International Food Research Journal 20(6): 3047-3052 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

The effect of peptide (Asp – Glu) synthetic base on sterilized fermented soymilk on lipid profile of Sprague Dawley rats

[1*]Fatma, Z. N., [1]Bety Wahyu, H. and [2]Waluyo

[1]Health and Nutrition Departement, Medicine Faculty, Universitas Gadjah Mada,
[2] Nutrition Department, Health Polytechnic Yogyakarta

Article history

Received: 4 April 2013
Received in revised form: 9 July 2013
Accepted: 12 July 2013

Keywords

Peptide
Asp-Glu
Soyghurt sterilization
Hypercholesterolemia
Lipid profile

Abstract

Death due to coronary heart disease is caused by coronary artery plugging known as atherosclerosis. High blood cholesterol concentration is the risk factor of atherosclerosis. Soy protein in animal and human studies has been shown to have effect in lowering blood cholesterol concentration. Soymilk made from soy is presumed to possessed hypocholesterolemic effect due to the soy protein biogenic peptide activity. Asp-Glu peptide is a sterilized soymilk fermentated peptide which is known to bind cholesterol in vitro, making it a potential candidate for lowering cholesterol in vivo. In this study, the effects of peptide (Asp-Glu) on rat lipid profile were determined using 24 three months old Sprague dawley male rats. In the study, standard diet adaptation was given in the first week; for the second week high cholesterol diet was given; and in the last three week rats were divided into four groups consisting of four different diets: K(-) (diet with no intervention); K(+)(diet with ezetimibe); SKFS (diet with sterilized fermented soymilk 1 ml) and P1 (diet with Asp-Glu peptide). The results obtained showed that after 3 weeks of treatment with total cholesterol, triglycerides and LDL concentration decreased significantly ($p < 0,05$), whereas HDL concentration increased significantly ($p < 0,05$) and high concentration of cholesterol was present in the rats feces. These observations suggested that peptide Asp-Glu has the ability to reduce blood cholesterol concentration in hypercholesterolemia rats.

Introduction

Cardiovascular disease is one of the major causes of death in late 20th century, accounting for about 30% of total world mortality. In Indonesia, death caused by cardiovascular diseases was 26.3% in 2001 and death due to heart disease at hospitals in 2005 was 16.7% (Arie, 2007). Coronary heart disease is caused by coronary arteries obstruction due to fatty buildup (atherosclerosis) since young age. Hence, high blood cholesterol levels is reported as the risk factor for atherosclerosis (Berenson and Srinivasan, 2003; Mahan and Stump, 2004).

Soy proteins in various animal and humans studies have been proven to reduce blood cholesterol levels and daily intake of soy protein and soy-based food products have been associated with lower incidence of coronary heart disease (Bakhit *et al.*, 1994; Greaves *et al.*, 2000). Soy milk processed from soy is expected to have hypocholesterolemic effect (Nisa' *et al.*, 2006) and the fermented soy milk and soy milk sterilization fermentation have been proven capable of binding to cholesterol *in vitro* and capable of lowering cholesterol *in vivo* in experimental animals.

Cholesterol reduction mechanism by soy proteins is uncertain, but it is believed that decreasing cholesterol absorption due to cholesterol inhibition and bile acids by components found in soy milk such as saponins, isoflavones, phytic acid and peptide fraction or acidic amino (Potter, 1995). Peptide as one of soya milk components associated with decreasing the incidence of heart disease through an antihypertensive effect by inhibiting ACE and therefore reduces the blood pressure (Masuda *et al.*, 1996; Maeno *et al.*, 1996; Erdmann *et al.*, 2008). Studies of peptides that have hypocholesterolemic effect has not been done, as previous studies are more focus on research to prove the hypocholesterolemic effects of undigested soy protein in the gastrointestinal tract.

