Polymerization of meat and tempeh protein using transglutaminase and their potency as an antihipertency and antioxidant agent[1]

Yuny Erwanto, Jamhari, and Rusman

Department of Animal Products Technology, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

ABSTRACT: This research was done to explore meat and tempeh protein mixture as biopolymer materials and their potency as an antihipertency and antioxidant agent. Meat and tempeh protein contain many kinds of peptides which very useful for human health. Meat and fermented soybean protein can form crosslink bond using transglutaminase as a catalyst and will increase their ability as a food ingredients, antihypertency and antioxidant agent. Materials consisted of 5 groups of protein mixture between meat and tempeh (100 % meat : 0% tempeh, 95% meat : 5% tempeh, 90% meat : 10% tempeh, 85% meat : 15% tempeh and 80% meat : 20% tempeh, w/w) with 4 levels of transglutaminase enzyme concentrations (0.00; 0.02; 0.04 and 0.06%, w/w). Observed variables were protein polymerization, antihipertency and antioxidant activity. Biopolymer of meat-tempeh protein mixture was confirmed using electrophoresis method. Results showed meat-tempeh protein was able to form protein crosslinks using transglutaminase enzymes. Protein hydrolyzed of meat and tempeh mixture showed the ability to inhibit angiotensin converting enzyme, and both materials (meat and tempeh) had the potency as an antihipertency agent. The substitution of Meat protein with tempeh protein showed the increasing antioxidant activity with increasing tempeh addition level. In conclusion, tempeh contents protein and formed crosslinks bond with meat protein and the mixture protein was able to inhibit angiotensin converting enzyme and oxidations activity.

Key words: meat, tempeh, biopolymer protein, antihipertency, antioxidant

INTRODUCTION

Vegetable protein is an alternative good source of protein which wide application in meat products such as sausage and others. As far protein has developed to improve products texture in many food products and also has develop as an emulsifier agent.

Soybean protein has been applied for meat substitution to decrease fat content in meat products (Calderon *et al*., 2000; Cofrades *et al*., 2000; Muguruma *et al*, 2003). Soybean protein was added on sausage production in many kinds such as hydrolysates protein (Calderon *et al*., 2000) or soy fiber flour (Cofrades *et al*., 2000), soy protein isolate (Muguruma *et al*., 2003), and soybean modified protein (Ramezani *et al*., 2003). The reason using soybean protein was the specific functional properties of soybean protein in food system especially in the improvement of sausage properties (Hin *et al*., 2000).

Peptides from protein hydrolysis have been investigated for many years and show bioactive properties such as anti-hypertension, immunomodulation, antioxidation, antimicrobial action and antithrombosis (Arai *et al*. 2001). These peptides are inactive in the amino acid sequence of original proteins, but their bioactivity can be released by proteolytic enzymes during gastrointestinal digestion or food processing (Meisel, 1997). Thus, these peptides have a potential as health-enhancing nutraceuticals and may be used as components in functional foods or nutraceuticals. Previous research have studied fermented soybean and showed the fermented soybean resulted bioactive peptide compare soybean without fermentation (Okamoto et al., 1995). Fermentation process also produced more hydrolysates

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protein as a result of enzyme microbia activity. The interest one is Indonesia traditional fermented soybean is called tempeh. The soybean fermented was detected able to prevent cancer (Messina et al., 1994) and also can prevent the hipertency (Astawan, 2002).

Angiotensin converting enzyme (ACE) plays an important role in the regulation of blood pressure in the renin-angiotensin system. Hypertension is a worldwide problem of epidemic proportions that affects 15–20% of all adults. It is the most common serious chronic health problem in adults and carries a high risk factor for arteriosclerosis, strokes, myocardial infarction and end-stage renal disease. It is suggested that hypertension is closely related to food components (Jung *et al*. 2006). Hypertensive patients can control their blood pressure by taking medicine to inhibit ACE. It has recently been found that ACE inhibitory peptides can be produced from the enzyme hydrolysates of various food proteins (Cheng et al., 2008).

