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INFLUENCE VARIATION OF TEMPE GEMBUS (AN INDONESIAN FERMENTED FOOD) ON HOMOCYSTEINE AND MALONDIALDEHYDE OF RATS FED AN ATHEROGENIC DIET

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Abstract

Background and Aims*: Atherosclerosis has become a prominent health problem in Indonesia. Based on food as medicine concept, tempe gembus (a fermented food from Indonesia) is well known having the content of nutrient that influences atherosclerosis parameter. Research aimed to prove the influence of different variation of tempe gembus that was given without additional treatment (X1), with the steam blanching heating (X2), and was added the bromelain enzyme (X3) to the level of serum Homocysteine (Hcy) and Malondialdehyde (MDA)of rat's blood that was given the atherogenic diet.* ***Material and Methods****: The research of posttest randomized controlled group design on 35 Sprague dawley rats were divided into two main groups as follow; they were 2 control groups (called K– and K+) and 3 treatment groups. All of the treatment groups were given tempe gembus of 25 gram/ kg rat body weight.* ***Results:*** *The results showed that group variation of tempe gembus had a lower mean of Hcy and MDA levels than disease group (K+). However, a significant effect of tempe gembus was only decrease in MDA level (ANOVA test p = 0.001). Treatment X1 and X3 had meaningful differences to decrease MDA levels.* ***Conclusion:*** *Tempe gembus variation can decrease the MDA level significantly and decrease the Hcy level however, without statistical significance.*

key words: Tempe gembus, *fermented food, atherogenic diet, homocysteine (Hcy), malondiadehyde (MDA).*

Background and aims

Atherosclerosis is a disease caused by inflammation response on blood vessels and it causes the ossification of the arterial wall in which lead to rigidity and fragility. Atherosclerosis is the beginning process of coronary heart disease and stroke [1]. The deaths caused by cardiovascular diseases, especially the coronary heart disease and stroke, are estimated to increase up to 23.3 million deaths in 2030 with the current prevalence (2013) of coronary heart disease in Indonesia was 0.5% or about 883.447 people [2]. Stroke is a clinical disturbance that is caused by the loss of brain

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function due to the obstruction of blood supply to the brain [1]. According to the data of 2010 in the United States, stroke was the top third of the cause of fatality that reached 700 million people per year [3]. Meanwhile, stroke prevalence in Indonesia reached 12.1 per 1000 people [2].

The obstruction on the blood vessels happens because of the existence of blood solidification or called as a thrombus. The thrombus can be destroyed with thrombolysis mechanism (fibrinolysis) by activating the plasminogen to be proteolytic plasmin enzyme. This plasmin will change the shape of thrombus and limit the thrombosis development by digesting the proteolytic fibrin [4].

A research in 2014 was succeeded to isolate the microbe as producing protease fibrinolytic from *tempe gembus*, which was *Bacillus pumilus* 2.g (AB968524). Pure fibrinolytic enzyme from *B. Pumilus* 2.g was included in a group of protease serine a group of substilin that could degrade chain α and β from fibrinogen faster. Fibrinolytic enzyme was stable between *p*H 5 to *p*H 9 and the temperature was less than 60°C [5]. Hyperfibrinogenemia has important role of thrombosis creation and increase the aterogenesis because of fibrinogen will bind the receptor on thrombosis membrane and start the aggregation creation from thrombosis [6]. Besides hyperfibrinogenemia, the adhesive force and thrombosis aggregation also increase on high homocysteine level [7].

Homocysteine is an intermediate compound that has been produced on methionine metabolism. Homocysteine is considered as factor of independent risk for the cardiovascular disease and not constituent of normal diet. The only one homocysteine source is methionine, which is the essential amino acid that contains of sulfur. Homocysteine increases the production of oxidative stress that will increase the Reactive Oxygen Species (ROS) and another radical superoxide that damages the cardiovascular system [7,8].

The oxidative stress raising will spur on the peroxidase lipid. One of the indicators used for understanding the peroxidase lipid inside body is level of malondialdehyde (MDA). MDA is an aldehyde compound that is created by peroxidase lipid on cell membrane because of reaction to the free radical. The inflammation and thrombosis rising can explain the height of disease and fatality level because of cardiovascular disease [8].

