Research Article

DOI: 10.22146/ifnp.53535 https://journal.ugm.ac.id/ifnp

ISSN 2597-9388

Indonesian Food and Nutrition Progress

Effect of Temperature and Drying Time on Angiotensin Converting Enzyme (Ace) Inhibitor and Antioxidant Activity of Soybean *(Glycine max* L.) - Jackbean (*Canavalia ensiformis* L.) Mixed Grains Tempeh Flour

Diah Ayu Puspa Sari [1)*], Andriati Ningrum [1)] , Sri Anggrahini [1)]

[1)]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
[*)] Correspondence Email: diah.ayu.p@ugm.ac.id

ABSTRACT: The increase of hypertension sufferers encourages the development of functional foods. The presence of tempeh mixed grains of soybean and jackbean contains bioactive peptides, as antihypertensive and antioxidant agents, encouraging the development of functional food such as tempeh flour of mixed grains. The research was aimed to investigate the effect of temperature and drying time on functional properties, amino acids profile, and isoflavone compounds. The different drying temperatures (50 °C and 70 oC) and times (6; 8; and 10 h) were applied on soybean and jackbean tempeh (1:1 w/w) for making the mixed grains tempeh flour. The angiotensin converting enzyme (ACE) inhibitor activity, antioxidant activity, total phenolic, amino acid profile, and isoflavone content of the flour were analyzed. The result showed that the best tempeh flour of mixed grains was obtained by drying at 70 °C for 8 h with ACE inhibitor activity of 86.04%, antioxidant activity of 55.34% and total phenolic of 3.98 mg/g DB. This flour contained amino acids related to ACE-Inhibitor peptide precursors (glutamic acid and leucine), antioxidant peptide precursors (phenylalanine and histidine) as well as isoflavone compounds (daidzein and genistein). This finding reveals that drying temperature and time affected the functional properties, amino acids profile and isoflavone compounds.

Keywords: Angiotensin Converting Enzyme Inhibitor; Antioxidant; Isoflavone, Jackbean; mixed grains; Tempeh flour.

INTRODUCTION

According to the World Health Organization (WHO) and the International Society of Hypertension (ISH) in 2000, there were 600 million people with hypertension in the world and 3 million of them died each year. The WHO states that the number of hypertension sufferers is expected to increase by 29% with the increase of the population in 2025. Hypertension can be prevented and overcome by implementing a healthy lifestyle and fulfilling of balanced nutrition. Protein is important because its functions in growth, body development, and preventing disease (Philips et al., 2015). Foods with high protein content have the potency to produce antihypertensive and antioxidant bioactive peptides. Bioactive peptides can be produced from the mechanism of protein hydrolysis by the protease enzyme in the human digestive organs and food processing (Wijatnika, 2017).

Recently, the mixed grains tempeh from soybean (Glycine max L.) and jackbean (Canavalia ensiformis L.) with ratio 1:1 w/w that was fermented for 48 h with banana leaf package was introduced as a functional food. It contained high hydrophilic amino acids (Hesti, 2019) and bioactive peptides as ACE-inhibitors (antihypertension) and antioxidants (Harvian, 2018). This mixed grains tempeh had the same acceptance level with soybean tempeh ($p < 0.05$) and had higher soluble protein content (24.10% DB) than soybean tempeh (22.81% DB) (Hesti, 2019). However, mixed grains tempeh has a short shelf life, limiting application to the foods, and has low price. Therefore, mixed grains tempeh flour could be an alternative product which overcome the aforementioned challenges. However, tempeh flour mixed grains has not been widely developed in Indonesia and soy and jackbean flour with ratio 1:1 (b/b) has never existed.

In the making of tempeh flour involves the drying process. The parameters of the drying process, i.e., temperature and time, affect the food drying rate (Muchtadi, 2008 and Winarno, 1993). The drying process could change bioactive peptides and antioxidant compounds in food (Fadly, 2014, and Chang et al., 2007). Heating from the drying process causes the opening of protein folds from the original conformation so that enzymes easily bind and catalyze peptide bonds in proteins (Wijatnika, 2017). The heating also causes breaking of hydrogen bonds, hydrophobic interactions, salt bonds, and opening of folds (Winarno, 2004 and Li et al., 2018) that result in modification of the secondary, tertiary, and quaternary structure of protein molecules and simpler protein monomers such as peptides (Nemethy et al., 1966). However, the heating can also cause denaturation followed by aggregation which can affect functional properties of protein and protein solubility (Winarno, 2004). Proteins denatured at their isoelectric point can still dissolve at pH other than their isoelectric point (Poedjiadi, 1994). Heating could alter the content of soluble proteins, the degree of protein hydrolysis, and the functional properties of bioactive peptides such as ACE inhibitor and antioxidant activity (Fadly, 2014). The activity of ACE inhibitor and antioxidant peptides depends on the source of protein, the composition, and sequence of the amino acids that arrange the bioactive peptides. In addition, heating causes the release of phenolic compounds that bind to other components and increases the number of phenolic compounds in the sample. On the contrary, heating can also cause damage to phenolic compounds (Istiani, 2010).

This study was aimed to examine functional characteristics (ACE-inhibitor activity, antioxidant activity, and total phenolic) of mixed grains tempeh flour (soybean and jackbean with ratio 1:1 w/w) that were fermented for 48 h in banana leaf package, then dried at 50 °C and 70 °C for 6; 8; and 10 h. This study also examined the amino acid profile and the content of isoflavone compounds (daidzein and genistein) content of the best mixed grain tempeh flour, which has the highest functional characteristics.