Improving the functional properties of food ingredients is necessary in food industrial application. However their functional properties require some improvements, which can be made via enzymatic combination with others protein, protein mixing using enzymatic modification, and structure modification. These enhancements can contribute on functional and physical properties of some foods. Protein cross-links is one of the food ingredients modification through -(-glutamyl) lysine bonds using transgluminase enzyme. Muguruma et al. (2003) have used microbial transglutaminase for improving the texture and properties of chicken sausage. Research in the protein mixture of meat and tempeh using transgluminase and their properties have not yet been studied, thus the objectives of this research were to improve the protein mixture properties using transglutaminase and to investigate their potency as an antihypertency and antioxidant agent.

MATERIALS DAN METHODS

Materials

Beef, soy bean and tempeh yeast was purchased from local market, transglutaminase was obtained from Merck, *angiotensin converting enzyme* of rabbit lung (Sigma Chemical Co., USA), substrate *hypuril-histidil-leucine* (HHL) from Nacalai Tesque, borate buffer, acetate buffer, HCl, NaCl, polyacrylamide, SDS buffer and protein marker.

Methods

Meat and Tempeh Protein bond Preparation. Beef meat and tempeh were grounded and divided in five groups tempeh-meat ration (0:100, 5:95, 10:90, 15:85 and 20:80 %). Mixture protein (300 mg per tube) were placed into 25 ml conical tubes, supplemented with 7.5 ml buffer solution (100 mM SDS buffer, 50 mM NaCl) and 2.5 ml of distilled water and mixed well. Thus the final concentration of sample was 30 mg/ml (w/v wet basis). The samples were homogenized at 0^{o}C in an ice bath. Tranglutaminase enzyme was added to the sample solution in various concentrations (0.00, 0.02, 0.04 and 0.06%, weight per protein mixture weight). The samples were incubated at 40^{o}C for 1 h. After incubation, the enzyme reaction was terminated by heating at 100^{o}C for 10 min.

Biopolymer Confirmation by SDS-PAGE Analysis. To confirm that the intermolecular covalent cross-link was found by the enzyme treatment, samples were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis under reducing conditions was carried out on slab gels (4.5 % for stacking gel and 5-20% for separating gel) using an SDS-Tris-glycine discountnious buffer system according to the method of Laemmli (1970). Prior to electrophoresis, protein samples were heated at 95^{o}C for 5 min in the presence of 25 mM Tris-HCl (pH 6.8), 1 % SDS, 20% Glycerol, 0.01% Bromo Phenol Blue and 1% 2-mercaptoethanol. After running, the gel was stained with 0.25% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid, and it was destained with same solvent system without dye.

Analysis ACE Inhibition. Protein of sample was assayed using Biuret methods. Mixture protein of meat and tempeh was hydrolyzed using trypsine from bovine pacreas (Sigma Chemical Co., USA). ACE activity inhibition was analyzed according to method of Cushman and Cheung (1971) with slight modifications. Hydrolysate (6 ul) was added to the ACE solution (20 ul, with 200 mM borat acid and 50 mM sodium tetraborat buffer) and the reaction was started by the addition of 50 ul of 7.6 mM HHL. After incubation at 37°C for 30 min, the reaction was terminated by adding 554 ul of 0.1 N hydrochloric acid (HCl). The resulting hippuric acid was extracted with 1.5 ml of ethyl acetate. After being centrifuged (3000 *rpm*, 15 min), 1 ml of upper layer was transferred into a microcentrifuge tube and heated by dry bath (Type 17600; Thermolyne, Dubuque, USA) at 100°C for 10 min. The hippuric acid was dissolved in 1.0 ml of distilled water and the absorbance was read at 228 nm using spectrophotometer. Inhibitory activity was calculated using the absorbance of hippuric acid liberated from HHL by ACE. The IC50 value was defined as the concentration of peptide (mg/ml) required to inhibit 50% of ACE activity.

Antioxidant Activity Measurement. Antioxidant activity was analyzed based on the measurement of di-phenil-picryl-hydracyl (DPPH) and was calculated as percent antioxidant activity.