According to food as medicine concept, optimizing the functional food to help fighting down the diseases is important. One of the local functional foods is *tempe gembus* that known as food-stuff resulted from fermented waste-tofu by *Rhizopus oligosporus.* Although the basic material is the waste-tofu, but *tempe gembus* contains of essential fatty acids, such as linoleic acid (21.51%), linolenic acid (1.82%) and oleic unsaturated fatty acid (16.72%). The analysis of nutritional value of *tempe gembus* (dry weight per 100 g of edible portion) was consisted of energy 77.7 kcal, protein 4.07 g, lipid 0.23 g, total carbohydrate 14.25 g, fiber 4.69 g, ash 0.84 g, calcium 159.98 mg, phosphorous 59.69 g, iron 0.48 mg, and water 6 %. The isoflavone concentration in tempe gembus were daidzein 33.1 µg/g and genistein 57.1 µg/g. The level of fiber on *tempe gembus* is three times higher than soya bean tempe. Besides fat acid, *tempe gembus* also contains of isoflavonees that is bioactive that has activities as antioxidant, anti-hemolytic, anti-cholesterol and anti-cancer. Nutrient composition of *tempe gembus* is similar to soya bean *tempe*, especially those that influence the blood lipid profile, protein, polyunsaturated fatty acid (PUFA), fiber and calcium [9].

According to those backgrounds, this research was conducted to compare the different variation of more effective *tempe gembus* to

influence homocysteine and malondialdehyde (MDA) level on *Sprague Dawley* rat as trial animal that was given the atherogenic diet. *Tempe gembus* giving on this research was done through three treatments, such as giving *tempe gembus* without additional treatment, *tempe gembus* with reheating, and *tempe gembus* that was added simple protease enzyme (bromelain). Variety of *tempe gembus* with reheating process is caused by pure fibrinolytic enzyme from *B. Pumilus* 2.g that is not stand for high temperature treatment, whereas public's habit consuming gembus after being cooked [5]. Adding the simple protease enzyme which is bromelain hopefully can hydrolyze the polypeptide so it will result the decomposition product (hydrolysate) as simple compound, such as peptide and amino acid that are easier to absorb and activities of fibrinolytic bromelain from pineapple is able to increase the plasminogen conversion becomes plasmin so it can increase the fibrin degradation. The given dose on each treatment arm was 25 gram *tempe gembus* per kg of rat's weight for 28 days.

Material and methods

Research design

Tempe gembus was obtaines from the of local soybean seller that has been directly proceeded by the workers. This process is under researchers' control. The trial animals were strain *Sprague dawley* white rats (*Rattus Norwegicus*) in the age of 8-12 weeks, male, weight 150-200 grams. The materials of rat diet were shown in Table 1.

The research design was *True Experimental: posttest randomized controlled group design*. The number of samples were decided based on Federrer formula. There were 35 rats randomly divided in 5 groups. All care, treatment, and blood sampling was done in experimental animal laboratory of Medical Faculty, Diponegoro University. Homocysteine (Hcy) was checked with Hcy Elisa kit, a brand of *Bioassay Technology Laboratory*. Meanwhile, to check its absorbance, ELISA reader brand BIO-RAD 680 XR with the wave length 450 nm was used. It was conducted in Integrated Research and Testing Laboratory (*Laboratorium Penelitian dan Pengujian Terpadu* - LPPT), Gadjah Mada University, Yogyakarta. The malondialdehyde (MDA) was checked with TBARS (*thiobarbituric acid reactive substance*) method. To check its absorbance, it was measured by spectrophotometer with the wave length 532 nm that was conducted in Laboratory of Biochemical, Medical Faculty, Diponegoro University, Semarang.

Table 1. Rat Diet.