Identification of the types of peptides that have hypocholesterolemic effect from sterile fermented soy milk has been reported (Nisa', 2008). There are three fractions of peptides from sterilized fermented soy milk, fractions I: aspartic acid and glutamic acid; fraction II: aspargin, serine, glutamate, threonine and glycine, alanain, arginine and tyrosine; fraction III: tryptophan, phenylalanine, isoleucine, leucine, and lysine. Of the three fractions peptides, fraction I peptide (Asp-Glu) have the highest *in vitro*

*Corresponding author.
Email: *bety.hapsari@gmail.com*
Tel: +6281328425825

hypocholesterolemic effect with observations using cholesterol binding assay. However, specific studies on hypocholesterolemic effect of fermented soy milk sterile fraction I peptide *in vivo* has not been done. Thus, this study was carried out to determine the role of the peptide (Asp-Glu) *in vivo*.

Materials and Methods

The twenty four of Sprague dawley male rats used in this study for 5 weeks were treated as follows: in the first week, the rats were treated with standard diet adaptation; for the second week the rats were fed with high cholesterol diet. In the last third week the rats were divided into four groups and were given different diet as follows: K(-) (diet with no intervention); K(+)(diet with ezetimibe); SKFS (diet with sterilized fermented soymilk 1 ml) and P1 (diet with Asp-Glu peptide). Lipid profiles were analyzed in the 1st, 2nd and 5th weeks, whereas the lipid and digest profile were analyzed in the 3rd week.

During the study, the rats were given feed and water in ad libitum, while dietary intervention was done with force feeding of up to 25 g/day and the remaining feed were weighed on the following days, while the rats were weighed at the beginning study and once a week during the study. Hypercholesterolemia and standards feed are based on the standard formulation of AIN-9312. To make hypercholesterolemia feed, 180 g beef tallow / kg diet containing cholesterol 144.65 mg/100 g and 98.04% fat was added, to produce 260 mg cholesterol in 1000 grams of feed (Nurhayati *et al.*, 2003). Peptide used in the study (aspartic acid and glutamic acid) is a synthetic peptide of amino acid isolation SIGMA 22k9804 (LAA-21) was obtained from LPPT Universitas Gadjah Mada. Soy milk was made following the modified method of Pusbangtepa IPB to produced fermented soy milk using *Lactobacillus bulgaricus* and *Streptococcus thermophillus* (Koswara, 2003). Sterilization was done by autoclaving at 121°C for 15 minutes.

Total cholesterol levels in Sprague Dawley rat serum specimens were determined by the Chod-PAP15 method. The specimen was mixed with the reagent and distilled water into a test tube, then incubated for 20 minutes at 20-25°C or 10 minutes at 37°C. The blank which contains reagent absorbance was read at λ 500 nm or 546 nm filters with a spectrophotometer Hg Microlab 300 from Merck. Triglyceride levels were determined by the GPO-PAP16 method using Triglycerides FS as the reagent for analysis. HDL level was determined by the Chod-PAP17 method, in which the specimens were reacted with Phosphotungstic acid (PTA) and magnesium chloride to precipitate LDL and VLDL. The resulting supernatant was mixed with Cholesterol FS reagent and its absorbance read at λ 500 nm or 546 nm filter Hg with a spectrophotometer Microlab 300 from Merck to determine levels of HDL. Measurements of LDL were carried out using the formula = total cholesterol levels - HDL levels - Triglycerides levels / 5. Weight digest was measured by using analytic scales, pH digest was measured by pH meter, gravimetric method was used to measure water digest levels and cholesterol digest levels was measured by Liebermann-Burchard method (Plummer,1977). The specimens was reacted with Liebermann-Burchard reagent, then it absorbance with a spectrophotometer and compared with a standard curve.

One way ANOVA tests was used to determine differences between the treatment groups, followed by the continued Post Hock Tuckey statistical tests. T-test was used to determine the significance alteration of the lipid profile for each group at different times.

Results and Discussion

All the different groups of rats has mean intake of feed of 24.58 ± 0.042 g with mean compliance of 97.23% for feed intake. Rats body weight have no real difference ($p > 0.05$) in each week during the study and the percentage of rat's weight increased for about 11.92% from the beginning to the end study.

Total cholesterol (KT)

Total cholesterol levels of rats after 3 weeks treatment showed a significant different between groups ($p < 0.05$). Decreasing total cholesterol levels in descending order are as follows: K (+) 89.26%, P1 (56.70%), SKFS (52.54%) and K (-) (-2.07%), in which negative values indicate an increase in total cholesterol level. There was no real difference between Group P1 and group SKFS, which showed the same levels of reduction of cholesterol.