MATERIALS AND METHODS

Materials

Jackbeans (*Canavalia ensiformis* L) were obtained from Purworejo, Central Java. The specification of the jackbean was 9 - 12 months (old jackbean) with white color. The local Grobogan local soybean was obtained from Kulonprogo farmers. This study used commercial enzymes that included ACE (A_{6778}) (Sigma-Aldrich), pepsin (107185) (Sigma-Aldrich), and the Hippuril-His-Leu substrate (Sigma-Aldrich). The chemicals that were used are 20% Trichloroacetic Acid, 1% Bovine Serum Albumin, 5% Sodium Bicarbonate, 2 N Folin-Ciocalteu, and Gallic Acid (Sigma-Aldrich). Another ingredient was *Rhizopus oligosporus* tempeh yeast (Raprima). The tempeh package was Banana Leaf bought from a traditional market in Yogyakarta.

Methods

Preparation of Mixed Grains Tempeh

The making of tempeh of mixed grains was adopted from Hesti (2019) and Kusumawardhani (2015). Jackbean was soaked in CaCl2 solution for 72 h followed by dehusking. Subsequently, the jackbean was washed and cut into 8 parts followed by boiling for 30 minutes. It was then cooled at room temperature. The soybean was washed and soaked for 2 h. Subsequently the soybean was boiled for 30 minutes with the boiled water 100 °Cand soaked again for 24 h. The next step was peeling the husk followed by boiling for 30 minutes. It was then cooled at room temperature. Both soybean and jackbean were then inoculated with 0.2% (w/w) tempeh starter. The inoculated beans were then mixed with a 1:1 ratio (w / w) and packed with banana leaf. Each pack contains 150 g of beans. The mixed grain was then fermented at room temperatured for 48 h in the shelf to produce mixed grains tempeh.

Preparation of Mix Grains Tempeh Flour

Mixed grain flour was produced according to Ariyantoro et al. (2016). The mixed grains tempeh was sliced into 2 mm thickness and then dried in a cabinet dryer at temperature of 50 °C and 70 °C for 6, 8, and 10 h. The dried tempeh was mashed with a blender for 3 minutes. Subsequently, the flour was shifted with a sieve (60 mesh) to produce mixed grains tempeh flour.

Sample Preparation for ACE Inhibitor Analysis

The extraction of mixed grains tempeh flour was carried out using a method reported by Rusdah et al., (2016). One gram of the tempeh flour mixed with grains was dissolved in the aquadest with a ratio of 1:30 (w/v) in the beaker. The mixture was then homogenized with a blender for 1 minute and then the samples were placed on a water bath shaker at 60 rpm for 60 minutes at 30 ° C. Subsequently, the samples were placed in 2 ml eppendorf tubes and centrifuged in 20,000 g for 15 minutes at 25 °C. The supernatant of samples were taken with a micropipette and placed in the falcon tube. The extract of samples were then tested for ACE-inhibitor activity.

Protein Hydrolysis

Protein hydrolysis (in vitro) used gastrointestinal simulation digestions (Torres et al., 2012). The extract of each sample was added by HCl 0,1 M until reach pH 2. It was then incubated in a waterbath shaker at 350 rpm for 30 minutes at 39 ºC to mimic the stomach condition. Subsequently, pepsin (E/S 1:67) was added into samples and then re-incubated in a waterbath for 2 h at 350 rpm and 39ºC. Hydrolysis was stopped by adding NaOH 1 M to reach pH 7. The supernatants of the samples were stored for analysis of the ACE inhibitor activity.

ACE-Inhibitor Activity Analysis InVitro als

In this study, the ACE-inhibitor activity consisted of ACE-inhibitor activity without pepsin hydrolysis and ACE-Inhibitor activity with pepsin hydrolysis for 2 h. The method was adopted from Cushman and Cheung (1970) with a modification as reported by Puspitojati et al. (2019). ACE-inhibitor activity without pepsin hydrolysis was the activity of the ACE inhibitor of tempeh flour of mixed grains (soybean and jackbean ratio 1:1) without being hydrolyzed by pepsin. ACE-inhibitor activity with pepsin hydrolysis for 2 h was ACE-inhibitor activity of mixed grains tempeh flour after being hydrolyzed by pepsin for 2 h. ACE-inhibitor activity analysis used the ACE enzyme 25 mU/mL. The HEPES-NaCl buffer was made to HHL solvent. This buffer was made by NaCl 300 mM and HEPES 50 Mm then added HCl 1 M until pH 8.3. The substrate was made with the addition of 8 mM HHL to HEPES-NaCl buffer pH 8.3. In ACE inhibitor activity analysis, several complexes such as of A, B, and C were used as follows:

Complex A

Complex A consisted of the HHL substrate and ACE enzyme (control). While the preparation of complex A was incubation of 50 µL HHL at 37 °C for 10 minutes in an incubator. ACE enzyme (25 mU/mL) as much as 50 µL was added to HHL and mixed using a vortex for 30 seconds, then incubated at 37 °C for 30 minutes. The reaction was stopped by adding 200 µL HCl 1 M and then mixed using a vortex for 30 seconds. Subsequently, 1500 L ethyl acetate PA of 1500 µL was added to the mixture. The mixture was then homogenized for 1 minute, followed by centrifugation at 4000 g and 4 °C for 15 minutes. One thousand microliters of the supernatant was placed in the test tube followed by evaporation at 100 °C for 30 minutes. Subsequently, three thousand microliters of water were added to the tube. The absorbance was then measured at a wavelength of 228 nm using a UV-vis spectrophotometer.