Standard Diet	Atherogenic Diet
Hi Provite 594 100%	Hi Provite 594 55%
	Flour 28%
Water at sufficiency	Yolk 6%
	Cholic acid 0.2%
	Pig Oil 10%
	Coconut Oil 1%
	Water at sufficiency

Source: Nur Kholis, Veni. 2011 [4]

Preparation period

The preparation period was carried out 3 days to acclimatize the rats to the experimental condition, by ad libitum consumption of standard food (merk Hi Provite-594), followed by pre-experimental period of 28 days. The rats were randomly divided into five groups. Those which was given purified diet based on Hi Provite-594, was laterrated as negative control (K -) and four groups were given atherogenic diet (K +, X1, X2, X3). Atherogenic diet composition was following the previous studies (Table 1).

Experimental period

After the pre-experimental period, the experimental period was conducted in 4 weeks.

Then, the atherogenic diet was stopped. Two control groups (K - & K +) were continued with a standard diet for 28 days. Three treatment groups (X1, X2, X3) were given tempe gembus of 25 gram/ kg rat body weight. *Tempe gembus* was obtained from local waste-soybean that was processed by the worker and controlled by researchers. The three treatment groups were divided into three types of giving treatment: (1) the rat group that was given the standard diet + *tempe gembus* without any additional treatment, it was called as X1; (2) the rat group that was given the standard diet + *tempe gembus* with reheating method of steam blanching for 5 minutes, it was called as X2; and (3) the rat group that was given the standard diet + *tempe gembus* that was added by the bromelain enzyme 25 ppm, it was called as X3. The diet was made with the same calories and protein in each group.

Data analysis

The data resulted from this research was tested for normality using *Shapiro-Wilk* test. Since data was distributed normally to test the statistical differences between groups, one-way Anova test was udes followed by Post Hoc analysis if the result showed some influences to the confidence interval 95% ($p < 0.05$). Pearson correlation coefficient was used to assess the correlation' strengths. The data analysis was performed using SPSS program 16.00 for Windows.

Ethical clearance

Request ethical clearance has been reviewed and approved by the ethical clearance with the certificate number 1034 / EC / FK-RSDK / X11 / 2016.

Results

Body Weight

It was known that the mean of rat's body weight that was given the atherogenic diet was bigger than other rat's groups. The mean of body weight of treatment group was smaller than control group, it showed that group that was given *tempe gembus* had better ability on pushing out the body weight gain. The differences of body weight's mean of before and after treatment showed significant differences. (Table 2).

Table 2. Body weight before and after treatment.

Group	Body weight (gram)			p
	Before (Mean + SD)	After (Mean + SD)	Δ	
K(-)	208.57 ± 20.58	236.43 ± 21.86	27.86	0.001
K(+)	218.00 ± 26.54	250.71 ± 39.96	32.71	0.006
X1	244.71 ± 22.61	247.89 ± 23.19	3.18	0.473
X2	227.50 ± 26.19	241.89 ± 27.18	14.39	0.045
X3	234.85 ± 37.77	244.80 ± 36.60	9.95	0.041

Results are presented as means and standard deviations. P value was calculated using t-student test.
Table legend:
K(-) = Negative control group, without atherogenic diet (standard diet).
K(+) = Positive control group, the rats were given the atherogenic diet for 4 weeks without *tempe gembus* treatment.
X1 = Group with atherogenic diet first treatment that was given *tempe gembus* 25 gram/ kg body weight without additional treatment.
X2 = Group with atherogenic diet second treatment that was given *tempe gembus* 25 gram/ kg body weight with reheating using steam blanching method for 5 minutes.
X3 = Group with atherogenic diet third treatment that was given *tempe gembus* 25 gram/kg body weight + bromelain enzyme 25 ppm.

Homocysteine (Hcy)

It was resulted that group X3 given *tempe gembus* with additional bromelain enzyme had the lowest mean value of homocysteine level. Group that was given only atherogenic diet without treatment (K+) had the highest mean value of homocysteine. There were no significant differences of homocysteine levels between the research groups. (Table 3).

