peptides. The shorter the peptide, the more peptides are contained in the solution and the easier the material passes through the pores of the acrylamide gel, thus appearing on

the lower part of the gel. The protein band with a molecular weight of 45 kDa was identified as an actin fragment and the three bands of lower molecular weight may correspond to the myosin light chain (MLC) and a tropomyosin fragment. These results are consistent with the results of Martinez *et al.*[30] and Lin *et al.*[31]. The electrophoresis results from the BMPT treatment were unclear and barely visible. A similar result was observed by Katayama *et al.*[10]. This may have occurred because the concentration of hydrolysate in the lane was too low to either be stained by Commasie Brilliant Blue (CBB) or to remain in the gel.

The IC_{50} of buffalo meat could not be determined in this experiment. This is because the protein in the buffalo meat retains its complex protein structure and therefore, it has no inhibitory activity as do the bioactive peptides. The ACE-inhibitor is a protein that exists in the food in an inactive form and it therefore requires the enzymatic process to break down the proteins[12]. The hydrolysates had high ACE inhibitory activity ($IC_{50} = 133 \pm 18$ μg mL^{-1}), which is consistent with the results of Saiga *et al.*[32] (IC_{50} = 130 μg mL^{-1}). Moreover, this similarity was paralleled by the similarity in molecular weight of 30 kDa reported by Saiga *et al.*[32], which is comparable to the molecular weight of approximately 40 kDa documented in this study.

The ACE inhibitory activity of BMT (IC_{50} = 353 μg mL^{-1}) and BMPT (IC_{50} = 333 μg mL^{-1}) hydrolysates were lower than that of BMP. The ACE inhibitory activity can be affected by differences in the enzyme used for hydrolysis, which will have different effects on the structure of the protein and the products of the hydrolytic process such as the amino acid sequences or oligopeptides. This hypothesis is supported by Katayama *et al.*[9], who stated that different enzymes could produce different amino acid sequences and therefore, the activity of the resulting peptide could be different. Iwaniak *et al.*[33] further argued that the smaller peptides produced by digestion/hydrolysis resulted in stronger ACE inhibition than the larger peptides because they were more suitable for the ACE active sites and therefore, changed activities. Studies by Akillioglu and Karakaya[34] and Park *et al.*[35] stated that ACE inhibitors were proteins or peptides that were presented in foods but were generally not present in an active state. Therefore, enzymatic or fermentation processes are required to break down the proteins.

The purified hydrolysate had a higher ACE inhibitory activity ($IC_{50} = 75.8 \pm 20$ μg mL^{-1}) than the hydrolysate without purification. Yuliatmo *et al.*[14] also revealed that protein purification using SEP-PAK Plus C18 could increase ACE inhibitory activity. Thus, this research confirms the prior discovery that increased ACE inhibitory activity can be achieved using SEP-PAK Plus C18.

CONCLUSION

Buffalo meat, with its high percentage of crude protein (22.66%), is good source of protein for enzymatic digestion. Hydrolysates from BMP have potential as ACE inhibitors with an IC_{50} of 133 μg mL^{-1}. The inhibitory activity of ACE was enhanced in purified hydrolysates (IC_{50} 75.8 ± 20 μg mL^{-1}). Protein purification using SEP-PAK Plus C18 increased ACE inhibitory activity.

SIGNIFICANCE STATEMENT

This study demonstrates that hydrolysates containing peptides can inhibit the activity of ACE and therefore may be a beneficial antihypertensive drug ingredient. Previous studies have already shown that these peptides can be purified from foods, including porcine skeletal muscle proteins, salmon and chicken legs, but the study of buffalo meat as a source of hydrolysates and as a food that can function as an antihypertensive agent has not previously been performed. Current study also reported that buffalo meat is able to produce bioactive peptides and that SEP-PAK Plus C18 can be used to increase ACE inhibitory activity. Researchers can use this study as an initial reference for developing buffalo meat as a functional food, this study also confirms the utilization of SEP-PAK Plus C18 for the purification of hydrolysates to increase the activity of bioactive peptides.