Nisa *et al*. (2006) showed that sterile fermented soy milk has a cholesterol lowering effect, the decreased cholesterol levels was presumed to be derived from fermented metabolites in the form of peptide fragments. This is in agreement with the results of this study that decrease cholesterol levels has no real difference between sterilized fermented soy milk with Asp-Glu peptide group, although it is possible that the sterile fermented soy milk may contain many other components that also have hypocholesterolemic effects. However, from the results of this study we can conclude that the peptide Asp-Glu is capable of lowering cholesterol levels *in vitro* are also capable of lowering cholesterol levels *in vivo* in experimental animals.

Isolated peptide from soy protein has direct relationship to hypocholesterolemic effect. The amino acids mixture diet in animals study is evidently able to reduce cholesterol levels (Erdmann *et al.*, 2008). In *in vitro* assays, SAPH peptides from soy protein incubated with pepsin were able to lower cellular cholesterol solubility to 4%. From *In vivo* study using mice, the results shows that peptide from SAPH were able to lower LDL and VLDL 45% higher than placebo with high cholesterol feed, indicating that the peptide from SAPH have capability to reduce cholesterol level (Zhong *et al.*, 2007a).

Decreased cholesterol levels by peptide was believed to be related with solubility of the peptide in water (hydrophobic peptides) that are capable to binding bile in digestive tract and increases the cholesterol release from the body (Erdmann *et al.*, 2008; Zhong *et al.*, 2007a). Based on vitro studies, alkalase hydrolysates, isolated peptide from soy protein shown result that increasing cellular cholesterol absorption was equally the same with increasing hydrophobic peptide value, indicating that hydrophobic peptides have more effectiveness in cholesterol absorption. However, the hypocholesterolemic effect of peptide does not only depend on solubility in water but also the constituent of the amino acids composition.

Triglycerides (TG)

Triglyceride levels after 3 weeks of treatment also showed a significant decrease ($p < 0.05$) and significantly different between the treatment groups ($p < 0.05$). Sequential decrease in triglyceride levels from the highest were K (+) (46.83%); P1 (27.01%), SKFS (13.31%) and K (-) (-0.94%, negative values indicating no lowering effect). In addition, there were obvious difference in the decreasing levels of triglyceride between peptide and sterilized fermented soybean milk.

Blood triglyceride level changes along with diet changes, it indicates dynamic balance of the body's metabolism and directly showing changes in reserve energy (Chen *et al.*, 2003). Though the mechanism of reduction in the triglyceride level is not known, it was assumed that decreasing in serum cholesterol levels will be followed by a decrease in triglyceride levels. Decrease triglyceride levels is caused by the imbalance of certain amino acids such as lysine, which can lower plasma triglyceride levels, followed by decreased LDL hearts synthesis and thus reducing the lipoprotein synthesis in the liver.

Our data are in agreement with previous study, in which low ratio of lysine and arginine in soy

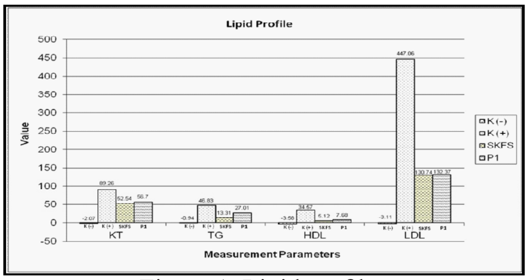

Figure 1. Lipid profile

protein will decrease insulin secretion and increase glucagon secretion (Potter, 1995). High ratio of insulin-glucagon is associated with increased risk of coronary heart disease, which are related to their lipogenesis stimulation. Glucagon is a hormone that works against insulin, its mechanism causing glycogenolysis thereby inhibiting lipogenesis, leading to possible reduction in the levels of trigliserida23. Increased triglycerides level is usually related with increased of VLDL, LDL and decreased of HDL (Murray *et al*., 2003). The absorption of VLDL, LDL and HDL are in unity micelles form and chilomikron (Astawan *et al.*, 2005).