Table 3. Mean Levels of Serum Homocysteine after Treatment

Group	Mean ± SD Level Hcy (µmol/mL)	p
K (-)	3.98 ± 0.58	0.751
K (+)	4.30 ± 0.37	
X1	4.19 ± 1.15	
X2	4.28 ± 1.04	
X3	3.73 ± 1.15	

Differences were analyzed using One-way ANOVA test.

Malondialdehyde (MDA)

It was known that the mean of positive group had the highest level of MDA. The one-way Anova test showed that at least there were not two groups that had different meaningful mean of MDA level. (Table 4).

Table 4. Mean Levels of Serum MDA after Treatment.

Group	Mean ± SD Levels MDA (nmol/mL)	p
K (-)	16.37 ± 0.69	0.001
K (+)	21.91 ± 2.71	
X1	15.49 ± 1.53	
X2	20.50 ± 3.09	
X3	17.18 ± 1.16	

Differences were analyzed using One way ANOVA.

So, we followed by Games-Howell's multiple comparison test (see Table 5) Furthermore, it was done the *post hoc* test to find out between the groups that had meaningful differences.

Table 5. Multiple Comparison Test Levels of Serum MDA.

	K(-)	K(+)	X1	X2	X3
K (-)	-	0.008*	0.658	0.063	0.542
K (+)	0.008*	-	0.002*	0.889	0.017*
X1	0.658	0.002	-	0.026*	0.211
X2	0.063	0.889	0.026*	-	0.152
X3	0.542	0.017*	0.211	0.152	-

Games-Howell's multiple comparison test $p < 0.05$*

There were no statistical significant differences of MDA levels between treatment group and negative control group (K-). On the first and third treatment (X1 and X3) had meaningful differences to decrease the MDA levels.

Correlation of Homocysteine and Malondialdehyde

According to Table 6, there was a correlation between homocysteine and MDA levels (r= 0.086) although statistically it was meaningless (p= 0.622).

Table 6. Correlation of Homocysteine and Malondialdehyde.

	MDA
Hcy	r = 0.086
	p = 0.622
	n = 35

Analyzed by Pearson Correlation Test

Discussions

According to Table 2, it was resulted that the differences of body weight mean of before and after treatment showed significant differences (p=0.001). The body weight's mean of treatment group was lower than control group, it showed that group with *tempe gembus* treatment had

better ability on decreasing the body weight gain. It was related to *tempe gembus* with the fiber level three times bigger than soy *tempe*. Food fiber has ability to restrain water and can form strong liquid in digestive track. By this ability, the dissolved fiber is able to postpone the discharge capacity from the flank, obstruct the mixture of digestive track's substances with the digestive enzymes, with the result that decreasing the absorption of nutritious in proximal. This mechanism causes the decreasing absorption of fat acid by soluble fiber. Besides, food contains relatively high fiber will give fullness so it decreases consumption [11]. Besides, fiber of *tempe gembus* has isoflavones as the research of Zhang et al., stated that isoflavones from soybean caused the body weight decreasing through increasing the level of cholecystokinin digestive hormone (CCK) as stimulator on decreasing the appetite [12].

Based on Table 3, the group that was given *tempe gembus* by adding the bromelain enzyme (X3) had lower mean value of homocysteine due to higher antioxidant that was produced by hydrolysate protein that was able to protect the endothelium from oxidative stress, so endothelium would produce *Nitric oxide* (NO) optimally. NO would detox homocysteine, which was bind to homocysteine and form S-NO-Hcy that was harmless compound and had a role as inhibitor thrombosis [13]. Fibrinolytic bromelain activities from pineapple are also able to increase the conversion of plasminogen becomes plasmin so it can increase the fibrin degradation [10].

Groups that were given only atherogenic diet without treatment (K+) had the highest mean level of homocysteine. The atherogenic diet increased the hyperlipidemia creation. It was primarily related to the form of oxidized Hcy, which was *thiolactone* that could be complex bind with LDL. LDL-*Hcythiolactone* was saved in a form of *thioco* and became *thioretinamide* that created proliferation and fibrosis from smooth muscle cells. As long as conversion from *thioco* became *thioretinamide* it was resulted plentiful ROS that caused endotel dysfunction. Hcy-*tiolactone* is a reactive thioester, reacted to LDL and forms the foam cells that have role in atherosclerosis [14]. *Thiolactone* can be degraded by *thiolactonase (paraoxonase)* enzyme which its level is correlated by HDL level. The related risk to the increasing of plasma homocysteine can decrease by the increasing of HDL cholesterol or Apo A1 so *Hcythiolactone* can decrease [15].

