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Study of the antihypertensive peptide from soy protein hydrolysate produced by steam blasting treatment

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ABSTRACT

The purpose of this study was to determine the effect of steam blasting on soybeans and to evaluate the soy protein hydrolysate (SPH) resulting from steam blasting on ace inhibitory activity. Steam blasting works on the principle of heat and pressure. The research conducted begins with a process of pressure treatment and the heating time of soybeans. The pressures used were 2, 3 and 5 bar with heating time (hydrolysis) of 10, 20, 30, 40, 50 and 60 min for each pressure. Furthermore, the soybean resulting from steam blasting was analyzed for the degree of hydrolysis (DH), dissolved protein concentration, protein profile, and also its fractions. Then the ace inhibitory activity was performed on the SPH fraction. The results showed that the higher the pressure and the longer the heating time, the soybean colour from the steam blasting would turn dark brown due to the Maillard reaction. The value of % DH and protein solubility increase with a higher pressure. The % DH values ranged from 76 - 96%. The steam blasting process for soybeans also eliminates anti-nutritional properties and off-flavour compounds in soybeans and cuts protein into peptides/polypeptides. The fractionation results showed that the SPH fraction produced from the soybean steam blasting process had the highest ace inhibitory activity value of 68%, produced by the SPH fraction of 2 bar of 40 min steam blasting treatment. The SPH produced from the steam blasting process can potentially be a source of protein/peptides that can lower blood pressure (hypertension).

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1. Introduction

Hypertension is a chronic medical condition because blood pressure rises above normal blood pressure and is the most devastating killer disease globally. One of the ways to reduce high blood pressure is by consuming food ingredients that contain bioactive protein/peptides. Soybean is a high-quality vegetable protein source containing 35% protein and complete composition of essential amino acids. The SPH containing peptides with the potential as angiotensin-converting enzyme (ACE) inhibitors can lower blood pressure. Several antihypertensive peptides have been produced from soybean, such as soy protein, soy sauce, soy paste, tempeh, natto, soy milk, soy yoghurt, and tofu (Norris et al. 2012; Saha and Hayashi 2001; Kitts and Weiler 2003; Handa et al. 2020). He et al. (2005) also concluded that protein supplementation soybean resulted in decreased systolic and diastolic blood pressure. Soybeans contain isoflavones called saponins and genistein, which play a role in blood pressure. These findings suggest that increasing soy protein intake may play an essential role in preventing and treating hypertension.

Bioactive protein/peptides are released from soybeans through various protein processing technologies such as hydrolysis and thermal treatments or biological processes, including gastrointestinal digestion and microbial fermentation (Ashaolu, 2020). Hydrolysis is a reaction, either by chemical, biological or physical means, of splitting (lysis) water (hydro) or a larger compound into smaller compounds/components, and molecules of water are added in this process (Ashaolu, 2020). In this experiment, soybean hydrolysis will be carried out using steam blasting. The steam blasting process principle is processing soybeans through two stages: 1) steaming stage and 2) blasting stage. On the steaming stage, high-pressure hot steam enters the closed tube meetings containing soybeans causes an increase in pressure and temperature in the tube. After reaching the desired number, pressure and temperature are kept stable up to a predetermined time. At the stage next, the blasting process is carried out. At this stage, the hot steam contained in the tube will be released suddenly so that the decompression process occurs explosively. This explosive decompression process will produce a thermomechanical force that can destroy the biomass structure (Datar et al., 2007). Furthermore, this experiment aimed to investigate the effect of heating time and pressure treatment of SPH produced by steam blasting treatment on ACE inhibitory activity.

As a protein source, soybeans are rich in bioactive substances, such as essential amino acids, isoflavones, and flavonoids. However, soybean also has antinutrient factors such as antitrypsin, lectins, hemaglutamine, tannin, and phytic acid. These anti-nutritional factors can be reduced by heat treatment during food processing (de Carvalho et al., 2013). By applying the steam blaster's appropriate pressure and heating time, various soybean derivatives' nutritional value and organoleptic character can be maintained. So, the benefits of soybeans with different bioactive contents can be followed by good physical characteristics.

2. Materials and methods

2.1. Chemicals and reagents

The materials used in this study were soybean, Hippuril-L-histidyl-L-leucine hydrate (HHL) (Sigma), Angiotensin-converting enzyme (Sigma), Sephadex G-25 was obtained from GE Healthcare (Piscataway, NJ, USA). Other chemical reagents and solvents used in this study were of analytical grade and commercially available.

2.2. Production of SPH by steam blasting treatment

The pressures chosen for these experiments, 2 bar, 3 bar, and 5 bar, were selected to hydrolyze the soybean. The hydrolysis time at three different pressure was carried out from 10, 20, 30, 40, 50, and 60 min. So, there were 18 treatments with various pressure and hydrolysis time. For each treatment, 250 g of wet-peeled soybean was loaded into the steam blaster chamber (Fig. 1). The SPH was freeze-dried and then ground using a grinder. As much as 0.02 g of SPH was dissolved in 1 ml of buffer solution of Tris HCl pH 8.5. The suspension was shaken for 1 hour using an incubator shaker at 200 rpm, then centrifuged at 8000 rpm for 30 min. After that, the supernatant was separated and centrifuged at a speed of 13000 rpm for 5 min. The supernatant was analyzed for protein concentration using Bradford's reagent and analyzed the protein profiles using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Measurement of the degree of hydrolysis was also carried out. The bioactive peptide was fractionated for the selected SPH using column chromatography, then ACE inhibition activity was determined from the SPH fraction.