HDL

HDL levels increased significantly and were different in each group ($p < 0.05$) with sequentially increase of HDL as follows: K (-) (-3.58%), SKFS (5.12%), P1 (7.68%) and K (+) (34.57%). The increased in the HDL percentage levels are shown in Figure 1. The largest content of HDL was protein, therefore HDL are able to collect excess cholesterol from tissues to liver. The soy milk contained 15.27% protein, whereas fermented soy milk has higher protein content than soy milk (18.26 to 22.78%) (Yusmarini and Efendi, 2003). The results obtained in this study conforms to those reported in previous study (Liu *et al*., 2006), fermented soy milk with kefir tested in animals study (hamsters) showed decreasing levels of serum cholesterol and increasing HDL levels which were associated with high steroids content in animal feces.

In another study, Apo Al-mimetic peptides synthesized from D-amino acids can reduce the causes of atherosclerotic lesions by 79% through the protection mechanisms of LDL oxidation by HDL (Navab *et al*., 2002). Hence the increased in HDL levels after the treatments has potential benefits in reducing atherosclerosis risk and coronary heart disease. According to Kahl's (1999), increase of 1 HDL unit can reduce coronary heart disease risk to 2-3%.

LDL

The result of the LDL calculation showed a distinct

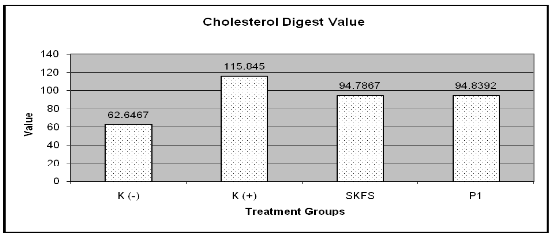

Figure 2. Cholesterol digest value

decrease ($p < 0.05$) and real differences of each group ($p < 0.05$). The LDL sequentially decreased were in the order of: K (+) (447.06%), P1 (132.37%), SKFS (130.74%) and least was for K (-) of (-3.11%).

LDL is the largest lipoprotein content of fat (Heslet, 1997). From the results obtained in this study, we can conclude that decreasing total cholesterol and triglycerides levels will be followed by LDL decreased level. Total cholesterol, triglycerides, and LDL have unidirectional disposition of the rise and fall, because 65% cholesterol is in LDL form. These results were supported by previous studies, which show that fermented soy milk diet supplement on animals study group can significantly reduce levels of total cholesterol, VLDL and LDL compared to group without supplementation. This were further corroborated by the observation on availability of bile levels in the feces. Bile acids are end products of cholesterol metabolism. High bile acids excretion will trigger more cholesterol conversion to bile acids for dissolve fat, resulting in total cholesterol serum and LDL decreased. Another study showed soy protein extract by ethanol in single dose was able to lowering blood cholesterol and LDL concentrations (Lovati, 1992).

HDL and LDL has opposite function, LDL is atherogenic because it carries high amount cholesterol in causing of calcification in coronary arteries. Otherwise HDL contain lots of protein, that have functions in the transport of cholesterol from tissues to liver preventing calcification (Heslet, 1997; Anwar, 2003; Murray *et al*., 2003).

Digest profile

The results from the measurements of weight digest, water content and pH digest showed no real difference ($p > 0.05$) between all treatment groups, but on cholesterol digest measurement, there are significant difference between treatment groups ($p < 0.05$) with cholesterol levels sequentially from the highest were: K (+) (115.84 mg / dL), P1 (94.83 mg / dL); SKFS (94.78 mg / dL), and the last K (-) (62.65 mg / dL). Cholesterol digest analysis was used to determine how much cholesterol can be bound and secreted through the feces by the active component in each treatment group. Results analysis for the digest cholesterol is shown in Figure 2.

In cecum weight measurements, it was found that sequentially mass cecum from the highest were: K (+), P1, SKFS and K (-) (Figure 2). A previous review suggested that the mechanisms of cholesterol binding by peptide in the gut are possibly the same as fiber, as in the binding mechanism by undigested soy protein peptides in gastrointestinal tract. The increased in the cecum weight were due to water binding and other organic compounds, such as fat, cholesterol, bile acids, vitamins, and mineral (Wolever *et al*., 1997).