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REFERENCES

1. Copley, J.B. and R. Rosario, 2005. Hypertension: A review and rationale of treatment. Disease-a-Month, 51: 548-614.
2. Forouzanfar, M.H., P. Liu, G.A. Roth, M. Ng and S. Biryukov *et al.*, 2017. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015. JAMA, 317: 165-182.
3. Hanif, K., H.K. Bid and R. Konwar, 2010. Reinventing the ACE inhibitors: Some old and new implications of ACE inhibition. Hypertens. Res., 33: 11-21.

4. Ondetti, M.A., N.J. Williams, E. Sabo, J. Pluscec, E.R. Weaver and O. Kocy, 1971. Angiotensin-converting enzyme inhibitors from the venom of *Bothrops jararaca*. Isolation, elucidation of structure and synthesis. Biochemistry, 10: 4033-4039.
5. Cheng, F., T. Wan, Y. Liu, K. Lai, L. Lin and R. Sakata, 2008. Attenuating development of cardiovascular hypertrophy with hydrolysate of chicken leg bone protein in spontaneously hypertensive rats. Asian Aust. J. Anim. Sci., 21: 732-737.
6. Katayama, K., H. Fuchu, M. Sugiyama, S. Kawahara, K. Yamauchi, Y. Kawamura and M. Muguruma, 2003. Peptic hydrolysate of porcine crude myosin has many active fractions inhibiting angiotensin I-converting enzyme. Asian Aust. J. Anim. Sci., 16: 1384-1389.
7. Arihara, K., Y. Nakashima, T. Mukai, S. Ishikawa and M. Itoh, 2001. Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. Meat Sci., 57: 319-324.
8. Nakashima, Y., K. Arihara, A. Sasaki, H. Mio, S. Ishikawa and M. Itoh, 2002. Antihypertensive activities of peptides derived from porcine skeletal muscle myosin in spontaneously hypertensive rats. J. Food Sci., 67: 434-437.
9. Katayama, K., M. Tomatsu, S. Kawahara, K. Yamauchi and H. Fuchu *et al*., 2004. Inhibitory profile of nonapeptide derived from porcine troponin C against angiotensin I-converting enzyme. J. Agric. Food Chem., 52: 771-775.
10. Katayama, K., T. Mori, S. Kawahara, K. Miake and Y. Kodama *et al*., 2007. Angiotensin I converting enzyme inhibitory peptide derived from porcine skeletal muscle myosin and its antihypertensive activity in spontaneously hypertensive rats. J. Food Sci., 72: S702-S706.
11. Katayama, K., H.E. Anggraeni, T. Mori, A.M. Ahhmed and S. Kawahara *et al*., 2007. Porcine skeletal muscle troponin is a good source of peptides with angiotensin-I converting enzyme inhibitory activity and antihypertensive effects in spontaneously hypertensive rats. J. Agric. Food Chem., 56: 355-360.
12. Darewicz, M., J. Borawska, G.E. Vegarud, P. Minkiewicz and A. Iwaniak, 2014. Angiotensin I-converting enzyme (ACE) inhibitory activity and ACE inhibitory peptides of salmon (*Salmo salar*) protein hydrolysates obtained by human and porcine gastrointestinal enzymes. Int. J. Mol. Sci., 15: 14077-14101.
13. Nahariah, N., A.M. Legowo, E. Abustam and A. Hintono, 2015. Angiotensin I-Converting enzyme inhibitor activity on egg albumen fermentation. Asian-Aust. J. Anim. Sci., 28: 855-861.
14. Yuliatmo, R., N.A. Z. Fitriyanto, B. Jamhari and Y. Erwanto, 2016. Increasing of angiotensin converting enzyme inhibitory derived from Indonesian native chicken leg protein using *Bacillus cereus* protease enzyme. Int. Food Res. J., 24: 1799-1804.
15. Lee, K.J., S.B. Kim, J.S. Ryu, H.S. Shin and J.W. Lim, 2005. Separation and purification of angiotensin converting enzyme inhibitory peptides derived from goat's milk casein hydrolysates. Asian-Aust. J. Anim. Sci., 18: 741-746.
16. Liepke, C., H.D. Zucht, W.G. Forssmann and L. Standker, 2001. Purification of novel peptide antibiotics from human milk. J. Chromatogr. B: Biomed. Sci. Applic., 752: 369-377.
17. Yust, M.M., J. Pedroche, J. Giron-Calle, M. Alaiz, F. Millan and J. Vioque, 2003. Production of ace inhibitory peptides by digestion of chickpea legumin with alcalase. Food Chem., 81: 363-369.
18. Jamhari, J., L.M. Yusiati, E. Suryanto, M.N. Cahyanto, Y. Erwanto and M. Muguruma, 2013. Purification of angiotensin converting enzyme inhibitory peptide derived from Kacang goat meat protein hydrolysate. J. Indonesian Trop. Anim. Agric., 38: 239-246.
19. Mohammad, N.P., M.K. Chatli, D.K. Sharma and J. Sahoo, 2010. Effect of cooking methods and fat levels on the physico-chemical, processing, sensory and microbial quality of buffalo meat patties. Asian-Aust. J. Anim. Sci., 23: 1380-1385.
20. Baco, S., M. Yusuf, B. Wello and M. Hatta, 2013. Current status of reproductive management in Bali cows in South Sulawesi Province, Indonesia. Open J. For., 3: 2-6.
21. AOAC., 2012. Official Methods of Analysis of AOAC International, Volume 1. 19th Edn., AOAC International, USA., ISBN: 9780935584837.
22. Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.
23. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
24. Cushman, D.W. and H.S. Cheung, 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol., 20: 1637-1648.
25. Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edn., McGraw-Hill Co., New York, USA., ISBN: 9780070610286, Pages: 666.
26. Kandeepan, G., S.K. Mendiratta, V. Shukla and M.R. Vishnuraj, 2013. Processing characteristics of buffalo meat: A review. J. Meat Sci. Technol., 1: 1-11.
27. Muhlisin, P., S.J. Lee, J.K. Lee and S.K. Lee, 2014. Effects of crossbreeding and gender on the carcass traits and meat quality of Korean native black pig and *Duroc crossbred*. Asian-Aust. J. Anim. Sci., 27: 1019-1025.
28. Muhlisin, C.S.S., Y.J. Rhee, Y.H. Song and S.K. Lee, 2016. Effects of direct-fed microbial and pine cone extract on carcass traits and meat quality of Hanwoo (Korean native cattle). Asian-Aust. J. Anim. Sci., 29: 722-730.
29. Wells, L., K.A. Edwards and S.I. Bernstein, 1996. Myosin heavy chain isoforms regulate muscle function but not myofibril assembly. EMBO J., 15: 4454-4459.