Complex B

Complex B consisted of a sample, HHL substrate, and ACE enzyme. Sample extract of each treatment was prepared as much as 50 µL then added to eppendorf which already contained 50 µL HHL. The mixture was then mixed using a vortex for 30 seconds and pre-incubated at 37 °C for 10 minutes. The ACE enzyme (25 mU /mL) as much as 50 µL was then added up to 50 L to the mixture and then mixed using a vortex for 30 seconds followed by incubation at 37 °C for 30 minutes. The reaction was stopped by adding 200 L of 200 µL HCl 1 M and mixed using a vortex for 30 seconds. The next steps were the same as the steps in complex A. The absorbance was measured at a wavelength of 228 nm using a UV-Vis spectrophotometer.

Complex C

Complex C consisted of the HHL substrate and ACE enzyme (blank). The water injection of 50 µL was added to the eppendorf which already contained 50 µL HHL. Subsequently, the mixture was added HCl 1 M up to 200 µL and preincubated at 37 °C for 10 minutes in the incubator, then the mixture was added 50 µL ACE enzyme and mixed using a a vortex for 30 seconds and incubated at 37 °C for 30 minutes in the incubator. The next steps were the same as the steps in complex A and B. The absorbance was measured at a wavelength of 228 nm with a spectrophotometer UV-Vis.

$$\% \text{ ACE inhibitor Activity} = \left(\frac{A-B}{A-C}\right) \times 100\% \quad (1)$$

Note :
A (Control Absorbance)
B (Sample Absorbance)
C (Blank Absorbance)

Antioxidant Activity Analysis with DPPH

Antioxidant activity was analyzed using the DPPH method (Chong et al., 2015). 0.5 grams of tempeh flour mixed with grains mixed with grains was placed in an erlenmeyer, then the sample was dissolved with 80 ml of PA (absolute) methanol methanol and sonicated for 30 minutes at 30 °C. Subsequently, one milliliters of sample extract was taken and placed in a test tube. The sample extract was added 3 ml of methanol PA (absolute) and 1 ml of DPPH solution and then incubated for 30 minutes. The absorbance of the sample was measured by a spectrophotometer UV-Vis at a wavelength of 514 nm.

$$\% \text{ ACE inhibitor Activity} = \left(\frac{\text{Blank Absorbance - Sample Absorbance}}{\text{Blank Absorbance}}\right) \times 100\%$$

Total Phenolic Analysis

Total Phenolic Analysis was carried out using a method reported by Hayastika et al., (2017). Two milliliters of the mixed grains tempeh flour extract sample was added by 5 ml of distilled water and 0.5 ml of Folin Ciocalteau reagent (50%) then incubated for 5 minutes. The sample solution was added 1 ml of 5% Na2CO3 solution, shaken out until homogeneous, and incubated at room temperature for 90 minutes, then the absorbance was measured at 725 nm wavelength.

$$C = \frac{C\ GAE \times V}{G} \times 100\% \quad (3)$$

C: Total Phenolic Content (mg/ml)
CGAE : Total Phenolic Content in Galat Acid Equivalent (mg/ml)
G : Material Mass (mg)
V: Extract volume (ml)

Amino Acids Profile Analysis with Liquid Chromatography Mass Spectrometry / Mass Spectrometry (LCMS / MS)

Amino acid analysis was carried out using a method reported by AOAC et al. (1999). Two grams of mixed grain tempeh flour was placed in 50 ml tubes. Twenty milliliters of 6 N HCl was added to the tube to hydrolyze the mixed grains tempeh flour protein. The mixture was then heated at 110 °C for 12 h. Neutralization was carried out by adding a strong base of 50 ml of 6 N NaOH. Subsequently, the hydrolysate was entered into 50 ml vial. Next, the hydrolysate sample was centrifuged for 10 minutes in 7000 rpm. Filtering was carried out with a 0.22 µM Whatman syringe filter. The dilution was carried out 50 times for the amino acid profile analysis with LCMS/MS.

The detector was The Xevo TQD Waters (Tandem Duadrupole Detector), the flow temperature was 500 °C with a speed of 1000 L/h, the sample injection volume was 2 µL. The column was C18 with the mobile phase of mobile phase A (0.1% Pentadecafluorooctanoic Acid (PDFOA) 99.5% : 0.5% ultrapure water/CH3CN with 0.1% Formic Acid (v/v/v/v)) and mobile phase B (0.1% PDFOA 10% : 90% ultra pure water/CH3CN with 0.1% Acid Format (v/v/v)). The mobile phase flow was 0.6 ml / min. The initial ratio of mobile phases A and B was 90:10, followed by 5:50 for 5 min, 90:10 for 5 min, and 90:10 for 7 min.

Isoflavone Compounds (Daidzein and Genistein) Content Analysis

Isoflavone compounds content analysis was carried out using a method reported by Sulistyowati et al, (2018). The standards used daidzein and genistein with a series of concentrations of 0.02; 0.04; 0.06; 0.08; and 0.1 mg / ml. Samples were macerated with 50% methanol in a ratio of 1:10. The samples' extract was filtered two times with Whatman no 1 filter paper, then centrifuged for 10 minutes. The filtrate of samples were filtered again using a 0.45 µm membrane filter (Celullose nitrate), then sonicated for 10 minutes. The HPLC system used the immobile phase TMC-18 (150mm x 4.6 mm, 5 µm) and the mobile phase was methanol: water containing 0.1% glacial acetic acid (v/v) with a ratio of 53:47. The detector was photo diode-array (PDA). The sample was injected using an autosampler with a volume of 10 µL and a flow speed of 1.0 ml / min.

The Experimental Design

The experimental design of this study was a randomized factorial block design with two factors. The factors were temperatures (50 °C and 70 °C) and drying times (6; 8; and 10 h). The determination of the best sample used priority matrix that based on the average of sample rank on all dependent variables in this study (ACE-Inhibitor activity, antioxidant activity, and total phenolics). The best mixed grains tempeh flour was sample with high functional characteristics (ACE-Inhibitor activity, antioxidant activity, and total phenolics). The best mixed grains tempeh flour would be used in the analysis of amino acids profile and isoflavone compounds (daidzein and Genistein) content.