RESULTS AND DISCUSSION

Biopolymer Protein of Meat and Tempeh

The formation of protein mixture polymer through the intermolecular crosslinks was analyzed by SDS-PAGE. The protein profile of different ratio concentration between meat and tempeh protein is shown in Figure 1. The sample without transglutaminase showed beef and soybean protein had different profile. Soybean protein showed the low molecular weight but beef had higher molecular weight (line b and c). The mixture protein profile (Fig.1 line d-g) showed similar patterns and there were no intermolecular crosslinks, although the tempeh concentration level was extended to 20%.

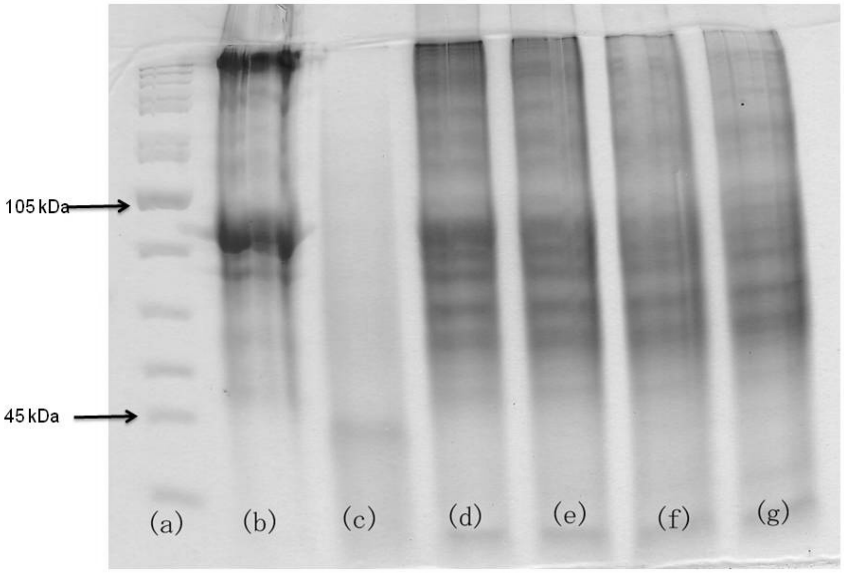

Figure 1. Protein mixture patterns of various meat-tempeh ration : (a) marker, (b) meat, (c) tempeh, (d) meat:tempeh 95:5, (e) meat:tempeh 90:10, (f) Meat:tempeh 85:15, (g) Meat:tempeh 80:20

The research showed there was protein mixture reaction between meat and tempeh with transgltaminase addition (Figure 2). The structure of native meat and soybean protein were not capable of incorporating the intermolecular bond of glutamine and lysine. When the samples were treated with transglutaminase addition, there were bands changes, including the appearance of the new bands around 100 kDa (figure 2 line b, f and g). The high molecular weight biopolymer bands, which appear around 100 kDa showed the new patterns of protein. This result indicated that the reaction of meat and tempeh protein with transglutaminase was affected the protein formation but the transglutaminase levels were not significantly affect the reaction. The increasing transglutaminase level did not increasing the biopolymer band. The similar pattern of protein was shown in 0.02 % and 0.06% transglutaminase level.