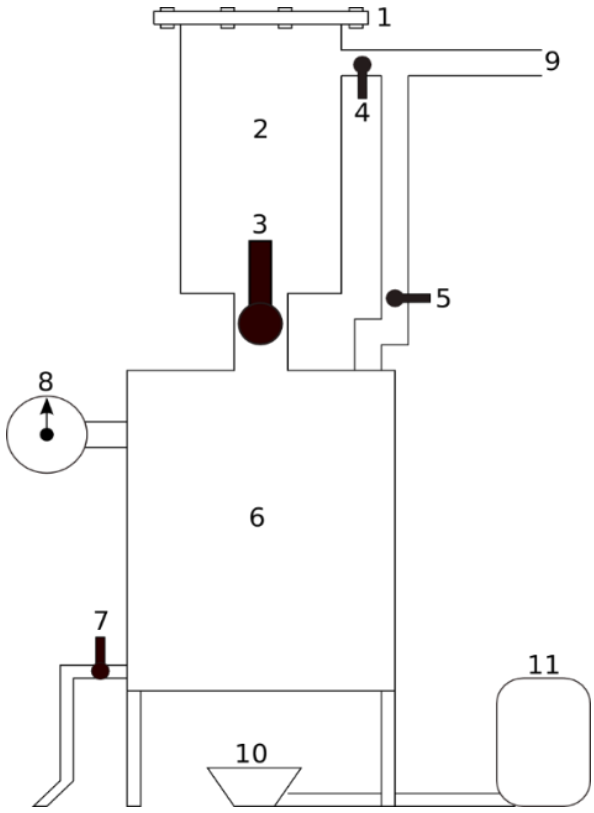

Fig. 1. Steam blaster configuration: 1. chamber cover, 2. chamber, 3. steam valve from the boiler to the chamber, 4. steam blasting valve, 5. steam exhaust valve, 6. Boiler, 7. water drain valve, 8. pressure gauge, 9. steam outlet, 10. stove blower, 11. LPG gas (Laily et al. 2011)

2.3. Determination of degree of hydrolysis

The degree of hydrolysis was determined by measuring dissolved nitrogen from a sample mixed with 10% trichloroacetic acid (TCA) (Kim et al., 1990). A total of 2 g of hydrolysate were dissolved in 100 ml of tris HCl buffer (pH 8.5), added with TCA 20% (1:1), then centrifuged at 10,000 g for 15 min. Dissolved nitrogen in the supernatant and total nitrogen in the sample were measured using a UV-Vis spectrophotometer at a wavelength of 280 nm. The equation expresses the percentage of DH:

$$\%DH = \frac{N_{total}\ in\ 10\%TCA}{N_{total}\ in\ samples}\ x\ 100\%$$

2.4. Measurement of protein concentration

Measurement protein concentration from SPH and SPH fraction was performed using the Bradford method. The standard used is the bovine serum albumin (BSA) (Sigma). Every 10 µl of BSA concentration and the sample was placed on a microwell plate reader, and 260 µl of Bradford (Biorad) reagent was added and incubated for 5 min, at 37 °C. The absorbance was measured at wavelengths of 450 and 595 nm (Ernst and Zor, 2010). The BSA standard concentrations were 50, 100, 200, 300, 400, and 500 µg/ml. The standard curve is a linear regression line equation (y = a + bx, with R-value close to one) between the absorbance ratio 595/450 as ordinate and BSA protein concentration as axis. The protein concentration was then calculated by entering the absorbance ratio 595/450 on the BSA standard curve linear regression equation.

2.5. Measurement of molecular weight of proteins/peptides

SDS-Page was performed to determine the molecular weight. This method used two gel parts, namely top gel (stacking gel) and bottom gel (separating gel), consisting of 4% stacking gel concentration and 12.5% separating gel. The composition of separating gel and stacking gel could be seen in Table 1.

Table 1. The composition of separating gel and stacking gel for performing SDS-Page

Materials	Separating gel, 12.5% (µl)	Stacking gel, 4% (µl)
Acrylamide 30%	2080	260
Tris buffer 1.5 M pH 8.8	1250	-
Tris buffer 0.5 M pH 6.8	-	500
Aquades	1580	1195
SDS 10%	50	20
TEMED	15	10
APS 10%	25	15

The amount of protein to be injected was 60 µg. The sample was diluted if the protein concentration is greater than 60 µg and concentrated if the protein concentration is less than 60 µg. The sample was diluted using Tris-HCl buffer 1 M pH 8.5. Ten µl diluted sample was added with 15 µl sample reducing buffer (SBR) 5x. The sample was concentrated by adding cold acetone in a ratio of 1:4 (sample: acetone) and concentrated for 1 hour in the freezer. Then centrifuged at speed 14,000 rpm, 4 °C for 10 min, and decanted to dry at room temperature until pellets were obtained. The pellets were added with 25 µl of SBR 2x, vortexed for 40 s, heated at 95 °C for 5 min in a water bath, vortexed again for 40 s, then centrifuged at a speed of 10,000 g for 5 min.