Statistical Analysis

Statistical analysis of the functional characteristics (ACE-Inhibitor activity, antioxidant activity, and total phenolics) was SPSS General Linier Model (Univariete) (0.05%; P<=0.05).

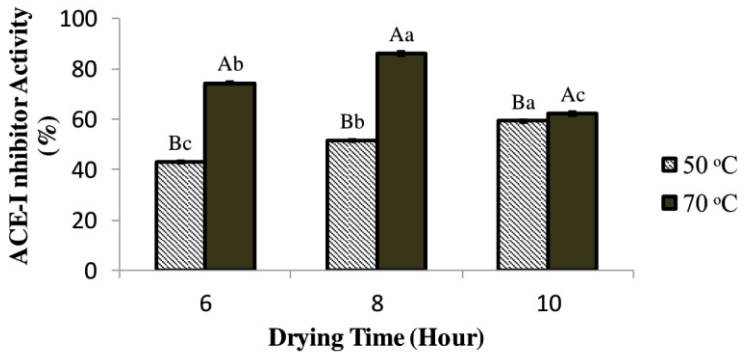
RESULT AND DISCUSSION

ACE Inhibitor Activity

Antihypertensive bioactive peptides play a role in the inhibition of the ANACE (ACE). The activity of ACE inhibitor peptides depends on the composition and sequence of the amino acids that arrange the bioactive peptides. ACE-inhibitor peptides directly relate to the active site of ACE (Harvian et al., 2018). In this study, an analysis of the activity of the ACE inhibitor was performed on tempeh flour mixed grains that were dried at different temperatures (50 °C and 70 °C) and time (6, 8 and 10 h). ACE inhibitor activity analysis consisted of ACE inhibitor activity without pepsin hydrolysis and ACE inhibitor activity with pepsin hydrolysis for 2 h.

Figure 1 shows that ACE-Inhibitor activity of mixed grains tempeh flour was affected by temperatures ($p < 0.05$) and drying times ($p < 0.05$). In general, the ACE-inhibitor activity increased with the incline of drying temperature and time. The samples that were dried at 50 °C had the lower degree of fermented hydrolysis (before samples were hydrolyzed by pepsin) with the increase of temperatures and drying times (data not shown). These samples may resulted in peptides that had higher ACE-inhibitor activity with the increase of temperatures and drying times. Meanwhile, samples that were dried at 70 °C for 6 and 8 h had the highest soluble protein content and the degree of fermented hydrolysis with

Figure 1. ACE-Inhibitor activity of soybean and jackbean mixed grains tempeh flour (1:1 w/w) without pepsin hydrolysis with different treatments of temperatures (50 °C and 70 °C) and drying times (6;8; and 10 h)



Note:
A-B Superscript showed significantly different between the drying temperatures ($p<0.05$).
a-c Superscript showed significantly different between drying times ($p<0.05$).

increasing temperatures and drying times (data not shown). Therefore, these samples resulted in higher ACE-inhibitor peptides contents with the increase of temperatures and drying times. In this case, the heating may cause the opening of protein folds that contained high hydrophilic amino acids from the protein original conformation. Heating can break hydrogen bonds, hydrophobic interactions, salt bonds, and opening of folds (Winarno, 2004 and Li et al., 2018) and results in simpler protein monomers such as hydrophilic peptide sequences (Nemethy et al., 1966). It caused high soluble protein content and degree of hydrolysis. The increase of soluble protein content and degree of hydrolysis resulted in the increase of the peptides content. These peptides can have potency as an antihypertensive peptides. The ACE-inhibitor activity is influenced by composition, sequences, and molecular weight of bioactive peptides (Ladesma et al., 2011).

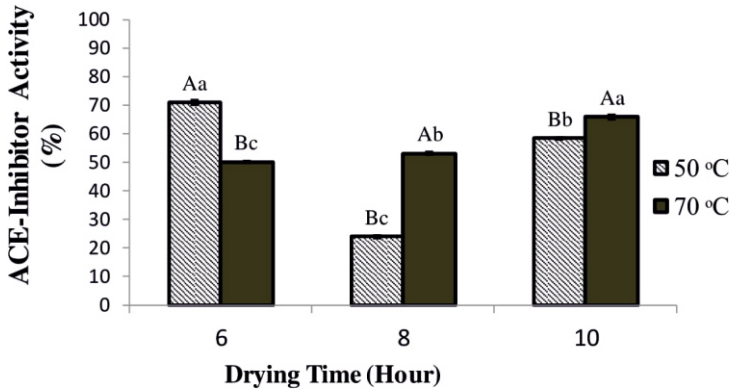
The sample that was dried at 70 °C for 10 h (62.21%) had ACE inhibitor activity lower than that dried for 6 h (74.41%) and 8 h (86.04%). The bioactive peptides that act as ACE inhibitors might be damaged by increased temperatures and drying times. The drying process by heating causes protein denaturation. Denaturation can alter the protein structure. Denaturated protein has low solubility that can precipitate easily (Nemethy et al., 1966 and Winarno, 2004). All variations, except the sample that was dried at 70 °C for 10 h, showed that higher drying temperatures and times resulted in higher ACE inhibitor activity.