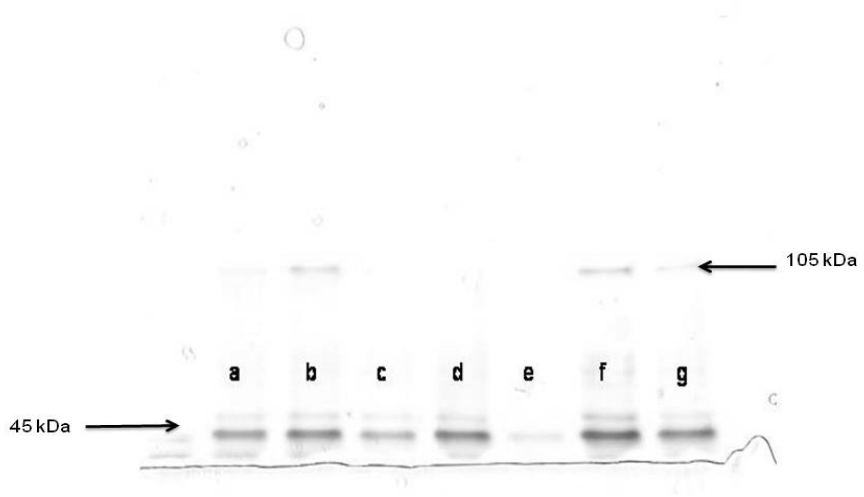

Figure 2. Protein mixture with transglutaminase addition : a – d, meat 90% and tempeh 10% with different transglutaminase addition. a) control without transglutaminase b) 0.02% c) 0.04%, d) 0.06%. Line e. f. g contain 20% tempeh with various transgluminase from 0.02 to 0.06%

Transglutaminase was wide apply to improve protein polymer such as in sausage propertiess by protein mix between globulin with chicken meat (Muguruma et al., 2003), turkey breast muscle and surimi polymerization (Ashie and Lanier, 1999) and surimi gel cross-linking with microbial transglutaminase (Jiang et al., 2000).

Angiotensin Converting Enzyme Inhibition Activity

Change in inhibition activity to angiotensin converting enzyme (IC_{50}) of protein mixture with and without transglutaminase were shown in figure 3, 4 dan 5. The result showed protein mixture of meat and tempeh with or without transglutaminase as a catalyst have the similar potency in ACE inhibition. The concentration of protein for angiotensin converting enzyme inhibition (IC_{50}) was 0,4 to 0,5 mg/ml.

Protein hydrolyzation using protease enzyme resulted more simple peptides. Some peptides has the ability to inhibit ACE activity and consequently the blood pressure reduces with the increasing of bioactive peptides. This result was coincided with Astawan (2002) that tempeh protein had high potency as an ACE inhibitor. The high potency of tempeh caused by fermentation process which could hydrolize of some protein into simple molecule of peptides. Meat protein hydrolized using protease enzyme also showed highly active in inhibiting ACE. The studies on the purification of ACE inhibitory activity have indicated that small peptides were highly active in inhibiting ACE. The inhibitory peptides of ACE showed high activity when the molecular weight was not above 5 kDa (Jung *et al*. 2006).

Arihara *et al*. (2001) reported that hydrolized protein from pork protein showed effects in antihypertency. The bioactive compound is miopentapeptides met-asn-pro-pro-lys and ile-thr-thr-asn-pro, and inhibiton activities were 945.5 dan 549.0 µM to IC_{50}. They also found met-asn-pro, asn-pro-pro, pro-pro-lys, ile-thr-thr, thr-thr-asn, and thr-asn-pro, which is a part of miopeptida. These sequences were founded in myosin heavy chain protein.

Moreover, short chains of peptide not only easily pass through the gastrointestine, but are also easily absorbed by the animal's body. This potential bio-activity was observed after the absorption (Robert *et al*. 1999). Cheng et al. (2008) reported that the IC_{50} value was consistent with hydrolysates of alcalase enzyme at 4 and 8 h showed lower IC_{50} values of 1.960 mg/mL and 0.945 mg/mL. However, even though higher ACE inhibitory percentage was found in hydrolysates of pepsin and trypsin at 6 and 8 h incubation, respectively, the result of IC50 (7.012 and 4.016 mg/mL) demonstrated its poor capacity to inhibit ACE compared with Alcalase hydrolysates.