The samples were put in the gel as well as much as 15 µl. The electrophoresis device was connected to the power supply at a voltage of 140 volts and a current of 100 amperes. After reaching the separating gel, the voltage was increased to 160 volts. The LMW (low molecular weight) marker (Biorad) was used with a 10-250 kDa molecular weight range. The gel staining process used 0.1% coomassie brilliant blue (CBB) R-250 (Sigma) in 40% methanol and 10% acetic acid. While de-staining gel was used 40% methanol and 10% acetic acid. If protein bands were produced from staining using CBB was unclear, then silver staining was performed.

2.6. Fractionation of ACE inhibitory peptide from SPH

The method used to separate molecules based on their molecular weight was the gel filtration method. The gel filtration method was carried out using ÄKTAprime with Sephadex G-25 as a matrix for gel filtration, and Phosphate buffer 0.05 N pH 7 was used as eluent with an elution rate of 0.2 ml/minute. The protein concentration used in gel filtration was 2 mg/ml, with a sample volume of 5 ml, and the protein fraction collected for each tube was 3 ml. The sample elution process was monitored at 280 nm of wavelength.

2.7. Assay for ACE inhibitory activity

The peptide samples' ACE inhibitory activity was measured by a UV spectrophotometer based on the formation rate of hippuric acid and L-histidyl-L-leucine (Chusman and Cheung, 1971) with some modification. For each measurement, 10 µl of the peptide sample and 10 µl of 50 mM HHL were incubated with 10 µl of 0.8 mU ACE at 37 °C for 45 min. The reaction was stopped by adding 100 µl of 1 N HCl, then 600 µl of ethyl acetate were added and homogenized using vortex for 30 s, and then centrifuged at 3000 xg for 10 min. One hundred µl of supernatant (top layer) was taken and dried at 140 °C for 10 min. The residue obtained was dissolved in 600 µl of 1 M NaCl. The absorbance was measured at a wavelength of 228 nm. The procedure for determining ACE inhibitory activity could be seen in Table 2.

Table 2. Procedure for assay of ACE inhibitory activity (Chusman and Cheung, 1971)

Materials	Volume (µl)		
	Blank	Control	Sample
Distilled water	10	10	10
NaCl 1.0 M	10	10	10
Borate buffer 0.1 M pH 8.3	50	50	50
Hip-His-Leu 50 mM	10	10	10
HCl 1.0 N	100	-	-
Borate buffer 0.1 M pH 8.3	10	10	-
Hydrolysate sample (ACE inhibitor)	-	-	10
ACE (0.8 mU)	10	10	10
Incubated at 37°C for 45 min			
HCl 1.0 N	-	100	100
Ethyl acetate	600	600	600
Homogenized by vortex for 30 s			
Centrifuged at 3000xg for 10 min			
Filtrat (upper layer)	100	100	100
Dried at 140 °C for 10 min			
NaCl 1.0 M	600	600	600
Measured at 228 nm			

The ACE inhibitor activity was expressed as percent (%) inhibition that calculated by the formula:

$$\% \text{ inhibition} = \frac{(Ac - As)}{(Ac - Ab)} \times 100\%$$

where,

Ac = absorbance of the control
As = absorbance of the sample
Ab = absorbance blank
ACE Inhibitor activity of peptide = 100 - % inhibition

3. Results and Discussion

3.1. Physical characteristic of SPH

The SPH was obtained by applied pressure and heating time using a steam blaster. The pressures used were 2, 3, and 5 bars. Simultaneously, the heating times were 10, 20, 30, 40, 50, and 60 min. The two parameters are combined to produce 18 combinations of pressure from the steam blaster and heating duration. The pressure during the steam blasting process was sometimes inconsistent. The wasted steam caused it at the opening of the valve chamber, and it could also be due to the fire, which caused changes in temperature in the boiler. This steam blaster could not show the temperature during the heating process. However, this temperature could be estimated using the pressure conversion table (bar) to saturated steam (°C) (The Engineering ToolBox, 2014).

Table 3. Conversion table of pressure (bar) to saturated steam temperature (°C) (The Engineering ToolBox, 2014)

Pressure (bar)	Saturated steam temperature (°C)
2	120.23
3	133.54
5	151.85

The steam blaster's pressure and the heating time's length resulted in changes in the physical soy hydrolysate flour's colour and texture. The colour changed occurs due to the Maillard reaction. This reaction occurred due to the interaction between carbohydrates, significantly reducing sugars with primary amine groups from amino acids in soybeans (Winarno, 2008). The reaction resulted in brown soybeans. The Maillard reaction is a non-enzymatic browning chemical reaction between reducing sugars and proteins or amino acids. Depending on the type of material and the course of the reaction, the colour change that occurred can be from weak yellow to dark brown. Many factors influence the Maillard reaction, such as temperature, water activities; pH; moisture content, and chemical composition of a material (Ogutu et al., 2017). The longer the hydrolysis time, the value of the Maillard soy protein hydrolysate product also increases. The Maillard reaction occurs because the longer the hydrolysis time, the more peptide bonds are hydrolyzed. The more primary amine groups are produced, the more intense the Maillard reaction (Subagio et al., 2002).