The ACE inhibitor activity of the samples with pepsin hydrolysis was significantly influenced by drying temperatures and times (Figure 2). The hydrolyzate of the tempeh flour sample of mixed grains that was dried at 50 ° C for 8 h had the lowest ACE inhibitor activity (23.98%) among other samples. Meanwhile, for the hydrolyzate that was dried at 70 ° C, the increase in duration resulted in an increase in the activity of the ACE inhibitor. The hydrolyzate dried at 70 oC had higher soluble protein content and degree of hydrolysis with the increase of temperatures and drying times (data not shown). As a consequence, the ACE-inhibitor activity was higher with the increase of temperatures and drying times. The mixed grains tempeh contained high hydrophilic amino acids (Hesti, 2019). In this case, the heating may cause the opening of protein folds that contained high hydrophilic amino acids from the protein original conformation and enzymes bind and catalyze easily on peptide bonds in proteins (Wijatnika, 2017). Heating can also break hydrogen bonds, hydrophobic interactions, salt bonds, and opening of folds (Winarno, 2004 and Li et al., 2018) and results in simpler protein monomers such as hydrophilic peptide sequences (Nemethy et al., 1966). It caused high soluble protein content and degree of hydrolysis. The increase of soluble protein content and degree of hydrolysis resulted in the increase of the peptides content. These peptides can have potency as an antihypertensive peptides. The activity of ACE-inhibitor peptides depends on the composition and sequence of amino acids that arrange the bioactive peptides (Fadly, 2014).

The hydrolyzate of the tempeh flour sample of mixed grains that was dried at 50 °C for 8 h (23.98%) had the lowest ACE inhibitor activity. This result was opposite with the degree of hydrolysis, in which the degree of hydrolysis of the sample dried for 8 h was higher than that of dried for 6 h (data not shown). Sample that was dried at 50 °C for 8 h may contain peptides that had the lower ACE-inhibitor activity. The ACE-inhibitor activity is influenced by composition, sequences, and molecular weight of bioactive peptides (Ladesma et al., 2011). The results showed that for the hydrolyzate of mixed grains tempeh flour dried at 70 °C, the longer the duration, the higher ACE-Inhibitor activity was obtained.

Figure 1 and Figure 2 show that the average ACE-inhibitor activity of mixed grains tempeh flour samples before hydrolysis (62.72%) were higher than after hydrolysis (53.83%). One explanation could be that the hydrolysate had bioactive peptides with lower ACE-Inhibitor activity. Harvian (2018) said that jackbean peptides (concanavalin) have the highest ACE inhibitor activity after hydrolyzing by digestive enzymes. The subsequent process of hydrolysis of protease enzymes in the body (intestine) has the potential to produce peptides with shorter sequences so that they effectively act as ACE inhibitor. The glucosidase enzymes in the body can hydrolyze glucoside isoflavones into aglycone isoflavones such as daidzein and genistein. Aglycone isoflavones can act as ACE-inhibitors (Sanjunkta et al., 2016). Therefore, the ACE-inhibitor activity of bioactive peptides and isoflavones might increase after hydrolysis.

Figure 2. ACE-Inhibitor activity of soybean and jackbean mixed grains tempeh flour (1:1 w/w) with pepsin hydrolysis for 2 hours with different treatments of temperatures (50 °C and 70 °C) and drying times (6;8; and 10 h)

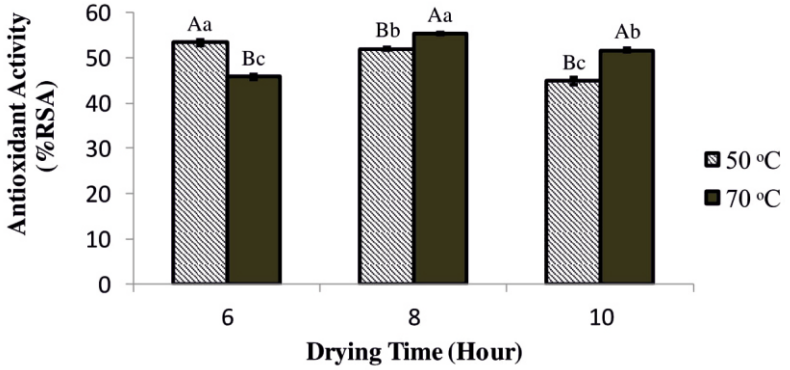


Note:
A-B Superscript showed significantly different between the drying temperatures ($p<0.05$).
a-c Superscript showed significantly different between drying times ($p<0.05$).

Antioxidant Activity

Antioxidants can protect the body from free radical attack, and a certain amount can inhibit the oxidation process (Haron et al., 2009). In this study, antioxidant activity analysis was carried out on mixed grains tempeh flour. As can be seen in Figure 3, antioxidant activity was significantly influenced by drying temperatures and times. In general, the longer drying time resulted in lower antioxidant activity. The lowest antioxidant activity was obtained from the sample that was dried at 70 °C for 6 h. The long drying time can damage antioxidant compounds (Sayuti and Yenrina, 2015). In addition, the high temperature and long drying time can increase the rate of oxidation and the increase of antioxidant compounds degradation (Dewi, 2006). The sample that was dried at 70 °C for 6 h had lower antioxidant activity than that dried for 8 h. This happened because increasing temperatures and drying times can release phenolic compounds that bind to other components such as fiber and carbohydrates and increasing antioxidant activity in the sample (Istiani, 2010). In addition, samples that were dried at 70 °C for 6 and 8 h had the higher antioxidant activity with the increase of drying times because they were expected to contain bioactive peptides acted as antioxidant. The samples were dried at 70 °C for 6 and 8 h, with a higher protein soluble content and a higher degree of hydrolysis with increasing temperatures and drying times (data not shown). The mixed grains tempeh contained high hydrophilic amino acids (Hesti, 2019). The heating may cause the opening of protein folds that contained high hydrophilic amino acids from the protein original conformation. Heating can break hydrogen bonds, hydrophobic interactions, salt bonds, and opening of folds (Winarno, 2004 and Li et al., 2018) and results in simpler protein monomers such as hydrophilic peptide sequences (Nemethy et al., 1966). It caused high soluble protein content and degree of hydrolysis. The increase of soluble protein content and degree of hydrolysis caused the increase of peptides content. These peptides have the potential as antioxidant peptides.