It also could explain one of the reasons of one way ANOVA statistic result p = 0.751, which meant there were no statistically significant differences between the homocysteine level of research groups. ELISA kit, which was used in this research, could not possibly measure oxidated Hcy, i.e. thiolactone that had been bind to LDL became LDL-Hcythiolactonnne. This kit could count the total of homocysteine. Total of homocysteine contained thiol-homocysteine, disulfide homocysteine, and cysteine homocystein [16].

The meaningless result is more feasible because of the homocysteine decreasing is more related to vitamin B6, B12, and folic acid than isoflavone [17-19]. Hcy was degraded by two ways, namely remethylation that created methionine and transsulfuration that produced cysteine. Hcy was conversed to cystathionine through transsulfuration pathway that was depended on vitamin B6 with *Cystathionine β Synthase* (CBS). Then, cystathionine was conversed to the cysteine and later it was excreted through urine. Remethylation happens on a part of cells with the rock 5-*methylene-tetrahydrofolate reductase* (MTHFR) with folic acid as co-substrate and methionine synthase and cobalamin (B12) as cofactor [20]. Based on

Selhub research as quoted by Welch, that found 2/3 of the researched hyperhomocysteinemia cases had one or more level of vitamin B that were inadequate [21]. Kang also found that there was reverse relationship between deficiency of vitamin B12 and accumulation of homocysteine and hyperhomocysteinemia on the sufferers of folic acid deficiency [22].

Based on Table 4, mean of control groups that were given atherogenic diet without *tempe gembus* (K+) had the highest MDA level (21.91±2.71 nmol/mL). Giving the high fat atherogenic diet could induce obesity, hyperglycemia, increasing the level of blood lipid and chronic inflammation that caused increasing the MDA level [23]. Based on Table 5, it is understood that MDA of first treatment group (X1) and third treatment group (X3) have difference meaning compared to group that is supposed to be sick (K+). Whereas second treatment group does not have statistical significant differences ($p > 0.05$) when compared to positive control group, supported by value of mean level of MDA X2 (20.50±3.09 nmol/mL) that almost closes to the mean of positive control group. It is possibly because of isoflavone inside *tempe gembus*, especially kind of daidzein and genistein are susceptible to the heat although the reheating that is done in this research is a kind of minimum reheating [24].

On the third treatment (X3), it had significant difference to decrease the MDA level due to the fragmentation *tempe gembus* protein by bromelain enzyme, so it formed digestible protein hydrolysate. Protein hydrolysate generally is used for flavor-lasing, but now its usage starts shifting becomes functional food product. Some researchers have reported that protein hydrolysate is more soluble protein source in human's body because of its simpler chemical structure (namely dimer or oligomer amino acid). Other researches show that the composition of amino acid in protein hydrolysate also has strong enough antioxidant activities [25].

According to the research of Borra et al, giving antioxidant, especially isoflavone is able to prevent oxidase lipid by free radical so it decreases the MDA formation of rat's liver. It is also related to the activities of SOD enzyme in the liver. Isoflavone is also able to defend the SOD enzyme activities probably because of isoflavone genistein's role to induce genetics that take charge of SOD enzyme synthesis [26]. Besides, isoflavone helps the task of superoxide dismutase in separating the free radical. Isoflavone works by rendering its one electron to the radical compound so its radical compound changes becoming non-radical compound or un-dangerous compound to the cells. Thus, isoflavone helps the task of superoxide dismutase so the enzyme level of superoxide dismutase in the cells can be defensible. As antioxidant, isoflavone compound can eliminate free radical and prevent further chain reaction to the cell membrane components so it reduces MDA formation as final product [27].