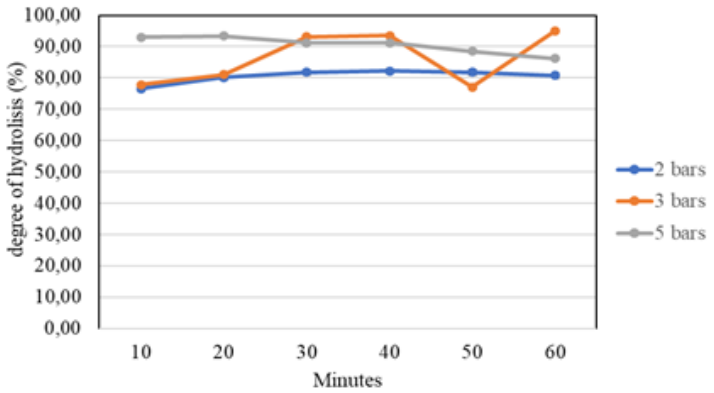

Fig. 2. Degree of hydrolysis (% DH) at a pressure of 2, 3, and 5 bar with hydrolysis time of 10, 20, 30, 40, 50, and 60 min

In addition to discoloration, the soybean from steam blasting also changes in texture. The high pressure of the steam blaster had made the soybean's texture was soft. The higher the pressure, the higher the steam temperature that was formed. High temperature destroys soy cotyledons (Marugkar, 2015). Therefore, the texture of soybeans becomes soft due to exposure to more saturated steam at higher temperatures. The taste of soybean tends to be more bitter.

3.2. Degree of hydrolysis (DH) SPH

DH was defined as the percentage of peptide bonds that are split (Adler-Nissen, 1979). Therefore, it was necessary to have a general method in determining DH from protein hydrolysate, especially for quality control. In this study, the degree of hydrolysis

was determined by the percentage of protein dissolved in trichloroacetic acid (TCA). The hydrolysate was mixed with TCA 20% (1: 1) to obtain soluble and insoluble fractions in TCA.

The data obtained from the measurement of %DH was shown in Fig. 2. At 2 bar pressure, the length of the pressure was applied resulted in a higher degree of hydrolysis, but there was a decrease in the 40 and 60 min. At 3 bar pressure, the degree of hydrolysis increased but decreased in the pressure treatment for 50 min. While at 5 bar pressure, it resulted in a high degree of hydrolysis, but the value decreases from 10 to 60 min. Increasing and decreasing the degree of hydrolysis was due to instability during the pressure application process. When the valve was opened, there was a possibility that the pressure indicated by the barometer might be decreased, or the amount of fire given can increase the pressure. Susanto (1997) stated that different protein and peptide formation would occur with increasing hydrolysis time so that more free amino acids are produced and fewer peptides are obtained.

Table 4. The flour color of the soy protein hydrolysate

Pressure (bar)	Heating time (min)	Flour Color
2	10	Light brown
	20	Light brown
	30	Light brown
	40	Dark brown
	50	Dark brown
	60	Dark brown
3	10	Light brown
	20	Brown
	30	Brown
	40	Dark brown
	50	Dark brown
	60	Very dark brown
5	10	Very dark brown
	20	Very dark brown
	30	Very dark brown
	40	Very dark brown
	50	Very dark brown
	60	Very dark brown

3.3. Protein concentration of SPH

Based on the dissolved protein concentration measurement, 5 bar pressure resulted in a higher protein concentration value than 2 bar and 3 bar (Fig. 3). The concentration of dissolved protein increases due to the denaturation of the protein. Heat breaks hydrogen bonds in protein molecules and reduces protein solubility due to damage to the sary, tertiary, and quaternary protein molecules (Winarno, 2008).

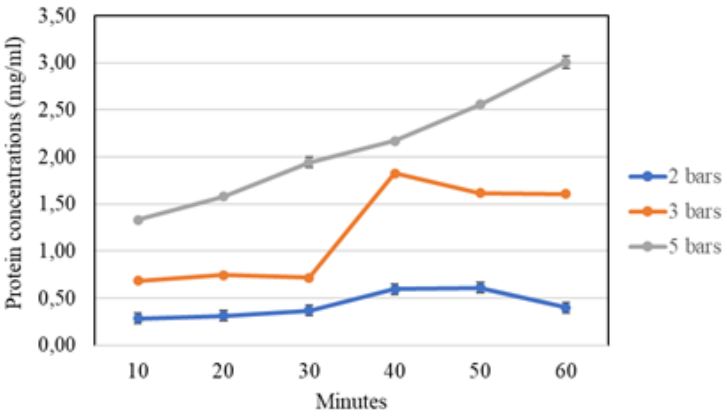

Fig. 3. The protein concentration of soy protein hydrolysate with pressure treatment at 2, 3, and 5 bar and hydrolysis time of 10, 20, 30, 40, 50, and 60 min

3.1. Protein profile of SPH using SDS-Page

SDS-PAGE analysis aimed to characterize soy protein resulting from steam blasting. The protein bands resulted from electrophoresis showed the characteristics of the polypeptides that made up the soy protein. The results of the SDS-page could be seen in figure 1-3. The different SDS page results were caused by two different staining, namely CBB staining and silver staining. CBB staining produces blue colour (Fig. 1 and Fig. 3), and silver staining produces a greyish-brown colour (Fig. 2). CBB is the most popular protein stain. It is an anionic dye, which non-specifically binds to proteins. CBB is an anionic dye, which non-specifically binds to proteins. The CBB structure contains non-polar compounds and is usually used in methanol solutions acidified with acetic acid. The protein in the gel is fixed by acetic acid and coloured simultaneously. Excess dye added to the gel can be removed by de-staining with the same solution without the dye. Proteins are detected as blue bands on a transparent background. A silver stain is used when a more sensitive detection method is required. CBB stain can usually detect 50 ng protein bands; silver staining increases the sensitivity, usually 10-100-fold more. Proteins were bound to gel with aqueous methanol solution, then incubated with a silver, nitric acid solution. The silver ion is reduced to its metallic form by formaldehyde at an alkaline pH. Acid solutions, such as acetic acid, stop their development (Ninfa et al., 2004).