Figure 3. Antioxidant activity of soybean and jackbean mixed grains tempeh flour (1:1 w/w) with different treatments of temperatures (50 °C and 70 °C) and drying times (6;8; and 10 h)



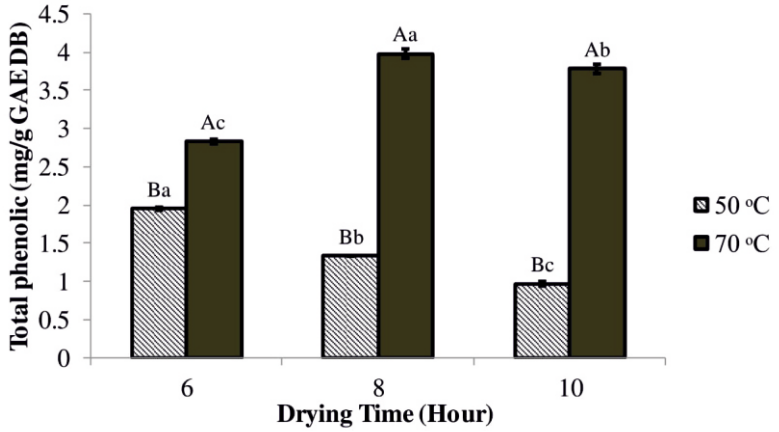
Note:
A-B Superscript showed significantly different between drying temperatures ($p<0.05$).
a-b Superscripts showed significantly different between drying times ($p<0.05$)

Total Phenolic

The phenolic compounds are very sensitive, unstable, and very susceptible to degradation. The degradation is caused by temperature, oxygen, and light (Tuminah, 2004). In this study, the total phenolic concentration was analysed. As can be seen in figure 4, the total phenolic was significantly influenced by drying temperatures and times. For samples dried at 50 °C, the increase in drying duration caused a decrease in total phenolic. The higher temperature and the longer drying time can damage the phenolic compounds (Sayuti and Yenrina, 2015). However, for samples dried at 70 °C, the increase in duration from 6 to 8 h increases the total phenolic compound. The increasing of temperature and drying time can release of phenol compounds which are bound by other components in the sample. It could increase total phenolic in the samples (Istiani, 2010).

Based on the results of ACE-inhibitor activity, antioxidant activity, total phenolic, and priority matrix, the best sample was obtained by drying at 70 °C for 8 h. There is a link between ACE inhibitory activity, antioxidant activity, and total phenolics. Peptides sequence can act as ACE-inhibitor and antioxidant (Harvian, 2018). Furthermore, flavonoid compounds can act as antioxidants and antihypertensive substances that can inhibit the action of the ACE enzyme (Carolia et al., 2016). The best sample contained high functional characteristics (ACE-Inhibitor activity, antioxidant activity, and total phenolics). The best mixed grains tempeh flour was then used in the analysis of amino acid profiles and isoflavone (daidzein and genistein) content.

Figure 4. Total phenolic of soybean and jackbean mixed grains tempeh flour (1:1 w/w) with different treatments of temperatures (50 °C and 70 °C) and drying times (6;8; and 10 h)



Note:
A-B Superscript showed significantly different between drying temperatures ($p<0.05$).
a-b Superscripts showed significantly different between drying times ($p<0.05$)

Amino Acids Profile

Table 1 presents the amino acid profile of the best mixed grains of tempeh flour obtained from the previous step. The flour contained hydrophilic and hydrophobic amino acids which generally function as ACE-inhibitor bioactive peptide precursors such as leucine (Pan et al., 2011), aspartic acid, glutamic acid, arginine, tyrosine, phenylalanine, valine, isoleucine, histidine, proline, and tryptophan (Harvian, 2018). The flour contained high hydrophilic amino acids. Based on results of amino acids profile analysis with LC-MS, the high ACE-Inhibitor activity of the best mixed grains tempeh flour was related to the high content of ACE-Inhibitor bioactive peptide precursors.

Tripeptides and dipeptide structures with hydrophobic amino acids, especially with aromatic amino acids and positively charged amino acids, bind to the active site of ACE. ACE is an exopeptidase that cuts the carboxyl side of the decapeptide (Angiotensin I) with histidine-leucine residues (his-leu) into octapeptides (Angiotensin II) (Pan et al., 2011). Harvian (2018) said that ACE has an active side that will be reactive to peptides with amino acid residues that have similarities to Angiotensin I, namely proline, phenylalanine, histidine, and leucine.

Table 1 also shows that tempeh flour tempeh flour mixed grains contained hydrophilic and hydrophobic amino acids that generally function as bioactive peptide precursors of antioxidants of antioxidants such as as tyrosine, phenylalanine, histidine and tryptophan (Harvian, 2018). Based on results of the amino acids profile analysis with LC-MS, the high antioxidant activity of the best flour because could be related to the high amino acids content as antioxidant bioactive peptide precursors. Bioactive peptides that act as antioxidant have several mechanisms in free radicals inhibiting like radical scavenging, mineral chelating, metal reducing agents, and protectors (Phelan et al. 2009). The antioxidant activity of a peptide are determined by its primary amino acid structure (Phelan et al. 2009). Elias et al. (2008) explained that histidine and hydrophobic amino acids have an important effect on antioxidant bioactive peptides. Histidine has an imidazole group that can function as a hydrogen provider, a peroxyl radical lipid-11 catcher, and a chelating metal. The antioxidant activity of bioactive peptides is regulated by the composition of peptide sequences.