The measurements result of digest water content by gravimetric method showed no significant difference among the group of rats tested. Digest water content consecutively from the highest were K (+), P1, SKFS and K (-). The digest water content is a reflection of the water-binding capacity of the dietary components, especially polysaccharides, as high-fiber diet can raise digest water levels. Digest water content between all treatment groups did not have real difference, high water content in digest plays an important role in increasing water feces volume (Wahjuningsih *et al*., 1999) since increasing levels of digest water content have direct relationship with increasing weight of digest.

In pH digest measurement, no real difference between all treatment groups and the pH digest consecutively from the highest were P1, K (-) and SKFS K (+). From this study, the highest pH was in Asp-Glu peptide, in agreement with the previous study that the increasing fecal nitrogen component in mice groups given non digestive protein isolate, leading to increase in pH digest.

The measurement of cholesterol digest showed real difference between treatment groups. This result is in agreement with Yang *et al.* (2007) who showed high levels of steroids in animal feces after given treatment of non-dialyzed soybean protein hydrolysate (NSPH) compared with control group (placebo). In another study, the group which were given additional casein feed have binding steroid feces lower than group with un-digestive soy protein isolate (USDP) (Chen *et al*., 2003). Soy protein which was separated using protease is divided into two fractions: high molecule insoluble fraction and low molecule soluble fractions. In insoluble fraction, when it given to rats can reduce total cholesterol levels with increasing steroids fecal excretion.

The possible mechanisms is when un-digestive soy protein isolate (USDP) to the gastrointestinal tract, USDP will be digested by digestive enzymes to further absorbed in the body. The un-digestive fraction will be able to bind several steroid and will be released through the feces (Chen *et al*., 2003).

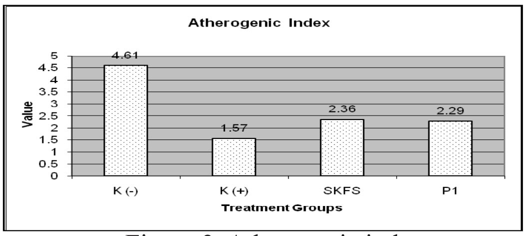

Figure 3. Atherogenic index

The binding mechanism is similar with binding mechanism for fiber. Peptides in soy milk have ability to bind cholesterol and bile acid uptake, thereby giving lowering cholesterol effect (Torres *et al.*, 2006).

Cholesterol secreted out by the body as bile, will send signal to the liver to provide cholesterol by increasing cholesterol synthesis. Increased excretion of cholesterol and bile acids in feces may decrease re-absorption of bile acids through enterohepatic circulation, thereby decreased bile acids deposits concentration (bile acid pool) and increased 7α-hydroxylase enzyme (bile acids marker in liver) activity. Increased bile acids synthesis will stimulate cholesterol metabolism in the body, then increased cholesterol use in liver and decreases serum cholesterol concentrations. Moreover, decreased liver cholesterol concentrations may increase 3-hydroxy-3 methylglytaryl coenzymeA reductase activity and increase hepatic cholesterol synthesis to keep homeostasis situation (Erdman Jr, 2000; Chen *et al*., 2003).

Atherogenic index

Atherogenic index (IA) measurement revealed IA value sequentially from the lowest were K (+) (1:57 ± 0.02), P1 (2:29 ± 0.04), SKFS (2:36 ± 0.05) and K (-) (4.61 ± 0.12). Result of the one way ANOVA test analysis revealed real differences between treatment ($p < 0.05$), with P1 and SKFS were not significantly different.

IA calculation results in this study have a direct relationship to total cholesterol and HDL level, IA ratio is very dependent on HDL level. IA ideal value for men is below 4.5, while for women is below 4.0. More high HDL value, more low IA value, and then it will reduce atherosclerosis risk (Baraas, 1994). From Ng *et al.* (1997) study, which evaluated HDL as an index of coronary heart disease risk factors in Malaysia showed that the levels of HDL are undoubtedly used as an assessment of coronary heart disease risk factors. Insoluble soy protein hydrolysate can reduce the ratio of atherogenic index significantly compared to placebo in rats with high cholesterol feed (Yang *et al*., 2007).