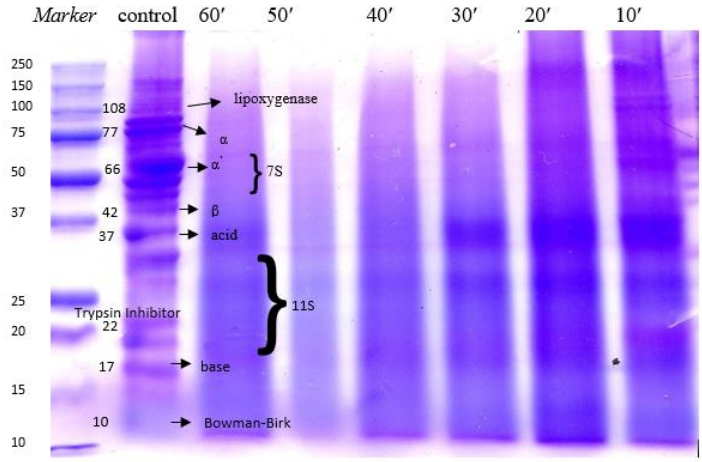

Fig. 4. SDS-page of protein from SFH at pressure 2 bar and at the hydrolysis time of 10, 20, 30, 40, 50, and 60 min

The effect of pressure and heating time on the protein profile of steam blasting showed protein bands with relatively different patterns. High pressure decreased the protein band, suggested that some protein from soy was damaged by heat treatment. Heat treatment of soybeans causes hydrophobic groups' exposure in soybeans, which are initially hidden on the protein molecule's inner side and form large molecular weight aggregates (Raikos, 2010). According to Nakornpanom et al. (2010), the compact soy protein structure requires temperatures above 80 °C to denaturation.

Meanwhile, Raikos (2010) stated that the dominant protein in soybeans is a globular protein type, which experiences aggregation at temperatures of more than 65 °C. Thus, was shown at a pressure of 2 bar (temperature at 120 °C); soy protein has begun to denaturation. Denaturation causes the opening of the hydrophobic protein folds to the outside so that protein solubility decreases. These hydrophobic bonds interact to form sizeable molecular weight aggregates (Raikos, 2010) and the 5-bar pressure treatment where the low molecular weight bands are no longer visible. These bands have undergone aggregation and denaturation.

Soybeans have various anti-nutritional substances and off-flavour compounds that can reduce the digestibility and quality of soybeans. Heat and pressure treated with a steam blaster could

eliminate anti-nutritional substances. One of the dominant anti-nutritional substances in soy is a trypsin inhibitor, divided into two types: Kunitz trypsin inhibitors and Bowman-Birk inhibitors. According to Murphy (2008), the Kunitz trypsin inhibitor's molecular weight is 21.5 kDa, while the Bowman-Birk inhibitor has a molecular weight of 9 kDa. At the treatment of 2 bar 30 min, 3 bar 50 min, and 5 bar pressure from 10 to 60 min, the Kunitz trypsin inhibitor was not identified. While the Bowman-Birk inhibitor was still identified at the pressure treatment of 2 bar to a treatment duration of 20 min, but it was not identified at the 3 bar and 5 bar pressure treatment.

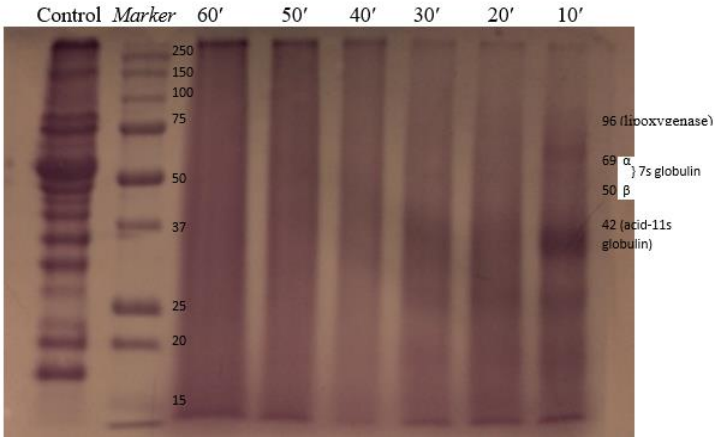

Fig. 5. SDS-page of protein from SFH at pressure 3 bar and at the hydrolysis time of 10, 20, 30, 40, 50, and 60 min

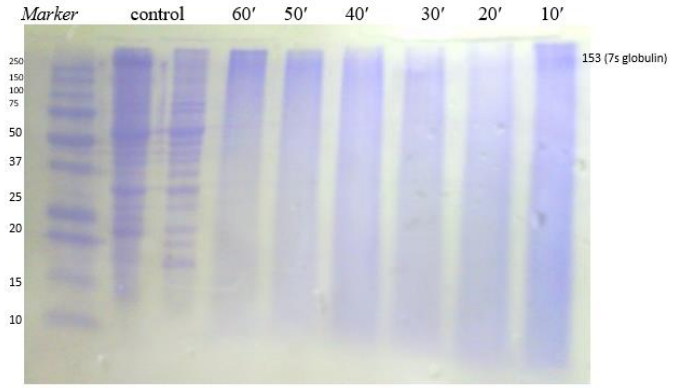

Fig. 6. SDS-page of protein from SFH at pressure 5 bar and at the hydrolysis time of 10, 20, 30, 40, 50, and 60 min

Soybean seeds contain large lipoxygenase. Lipoxygenase is an enzyme that acts as a flavour distortion or off-flavour in soybeans. Murphy (2008) stated that soy lipoxygenase has a molecular weight of around 102 kDa. Based on this study, lipoxygenase was still found at a pressure treatment of 2 bar with a time of 10 min at 108 kDa and at a pressure of 3 bar with a time of 10 min at 96 kDa, and it was not identified in the treatment of higher pressure and more extended time.