According to Pearson test (Table 6), there are positive correlations between the level of homocysteine and MDA ($r = 0.086$) although not statistically significant ($p = 0.622$). Hyperhomo-cysteinemia increases the oxidative stress with auto oxidation homocysteine that results disulfide oxidase, decreases the intracellular antioxidant (GPx) and cytotoxic directly to the endothelium cell that stimulates ROS formation [28,29].

The MDA increasing is possibly caused by the existence of other peroxidation lipid due hyperhomocysteinemia. According to a research by Rahayu, there was not only homocysteine that could cause peroxidation lipid because of the existence of other condition that could cause oxidative stress, such as hyperglycemia that

caused autooxidising glucose that faster the free radical forming [30]. Besides hyperglycemia, obesity also can trigger the oxidative stress. Adipose tissue in addition to being a reserved lipid but also has a role to produce bioactive substances called adipokine. Adipokine can induce increased production of Reactive Oxygen Species (ROS). The increasing ROS can connect to the cells damage, include membrane cell oxidation and protein that is conjugated to the disturbance of cellular redox homeostasis. This reaction can cause lipid peroxidation [31]. It was supported with a research by Yesibursa on an obese person, it could happen the increasing of endogen lipid peroxidation by found higher level of MDA blood compared to someone who had normal body mass index [32].

Conclusions

Variation of giving *tempe gembus* can decrease the MDA level significantly and decrease the homocysteine level although without statistical significance in atherosclerosis rats. Groups that are given *tempe gembus* without additional treatment (X1) and that are given bromelain (X3) meaningfully have lower MDA level compared to the groups that are given *tempe gembus* by reheating process. There are positive correlations between homocysteine and malodialdehyde although statistically it is meaningless.

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REFERENCES

1. Nelms M, Sucher KP, Lacey K, Roth SL. Diseases of the cardiovascular system. *Nutrition Therapy and Pathophysiology* 2: 283-285, 2010.

2. Badan Litbang Kesehatan Departemen Kesehatan RI. *National Institute of Health Research and Development Indonesia. National Basic Health Research (Riskesdas)*. Jakarta. 2013.

3. World Health Organization. *World health organization report*. World Health Organization, Geneva, Switzerland. pp 9-31, 2014.

4. Kholis N, Yanti VAD. Therapy of endogenous thrombolysis with dietary natto based on inferior local beans in atherogenic rat (Rattus norvegicus) model. *Jurnal Teknologi Pertanian* 12: 8-15, 2011.

5. Afifah DN, Sulchan M, Syah D, Yanti, Suhartono MT, Kim JH. Purification and characterization of a fibrinolytic enzyme from Bacillus pumilus 2.g isolated from Gembus, an Indonesian fermented food. *Prev Nutr Food Sci* 19: 213-219, 2014.

6. Ariëns RAS. Elevated fibrinogen causes thrombosis. *Blood* 117: 4687-4688, 2011.

7. Eldibany MM, Caprini JA. Hyperhomo-cysteinemia and thrombosis: an overview. *Arch Pathol Lab Med* 131: 872–884, 2007.

8. Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential marker of oxidative stress in stroke. *Free Radic Biol Med* 39: 841-852, 2005.

9. Sulchan M, Rukmi I. Effect of tempe gembus on cholesterol profile in hyperlipidemic rats. *Med J Indonesia* 16: 205-211, 2007.

10. Ovianto E. Fibrinolytic Activity Test Earthworm (Lumbricus rubellus) In vitro and Evaluation of Atherosclerosis Effect on Multiple Parameters in Long Tailed Monkey (Macaca fascicularis) [Scientific papers]. Bogor: Faculty of Agricultural Technology Bogor Agricultural University, 2004.

11. Ludwig D, Pereira M, Kroenke C et al. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA* 282: 1539-1546, 1999.

12. Zhang Y, Na X, Zhang L, Li L, Zhao X, Cui H. Isoflavonee reduces body weight by decreasing food intake in ovariectomized rats. *Ann Nutr Metab* 54: 163–170, 2009.