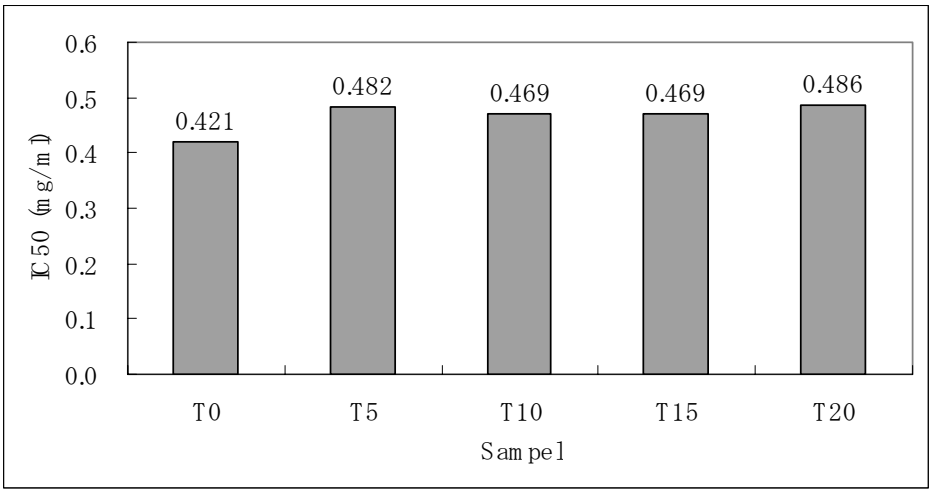

Figure 3. Nilai IC_{50} value of protein mixture with different tempeh and meat level: 0, 5, 10, 15 and 20% before polymerization by transglutaminase enzyme (mg/ml).

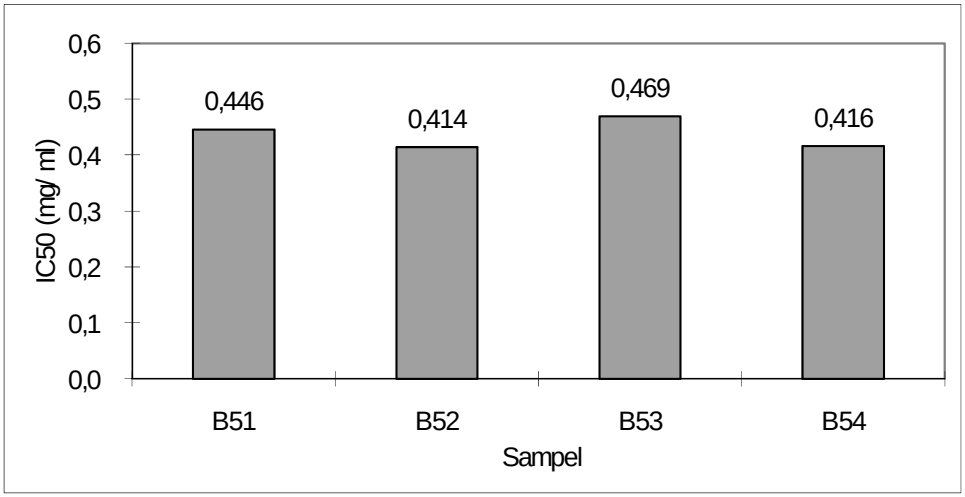

Figure 4. IC_{50} value of meat 95% and tempeh 5% protein mixture with various levels transglutaminase enzyme polymerization: B51) 0.01, B52) 0.02, B53) 0.03 and B54) 0.04 % (mg/ml).