Beta-conglycinin (7S globulin) and Glycinin (11s globulin) are the two main soybean protein components. The two fractions are called reserve proteins because they have no biological activity except reserve amino acids for seed germination (Murphy, 2008). Murphy (2008) states that the three subunits' molecular weights are 72, 68, and 52 kDa, respectively. The SDS-PAGE results in this study showed that the α' subunit was identified in the 2-bar treatment and the pressure duration of 10 min was 77 kDa, while the 3 and 5 bar pressure treatments were no longer identified. The α subunit was identified in the 2-bar pressure treatment with a pressure duration of 10 to 30 min at 66 kDa. The 3-bar pressure treatment was still identified at 69 kDa with a pressure duration of 10 to 20 min and was no longer identified at 5 bar pressure. The β subunit was still identified at a pressure treatment of 2 bar 10 to 30 min with a molecular weight of 42 kDa. At a 3-bar pressure, it was only identified in the 10-minute treatment of 50 kDa. β-conglycinin was denatured slowly with increasing temperature from about 70 °C (Maruyama et al. 1998).

Glycine (11s globulin) is a hexamer with a molecular weight of about 360 kDa. It consists of 12 polypeptides, namely six acidic polypeptides with a molecular weight of 34-44 kDa and six essential polypeptides with a molecular weight of about 20 kDa (Murphy and Resurrección, 1984). There was still an acid peptide subunit in the 2-bar pressure treatment, which was 37 kDa. At 3 bar pressure, it was also still identified at 42 kDa, only up to 30 min of treatment. While at 5 bars, it was no longer identified. The essential peptide subunit was showed at a pressure result of 2 bar, namely with an extensive range of 17-20 kDa. Glycine begins to denature at a temperature range of 90 °C (Damodaran, 2017). Barać et al., (2004) explained that the composition and structure between β conglycinin (7s globulin) and glycine (11s globulin) have differences in nutritional quality and functional characteristics. Globulin 7s protein has more heat stability than 11s globulin.

3.2. Fractionation of ACE inhibitory peptide from SPH

Each pressure treatment result was taken once each to fractionate ACE inhibitory peptide using gel filtration chromatography and Sephadex G25 as a matrix. Sephadex G-25 has a fractionation range for globular proteins of molecular weights (Mr) 1000 to 5000, with an exclusion limit of approximately Mr 5000. Therefore, protein and peptides larger than Mr 5000 are easily separated from molecules with molecular weights of less than 1000. The result of a pressure of 2 bar with a duration of 40 min represents a low-pressure treatment with longer heating time, a pressure of 3 bar with a duration of 20 min represents a medium pressure and time, while a pressure of 5 bar with a duration of 10 min represents a high-pressure treatment with a short time.

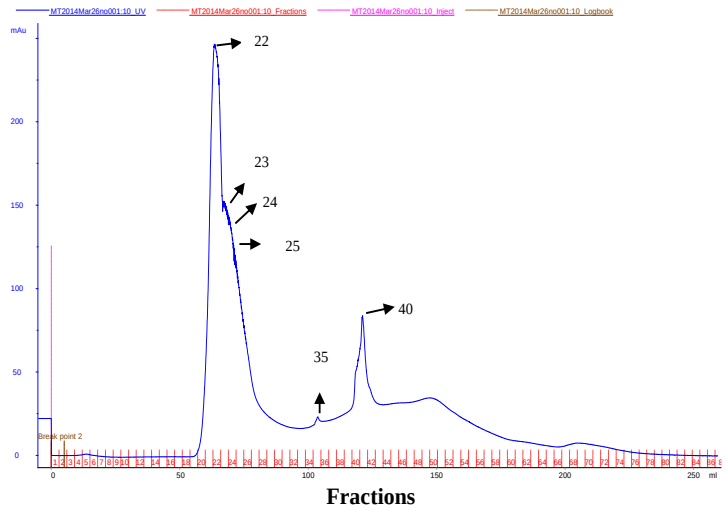

Fig. 7. Chromatogram of soybean with steam blasting treatment 2 bar for 40 min

The SPH fractionation with 2 bars at 40 min treatment resulted in six peaks, where the first peak had a more significant shape than the other peaks. Two large visible peaks were produced in fraction 22 and fraction 41, and one peak was quite large at fraction 35. Meanwhile, there were three small peaks at fractions 23, 24, and 25. From the refining result, the pressure of 3 bar in 10 min produced seven peaks. One peak is in fraction 22, and four small peaks are in fraction 23 to fraction 26. While one peak between fractions 38 and 39, and one peak between fractions 52 to 55. While fractionation resulted from 5 bar' pressure within 10 min, it

produced four peaks: three prominent peaks in fractions 22, 34, and 40, and one small peak in fraction 23. During the fractionation of the sample protein, there might be a possibility of decreasing protein value. Loss of protein value during purification can occur due to autolysis (Scopes, 1987), and this occurs due to dilution effects. From each purification result, the first peak produced has a more significant shape than the other peaks. The larger molecules exit first, while the smaller ones are trapped in the three-dimensional dextran (Sephadex) network. Then each fraction that produced a peak was calculated for its dissolved protein content. The fraction that had the peak was then analyzed to inhibit the ACE enzyme level by analyzing the ACE inhibitor in vitro.