13. Purnama RS. Effect of fruit seed extract Pinang (Areca catechu) to decrease total homocysteine levels of plasma at the white aat (Rattus novergicus strain Wistar) Model of Atherosclerosis [Thesis]. Malang: Muhammadiyah University. 2013.

14. Yadav AS, Bhagwat VR, Rathod IM. Relation of plasma homocysteine with lipid profile parameters in ischemic hearth disease. *Indian J Clin Biochem* 21: 106–110, 2006.

15. Daly C, Fitzgerald AP, O'Callaghan P, Collins P, Cooney MT, Graham IM. Homocysteine increases the risk associated with hyperlipidaemia. *Eur J Cardiovasc Prev Rehabil* 16: 150-5, 2009.

16. NygaÊrd O, Vollset SE, Refsum H, Brattstro L, Ueland PM. Total homocysteine and cardiovascular disease. *J Intern Med* 246: 425-454, 1999.

17. Jenkins DJ, Kendall CW, Jackson CJ et al. Effects of high- and low-isoflavonee soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am J Clin Nutr* 76: 365–372, 2002.

18. Mennen LI, de Courcy GP, Guilland JC et al. Homocysteine, cardiovascular disease risk factors, and habitual diet in the French supplementation with antioxidant vitamins and minerals study. *Am J Clin Nutr* 76: 1279–1289, 2002.

19. Jensen MK, Koh-Banerjee P, Franz M, Sampson L, Grønbæk M, Rimm EB. Whole grains, bran, and germ in relation to homocysteine and markers of glycemic control, lipids, and inflammation. *Am J Clin Nutr* 83: 275–283, 2006.

20. Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary atherosclerosis. *J Am Coll Cardiol* 27: 517-527, 1996.

21. Welch GN, Loscalzo J. Homocysteine and altherothrombosis. *N Engl J Med* 338: 1042-1050, 1998.

22. Kang SS, Wong PW, Norusis M. Homocysteine due to folate deficiency. *Metabolism* 36: 458-462, 1987.

23. Winarsih H. Natural antioxidants and free radicals. *Kanisius. Moulds V. Yogjakarta: Kanisius*; pp 26-30, 2011.

24. Huihua H, Hanhua L, Kin-Chor K. Effect of thermal processing on genistein, daidzein and glycitein content in soymilk. *J Sci Food Agric* 86: 1110–1114, 2006.

25. Kusumaningtyas E, Widiastuti R, Kusumaningrum HD, Suhartono MT. Antibacterial and antioxidant activities hydrolyzate goat milk protein hydrolysis results with bromelain crude extract. *J Teknologi dan Industri Pangan* 26: 179-188, 2015.

26. Borras C, Gambini J, Goez-Cabrera MC et al. Genistein, a soy isoflavonee, up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NFκappaB. *FASEB J* 20: 1476-1481, 2006.

27. Lee J, Renita M, Fioritto RJ, ST.Martin SK, Schwartz SJ, Vodovotz Y. Isoflavonee characterization and antioxidant activity of Ohio soybeans. *J Agric Food Chem* 52: 2647-2651, 2004.

28. Arab S, Khaza'ai H, Hambali Z, Ahmad Z. Homocysteine and malondialdehyde (MDA) levels associated with the occurrence of cardiovascular disease (CVD) in chronic renal failure (CRF) in Malaysia. *Global Journal of Health Science* 3: 119-127, 2011.

29. Moselhy SS, Demerdash SH. Plasma homocysteine and oxidative stress in cardiovascular disease. *Dis Markers* 19: 27-31, 2004.

30. Rahayu M. The influence of folate supplementation on serum homocysteine and lipid profil levels on diabetic rats [*Thesis*]. Semarang: Biomedical Sciences and Doctor of Clinical Pathology, Diponegoro University. 2011.

31. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, et al. Inflammation, oxidative stress, and obesity. *Int J Mol Sci* 12: 3117–3132, 2011.

32. Yesibursa D. Epidemiology of obesity in the Asia-Pacific region. *In The Asia Pacific Perspective: Redefining Obesity and Its Treatment*. Japan: WHO Western Pacific Region, International Association for Study of Obesity. p 9-13, 2000.