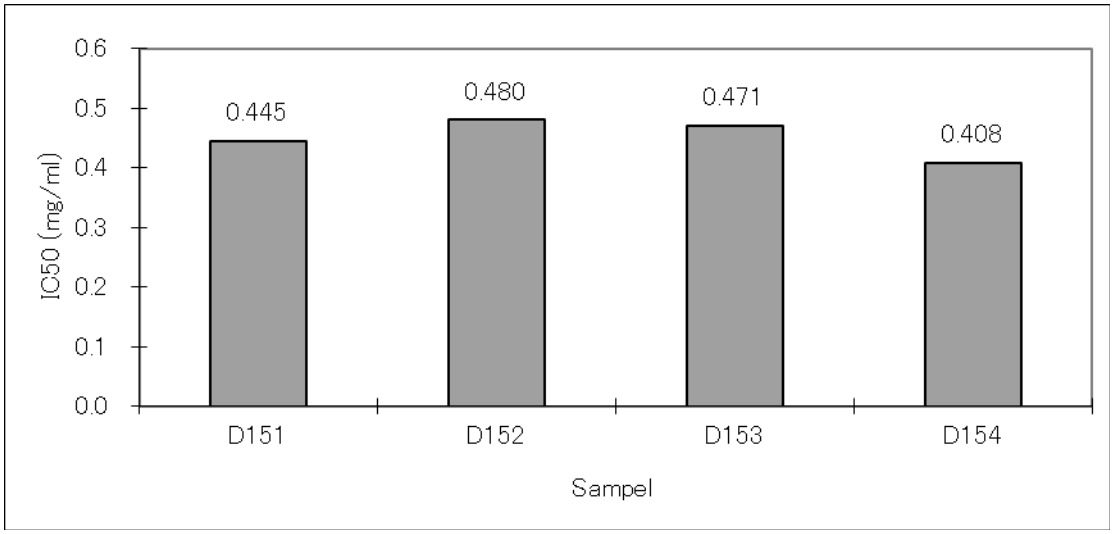

Figure 5. IC_{50} value of meat 95% and tempeh 5% protein mixture with various levels transglutaminase enzyme polymerization: D151) 0.01, D152) 0.02, D153) 0.03 and D154) 0.04 % (mg/ml).

Antioxidant Activity

Antioxidant activity was observed to investigate the influence of different level of tempeh on the protein mixture and their potency for antioxidant (Figure 6).

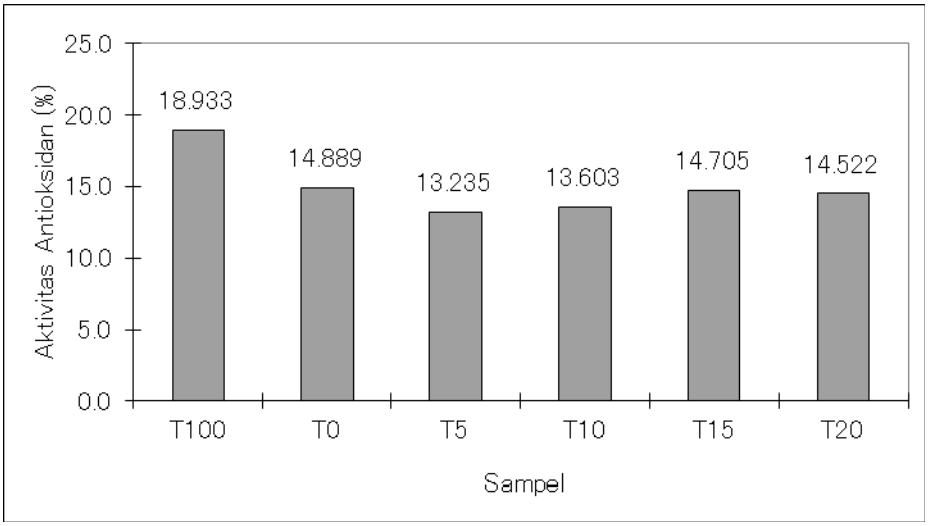

Figure 6. Antioxidant activity of meat-tempeh protein mixture in various levels of tempeh (0, 5, 10, 15 dan 20%) and 100 % tempeh as a control.

The result of the study showed meat substitution by tempeh protein to 10% did not affect the ability of antioxidant activity, while the increasing tempeh level to 15 and 20% showed the increasing of the antioxidant activity. Tempeh protein was hydrolyzed during fermentation and resulted simple oligopeptides which were able to inhibit the oxidation activity. Astawan (2002) reported that tempeh

protein was fermented and microbial activity during fermentation digested some protein into small molecular weight which was capable to inhibit oxidation. The low antioxidant activity in low tempeh concentration (5 and 10%, figure 6) showed the protein tempeh was significantly had the effects on oxidation activity.

Gibbs *et al*. (2004) studied bioactive compounds of soy bean on different treatment, one was fermented and the other one was hydrolyzed. The result showed that the fermented one had more bioactive compounds and had some activity such as: ACE inhibition, anti-thrombotic, surface active and antioxidant. They also found that *glycinin* and *ß-conglycinin,* were the domain protein in soybean biocative compound.

CONCLUSIONS

Protein mixture of meat and tempeh with transglutaminase addition resulted intermolecular protein cross-links. Protein mixture hydrolysate and meat individually showed similar inhibition activity for angiotensin converting enzyme. Antioxidant activity increased when the tempeh level was extended to 15 and 20% in protein mixture.

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