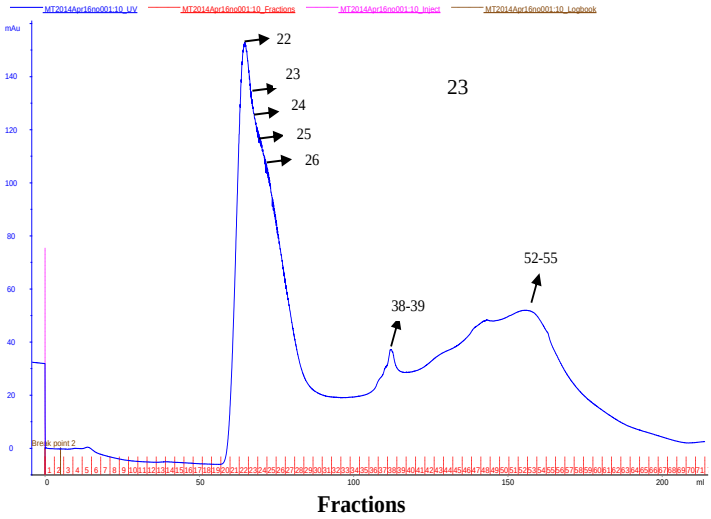

Fig. 8. Chromatogram of soybean with steam blasting treatment 3 bar for 20 min

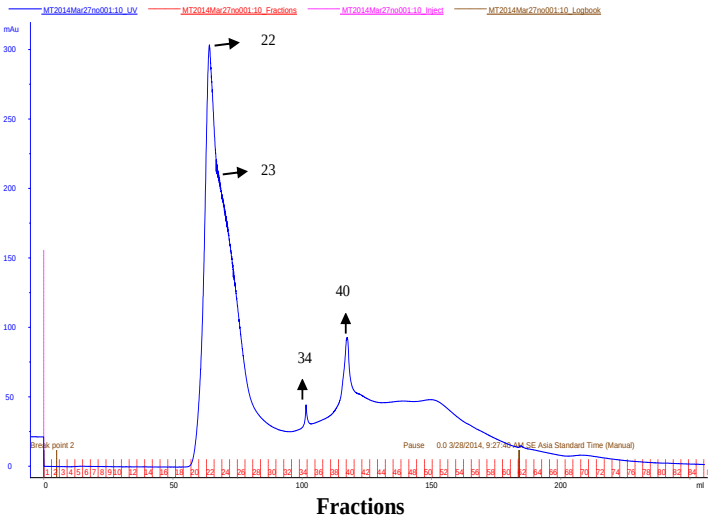

Fig. 9. Chromatogram of soybean with steam blasting treatment 5 bar for 10 min

3.3. ACE inhibition activity of SPH fractions

The inhibition of ACE enzyme activity was measured using a substrate of N-Hippuril-L-histidyl-L-leucine hydrate (HHL), which would be hydrolyzed to N-Hippuric acid and L-histidyl-L-leucine (Kasahara and Ashihara, 1981) (Fig. 10). The buffer used has a pH of 8.3 because it is the optimal condition for the HHL substrate for the ACE inhibition test. Hippuric acid (HA) from hydrolysis was extracted with ethyl acetate and calculated by measuring the absorbance spectrophotometry at 228 nm.

Analysis of the percent inhibition of ACE from SPH fractions with 2 bar 40 min treatment showed that the high ACE inhibitory activity resulted from fraction 25.68% with a protein concentration of 0.008 mg/ml. Simultaneously, the lowest was produced by fraction 40 with a protein concentration of 0.21 mg/ml (Fig. 11). For fractions 22 to 25, there was a high probability of potential as an ACE inhibitor; it needs to be tested further if fractions 22 to 25 were combined and purified again.

SPH's fractionation with 3 bar 20 min treatment showed a varying percentage of inhibition. The high percentage was shown in fraction 23 of 64% (Fig. 12) with a protein concentration of 0.1397 mg/ml. For factions 22 to 26, there was a high probability of another percent inhibition when collected and refined again.

SPH's fractionation with 5 bar 10 min treatment showed that ACE inhibitor activity was not that high, namely 11% in fraction 22 and 8% in fraction 23 with protein concentrations of 0.19 mg/ml and 0.09 mg/respectively (Fig. 13).