Conclusions

From this study we can conclude that peptide Asp-Glu has potential in lowering total cholesterol, triglycerides, LDL, raise levels of HDL, and that cholesterol were found in Sprague Dawley rats digest after extending intervention of Asp-Glu peptide. The results further suggested that the mechanism of binding of cholesterol by Asp-Glu peptide in gastrointestinal tract. This study used synthetic Asp-Glu peptide due to limited synthetic methods and tools to isolate peptide Asp-Glu directly from sterilized fermented soymilk. Further study on Asp-Glu peptide hypocholesterolemic effects to humans is needed to understand the effect of Asp-Glu peptide in human metabolism.

Acknowledgement

Authors acknowledge the DIKTI for their financial support in this research, PAU Laboratory, and Gadjah Mada University.

References

Anwar, T.B. 2003. Manfaat Diet Pada Penanggulangan Hiperkolesterolemi. Download from: *http://library.usu.ac.id/download/fk/gizi-bahri.pdf* on 6/5/2010.

Arie, A. 2007. Medical Aspects of Cardiovascular Disease. Proceedings of the National Scientific Meeting (PIN) III Association Dietisien Indonesia; 19 to 21 July 2007. Semarang.

Astawan, M., Wresdiyati, T. and Hartanta, A.B. 2005. Pemanfaatan Rumput Laut sebagai Sumber Serat Pangan untuk Menurunkan Kolesterol Darah Tikus. Hayati 12: 23-27.

Bakhit, R.M., Klein, B.P., Essex-Sorlie, D., Ham, J.O., Erdman, J.W. and Potter, S.M. 1994. Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. Journal of Nutrition 124: 213-222.

Baraas, F. 1994. Mencegah Serangan Jantung dengan Menekan Kolesterol. Jakarta: Gramedia Pustaka Utama.

Berenson, G.S. and Srinivasan, S. 2003. Cholesterol as a risk factor for early atherosclerosis : the Bogalusa Heart Study. Pediatric Cardiology 17: 113–123.

Chen, J.R., Chou, S.F., Suetsuna, K., Yang, N.Y. and Yang, S.C. 2003. Lipid Metabolism in Hypocholesterolemic Rats Affected by Feeding Cholesterol-Free Diets Containing Different Amount of Non-Dialyzed Soybean Protein Fractio. Nutrition 19: 676-680.

Erdman Jr., J.W. 2000. Soy Protein and Cardiovascular Disease. Circulation 102 : 2555-2559.

Erdmann, K., Cheung, B.W.Y. and Schröder, H. 2008. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. Journal of Nutritional Biochemistry. Download from: *www.sciencedirect.com.*

Greaves, K.A., Wilson, M.D., Rudel, L.L., Willliams, J.K. and Wagner, J.D. 2000. Consumption of Soy Protein Reduces Cholestreol Absorbtion Compared to Casein Protein Alone or Supplemented with an Isoflavone Extract or Conjugated Equine Estrogen in Ovariectomized Cynomolgus Monkeys. Journal of Nutrition 130: 820-826.

Heslet, L. 1997. Kolesterol. Jakarta : Kasaint Blanc.

Kahl's, P. 1999. Why HDL is important to your health. Download from: *http//www.zoneperfect.com./kahl_intro.html* on 05/31/2009.

Koswara, S. 2006. Susu Kedelai Tak Kalah Dengan Susu Sapi. Download from : *http://www.indomedia.com/intisari/2006/agustus/susu.htm* on 6/2/2009.

Liu, J.R., Wang, S.Y., Chen, M.J., Chen, H.L., Yueh, P.Y. and Lin, C.W. 2006. Hypocholesterolaemic effects of milk-kefir and soyamilk-kefir in cholesterol-fed hamsters. British Journal of Nutrition 95: 939–946.

Lovati, M.R., Manzoni, C., Corsini, A., Granata, A., Frattini, R., Fumagalli, R., Sirtori, C. R. 1992. Low-density lipoprotein receptor activity is modulated by soybean globulins in cell culture. Journal of Nutrition 122:1971–1978.

Maeno, M., Yamamoto, N. and Takanp, T. 1996. Identification of an Antihypertensive Peptide from Casein Hydrolysate Produced by a Proteinase from Lactobacillus helveticus CP790. Journal of Dairy Science 79: 1316-1321.