Table 1. Amino Acids Profile of The Best Mixed Grains Tempeh Flour (Soybean and Jackbean with ratio 1:1 w/w)

Parameter	*Mixed Grains* Tempeh Flour g/100g Protein (DB)
Hydrophilic	
Glutamate acid [AI]	2,74 ± 0,035
Aspartate acid [AI]	2,57 ± 0,001
Arginine [AI]	1,47 ± 0,003
Lysine	2,10 ± 0,146
Histidine [AI, A]	1,12 ± 0,114
Serine	1,19 ± 0,016
Threonine	1,32 ± 0,001
Tyrosine [AI, A]	0,75 ± 0,004
Hydrophobic	
Alanine	1,49 ± 0,015
Glisine	0,93 ± 0,085
Valine [AI]	1,43 ± 0,005
Leucine [AI]	2,25 ± 0,011
Isoleucine [AI]	1,29 ± 0,005
Proline [AI]	0,99 ± 0,007
Phenilalanine [AI, A]	1,37 ± 0,006
Metionine	0,21 ± 0,002
Sisteine	0,15 ± 0,001
Tryptophan [AI, A]	0,04 ± 0,001

Note:
Pan et al. (2011): (AI): Precursor of ACE-Inhibitor peptides and (A):Precursor of antioxidant peptides.
Each value is expressed as an average in SD (n = 2)

Isoflavone Compounds (Daidzein and Geneistein) Content

The determination of the content of daidzein and genistein in the best tempeh flour mixed with grains (dried at 70 °C for 8 h) used the interpolation of the standard curve. The best tempeh flour mixed grains contained 0.03 mg/g daidzein and 0.37 mg/g genistein. The genistein content in the best mixed grains was higher than that of daidzein. Istiani (2010) stated that the content of genistein in a chopped jackbean and soybean tempeh fermented for 48 h (0.71 mg/g and 5.86 mg/g, respectively) was higher than daidzein (0.36 mg/g and 8.56 mg/g, respectively). Tempe flour had lower isoflavone compounds than tempeh because the drying process by heating causes a decrease in the content of isoflavone compounds. Isoflavone compounds are sensitive to heating (Muchtadi, 2012).

Based on the results of isoflavone analysis, the high antioxidant activity of the best mixed grains tempeh flour (55.34%) was supported by the high content of daidzein and genistein. According to Wang and Murphy (1994), the high or low range of isoflavone content is caused by various factors, namely the temperature and solvent that was used for extraction, the level of maturity, the temperature and climate of the growing places, the soil conditions and the characteristics of the isoflavone compounds. Isoflavone compounds are very reactive and susceptible to oxidation so it can bind easily with other compounds and make the new compounds (Istiani, 2010).

CONCLUSION

The ACE-inhibitor activity, antioxidant activity, and total phenolic compound were influenced by the drying temperature and time. The best mixed grains tempeh flour was obtained by drying at 70 °C for 8 h.

The best mixed grains tempeh flour contained amino acids that can act as bioactive peptide precursors (ACE) inhibitors (e.g. glutamic acid, aspartic acid, leucine, arginine, phenylalanine, valine, isoleucine histidine, proline, tyrosine and tryptophan), as antioxidant bioactive peptides precursors (e.g. phenylalanine, histidine, tyrosine and tryptophan), and as well as isoflavone compounds (daidzein and genistein).

ACKNOWLEDGEMENT

The author thank to Ministry of Research and Technology as funder in Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) Program. The authors also thank to Department of Food Science and Technology, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta.

REFERENCES

AOAC. 2005. "Official Method of Analysis of The Association Analytical of Chemist". Ed 18th .The Association of Official Analysis Chemist, Washington, USA.

Ariyantoro, A.R., Rachmawati, D., and Ikarini, I. 2016. Karakteristik Fisikokimia Tepung Koro Pedang (*Canavalia ensiformis*) Termodifikasi dengan Variasi Konsentrasi Asam Laktat dan Lama Perendaman. Jurusan Ilmu dan Teknologi Pangan, Universitas Sebelas Maret, Surakarta.

Chang, S.K.C. and Xu, B.J. 2007. Comparative Analyses of Phenolic Composition, Antioxidant Capacity, and Color of Cool Season Legumes and Other Selected Food Legumes. Journal of Food Science. 72 (2): 67-77.

Chong, N.V.W., Pindi, W., Chye F., Shaarani, S.M., and Lee, J. 2015. Effects of drying methods on the quality of dried sea cucumbers from sabah. International Journal of Novel Research in Life Sciences. 2(4):49-64.

Cushman, D. W. and Cheung, H. W. 1971, Spectrophotometric Assay and Properties of the *Angiotensin Converting Enzyme* of the Rabbit Lung. Biochemical Pharmacology. 20:1637-1648.

Fadly, R. 2014. Aktivitas Inhibisi Enzim Pengubah Angiotensin pada Hidrolisat Tempe Koro Pedang (*Canavalia ensiformis* L.) secara In Vitro [Tesis]. Departemen Biokimia, Fakultas MIPA, Insitut Pertanian Bogor, Bogor .

Harvian, Z.A. 2018. In silico approach in evaluation of Jackbean (*Canavalia ensiformis*) Canavalin Protein as Precursors of Bioactive Peptides with dual antioxidant and Angiotensin I Converting Enzyme Inhibitor [Tesis]. Fakultas Teknoligi Pertanian, Universitas Gadjah Mada, Jogja.

Hayastika, Ansharullah, and Asyik, N. 2017.Pengaruh Subtitusi Tepung Kedelai (*Glysine Max)* terhadap Aktivitas Antoksidan Roti Tawar. Jurnal Sains dan Teknologi. 2; (4); 684-691.