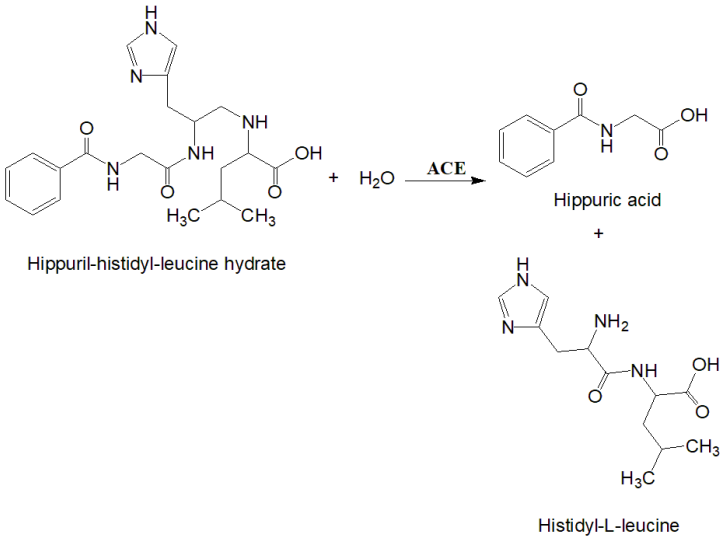

Fig. 10. Mechanism of formation hippuric acid formation in the presence of ACE (Kasahara and Ashihara, 1981)

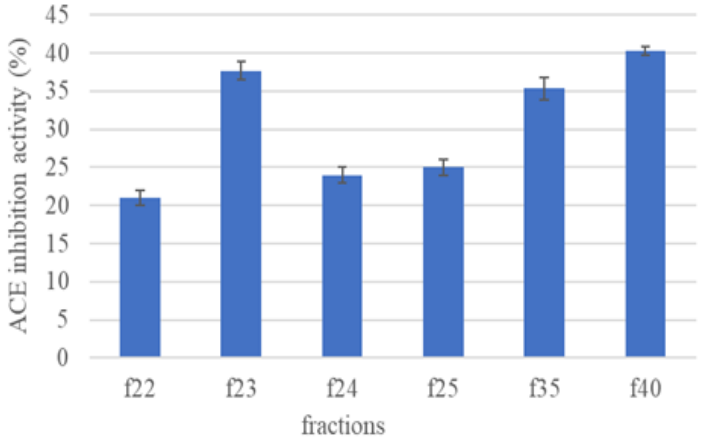

Fig. 11. ACE inhibition activity SPH fractions with 2 bar for 40 min treatment

The percentage of ACE inhibitory activity by captopril was shown by Hayes et al. (2007), which amounted to 100% with a captopril concentration of 0.005 mg/ml. This percentage value is higher than the results obtained in this study. The difference in the percentage of ACE inhibitory activity was probably due to differences in captopril's purity. Hayes et al., (2007) use the captopril from Sigma Co., which has a higher purity compared to commercial captopril, which contains various components of the medicinal mixture of raw material. A different percentage was also presented by Putra (2013) with soybean samples hydrolyzed using

the enzyme bromelain, which is 89.17% with a protein concentration of 10 mg/ml.

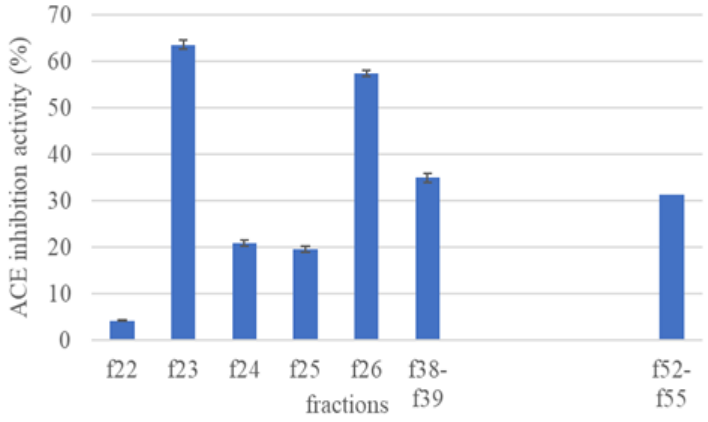

Fig. 12. ACE inhibition activity SPH fractions with 3 bar for 20 min treatment

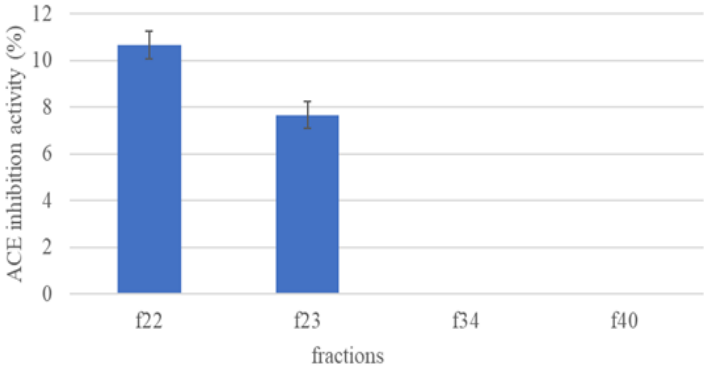

Fig. 13. ACE inhibition activity SPH fractions with 5 bar for 10 min treatment

4. Conclusion

Based on this study, the longer the pressure treatment and the variation in time resulted in the brownie soybean color, the color change occurred due to the Maillard reaction. Applied pressure by a steam blaster could eliminate the anti-nutritional properties and off-flavor compounds in soybeans; this could be seen when calculating the molecular weight using SDS-PAGE gel electrophoresis. Fractionation of protein samples containing bioactive peptides produced various peaks. The application of high pressure with variations in the hydrolysis time produced antihypertensive peptides. The highest antihypertensive activity was 68% produced in fraction 25 with the pressure treatment of 2 bar and hydrolysis for 40 min.

Conflict of interest

No potential conflict of interest was reported by the author.

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