30. Martinez, I., R. Slizyte and E. Dauksas, 2007. High resolution two-dimensional electrophoresis as a tool to differentiate wild from farmed cod (*Gadus morhua*) and to assess the protein composition of klipfish. Food Chem., 102: 504-510.
31. Lin, W.F., Y.C. Lyu, Y.J. Wu, C.H. Lu and D.F. Hwang, 2012. Species identification of snapper: A food poisoning incident in Taiwan. Food Control, 25: 511-515.
32. Saiga, A.I., K. Iwai, T. Hayakawa, Y. Takahata, S. Kitamura, T. Nishimura and F. Morimatsu, 2008. Angiotensin I-converting enzyme-inhibitory peptides obtained from chicken collagen hydrolysate. J. Agric. Food Chem., 56: 9586-9591.
33. Iwaniak, A., P. Minkiewicz and M. Darewicz, 2014. Food-originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. Compr. Rev. Food Sci. Food Safety, 13: 114-134.
34. Akillioglu, H.G. and S. Karakaya, 2009. Effects of heat treatment and *in vitro* digestion on the angiotensin converting enzyme inhibitory activity of some legume species. Eur. Food Res. Technol., 229: 915-921.
35. Park, Y.W. and M.S. Nam, 2015. Bioactive peptides in milk and dairy products: A review. Korean J. Food Sci. Anim. Resour., 35: 831-840.