Hesti, I.S. 2019. Karakteristik Tempe *Mixgrain* dari Kedelai (*Glycine max*) dan Koro Pedang Putih (*Canavalia ensiformisL.*) sebagai Prekursor *Aktivitas Angiotensin Converting* (ACE) *Enzyme.*[Tesis]. Fakultas Teknologi Pertanian, Universitas Gadjah Mada, Jogja.

Istiani, Y. 2010. Karakterisasi senyawa bioaktif isoflavon dan uji aktivitas antioksidan dari ekstrak etanol tempe berbahan baku koro pedang (*Canavalia ensiformis* L.) [Tesis]. Universitas Sebelas Maret, Surakarta.

Khirzin, M. H., Sukarno, Yuliana, N. D., Fawzya, Y. N., and Chasanah, E. 2015. The activity of *Angiotensin Converting Enzyme* (ACE) *inhibitor* and collagen peptide antioxidant from Gama sea cucumber (*Stichopus variegatus*). Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan. 10(1):27-35.

Kusumawardhani, P. C. 2015. Pemanfaatan Kacang koro Pedang (*Canavalia Ensiformis*) sebagai Bahan Subtitusi dalam Pembuatan Tempe Kedelai. Departemen Ilmu dan Teknologi Pangan, Fakultas MIPA, Insitut Pertanian Bogor, Bogor.

Li, M., S. Xia, Y. Zhang, and X. Li. 2018. Optimization of ACE inhibitory peptides from black soybean by microwave-assisted enzymatic method and study on its stability. LWT-Food Science and Technology. 358-365

Ledesma, B.H., Contreras, M.D.M., and Recio, I. 2011. Antyhypertesive Peptides: Production Bioavaibility and Incorporation into Foods. Advances in Colloid and Interface Science. 165: 23-25.

Lowry, O.H., Nisa, J.R., and Lewis, F. 1951. Protein Measurement with the Folin Phenol Reagent. The Journal of Biological Chemistry. 193: 265-275.

Muchtadi, T., R. 2008. "Teknologi Proses Pengolahan Pangan". Ed 3rd. Bogor: Departemen Ilmu dan Teknologi Pertanian, Fakultas teknologi Pertanian, Institut Pertanian Bogor, Bogor.

Muchtadi, D. 2012. "Pangan Fungsional dan Senyawa Bioaktif". Alfabeta. Bandung.

Mulyani, S. 2013. Karakterisasi Tepung Tempe dari Empat Varietas Kedelai Impor dan Aplikasinya Menjadi Minuman [Skripsi], Fakultas Teknologi Pertanian, Insitut Pertanian Bogor, Bogor.

Nayla, 2012. Studi Pembuatan Tepung Formula Tempe. Fakultas Pertanian, Universitas Hasanudin Universitas, Makasar.

Nemethy, G., Leach, S.J. and Scherage, H.A. 1966.. The influence of amino acid side chains on the free energy of helix-coil tansition. J.Phys.Chem. 70: 998-1004.

Pan, D, Guo, H, Zhao, B, Chao, J. 2011. The molecular mechanisms of interactions between bioactive peptides and angiotensin-converting enzyme. Bioorg Med Chem Let. 21:3898-3904

Phelan, M., Aherne, A., Fitz G. R .J., and O'Brien, N.M. 2009. Casein-derived bioactive peptides: Biological effects, industrial uses, safety aspects and regulatory . Int Dairy J. 19:643–654.

Phillips, S.M., Fulgoni III, V.L, Heaney, R.P., Nicklas, T.A., Slavin, J.L., and Weaver, C.M. 2015. Commonly consumed protein foods contribute to nutrient intake, diet quality, and nutrient adequacy. Lebensm.-Wiss. U-Technology. 37 :59–67.

Puspitojati, E., Cahyanto, M.N., Marsono, Y., and Indrati, R. 2019. Production of Angiotensin-I-Converting Enzyme (ACE) *Inhibitor*y Peptides during the Fermentation of Jackbean (*Canavalia ensiformis*) Tempe. Pakistan Journal of Nutrition. 18(5):464-470.

Poedjiadi, Anna. 1994. Dasar-dasar Biokimia. Jakarta: UI-Press Rusdah. 2016. Antioxidative of tempe peptides from Indonesia. Department Food Science Technology, Bogor Agricultural University, pp:50.

Sayuti, K., dan Yenrina, R. 2015. "Antioksidan Alami and Sintetik". Andalas University Press, Padang.

Sulistyowati, E., Martono,S., Riyanto, S., and Luitaningsih, E. 2018. Analisis Daidzein dan Genistein pada Kedelai (*Glycine max L.* Merril) Varietas Anjasmoro, Argomulyo dan Dena 2 Menggunakan Metode KCKT. Media Farmasi Indonesia. 13(1): 1-6.

Torres, F.C., Alaiz, M., and Vioque, J. 2012. Iron chelating activity of chickpea protein hydrolysate peptides. Food Chem.134:1585-1588.

Wang, H., and Murphy, P.A. 1994. Isoflavone content in commercial soybean foods. J Agric Food Chem. 42: 1666-73.

WHO. 2000. Informasi Kesehatan. www.infokes.com. Diakses tanggal 7 November 2019 pukul 10.30 WIB.

Wijatnika, B. D. 2017. Perendaman, Pemanasan dan Hidrolisis dengan Enzim Alkalase Guna Meningkatkan Kualitas Gizi Protein Koro Pedang Putih (Canavalia Ensiformis) [Tesis]. Departemen Ilmu dan Teknologi Pangan. Fakultas Teknologi Pertanian, Universitas Gadjah Mada, Jogja.

Winarno, F.G., 1993. Pangan, Gizi, Teknologi dan Konsumen. Gramedia Pustaka Utama, Jakarta.

Winarno, FG. 2004. Kimia Pangan dan Gizi. Gramedia Pustaka Utama, Jakarta