Mahan, L.K. and Stump, S.E. 2004. Krause's : Food, Nutrition and Diet Therapy, 11th edition. Philadelphia : Saunders.

Masuda, O., Nakamura, Y. and Takano, T. 1996. Antihypertensive Peptides Are Present in Aorta after Oral Administration of Sour Milk Containing These Peptides to Spontaneously Hypertensive Rats. Journal of Nutrition 126: 3063-3068.

Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W. 2003. Biokimia Harper Edisi 25. Jakarta: EGC.

Navab, M., Anantharamaia, G.M., Hama, S., Garber, D.W., Chaddha, M., Hough, G., Lallone, R. and Fogelman, A.M. 2002. Oral Administration of an Apo A-1 Mimetic Peptide Synthetized From D-Amino Acids Dramatically Reduces Atherosclerosis in Mice Independent of Plasma Cholesterol. Circulation 105: 290-292.

Ng, T.K.W., The, C.B., Vidyadaran, M.K., Tee, E.S,. Thong, M.L., Kandiah, M. and Ehalid, A. H.. 1997. A critical evaluation of high density lipoprotein cholesterol as an index of coronary artery disease risk in Malaysians. Malaysian Journal of Nutrition 3: 61-70.

Nisa, F.Z, Marsono, Y. and Harmayani, E. 2006. Hypocholesterolemic Effect of Sterilized Fermented Soymilk in Animal Model. Agrosains 19: 41-53.

Nisa, F.Z. 2008. Identification of Hypocholesterolemic Peptide in Fermented Soymilk Sterelized. Paper presented at the International Graduate Conference on Engineering and Sciences. Johor: University Teknologi Malaysia.

Nurhayati, D.R., Harmayani, E. and Marsono, Y. 2003. Efek metabolit susu fermentasi terhadap profil lipid pada tikus sprague dawley. Agritech; 37-39.

Plummer, D.T. 1977. An Introduction to Practical Biochemistry. Bombay: Tata McGraw-Hill Pub. Co. Ltd.

Potter, S.M. 1995. Overview of Proposed Mechanisms for the Hypocholesterolemic Effect of Soy. Journal of Nutrition 125: 6065-6118.

Sugano, M., Yamada, Y., Yoshida, K., Hashimoto, Y., Matsuo, T. and Kimoto, M. 1998. The hypocholesterolmic action of the undigested fraction of soybean protein in rats. Atherosclerosis 72: 115-122.

Torres, N/, Villalvazo, I.T., Tovar, A.R. 2006. Reviews: Current Topics Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. Journal of Nutritional Biochemistry 17: 365–373.

Wahjuningsih, S.B., Marsono, Y. and Noor, Z. 1999. Konsentrat serta kedelai : preparasi dan pengaruhnya terhadap sifat fisik dan kimia digesta pada tikus. Agritech 25: 90-95.

Wolever T. MS, Hegele R.A., Connelly P.W., Ransom T.P.P., Story J.A., Furumoto E.J., and Jenkins D.J.A. 1997. Long-term effect of soluble-fiber foods on postprandial fat metabolism in dyslipidemic with E3 and apo E4 genotypes. American Journal of Nutrition 66: 584-590.

Yang, S.C., Liu, S.M., Yang, H.Y., Lin, Y.H. and Chen, J.R. 2007. Soybean Protein Hydrolysate Improves Plasma and Liver Lipid Profiles in Rats Fed High-Cholesterol Diet. Journal of the American College of Nutrition 26 (5): 416–423.

Yusmarini and Efendi, R. 2003. Evaluasi Mutu Soygurt yang dibuat dengan Penambahan Gula. Jurnal Natur Indonesia 8 (2): 104-110.

Zhong, F., Liu, J., Ma, J. and Shoemaker, C.F. 2007a. Preparation of Hypocholesterol Peptides from Soy Protein and Their Hypocholesterolmic Effect in Mice. Food Research International 40: 661-667.

Zhong, F., Zhang, X., Ma, J. and Shoemaker, C.F. 2007b. Fractionation dan Identification of A Novel Hypocholesterolemic Peptide Derived from Protein Alcalase Hydrolysates. Food Research International 40: 756-762.