



Original Research Article

The role of black soybean tempe in increasing antioxidant enzyme activity and human lymphocyte proliferation *in vivo*

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A B S T R A C T

Keywords

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In some hospitals, soybean porridge is used by nutritionists for children who have diarrhea therapy. This is likely related to the recovery of the immune system, both systemically and in the digestive tract. Rats, fed with black soybean tempe diet for 35 days, had higher T cell proliferation index than standard diet rats. This study aims to determine the potential of black soybean tempe in modulating antioxidant enzyme activity and proliferation of human lymphocytes *in vivo*. A total of 21 respondents were divided into three groups, namely placebo consumption group, tempe extract capsules consumption and tempe consumption group. Interventions were conducted for 28 days in respondents' resident. During the intervention all respondents only consumed provided food by researchers. Each respondent in the treatment group consumed 100 grams tempe per day, while those in soybean extract group consumed 3 capsules. Black soybean tempe consumption in humans could enhance the immune system by increasing T cell proliferation and lymphocytes resistance to hydrogen peroxide. Consumption of black soybean tempe tends to enhance B cell proliferation, enzyme activity of SOD, catalase and glutathione peroxides. It can be concluded that consuming tempe could enhance the immune system, especially the cellular immune system.

Introduction

Tempe is traditional Indonesian food that has been known globally. Tempe is made from soybeans fermented by the fungus of *Rhizopus* spp such as *R. oligosporus*, *R. stolonifer* and *R. oryzae* with some characteristics such as white colored, compact texture and distinctive flavor mixed from mushrooms and soy flavor. The food is favored by Indonesian people as side dishes or snacks that taste distinctive and delicious, and a source of

protein in the daily food. In general, tempe is made from yellow soybeans, tempe can also be made from other raw materials, namely black soybeans. Research on soybean that has been published is generally from yellow soybeans, but very few from black soybean tempe. Black soybean tempe has potential of functional characteristics, it is because black soy contains of phenolic, tannins, anthocyanins and isoflavones and higher

antioxidant activity than yellow soybeans (Xu and Chang, 2007). According to Nurrahman, *et al.*, (2012) black soybean tempe has the same level of preference as yellow soybean tempe and higher antioxidant activity.

The fermentation process causes tempe has several advantages compared to soybeans, which can be seen from the composition of nutrients in general, the digestibility of protein and higher essential amino acid content, anti-nutrient substance named antitrypsin and phytic acid which are much lower than soybean. In Tempe, there are decomposing enzymes produced by tempe fungus, so the protein, fat and carbohydrates are more digestible (Suparmo and Markakis, 1987).

Tempe contains higher daidzein and genistein than other soy products, both are isoflavones with antioxidant characteristics (Haron, *et al.*, 2009). Other isoflavones are glycitein and factor II, factor II only exist in tempe. Isoflavones can act as an anti-tumor or anti-cancer, it is due to its antioxidant characteristic that can protect DNA from free radical attack. Daidzein and genistein are phytoestrogen which have estrogenic, antiestrogenic, anticarcinogenic, antiviral, antifungal and antioxidant characteristics (Mazur, 1998). Hermana, *et al.*, (1996) and Sudigbia (1996) reported that toddler patients of malnutrition and chronic diarrhea who were fed with tempe formula experienced nutritional improvement, weight gain and diarrhea recuperation in a relatively short time. In some hospitals, tempe porridge is used by nutritionists for child who has diarrhea therapy. This is likely related to the recovery of the immune system, both systemically and in the digestive tract.

Food has an important role in increasing

body's immune system. Food supply important components, such as carbohydrates, proteins, fats, vitamins and minerals, for the formation of cells and antibodies are involved in the immune system. Food's nutrients affect the body's defense system through immune response as needed for immediate mobilization to make the lymphocytes in an activated, proliferates and differentiates condition. Some components in food also served to increase the activity of the immune system. Nurrahman *et al.*, (1999), found that the consumption of 250 ml of ginger juice (made from extracted ginger with water (1:3)) for 30 days influenced the increase on T cell activity and lymphocyte resistance to paraquate. Consuming ginger juice also has a tendency to increase B cell activity. According to Zakaria-Rungkat *et al.*, (2003) and Tejasari (2007), components of gingerol in ginger can enhance the activity of T and B cell proliferation. Sasmito *et al.*, (2006a) found the elevated levels of IgG and IgA in rats fed with fermented mare's milk.

Aglycone isoflavones genistein is thought to play role in the immune system (Wang *et al.*, 2008). Isoflavones are flavonoid compounds abundant in soy and soy products. According to Sasmito *et al.*, (2006a), rats that fed soy milk can increase the levels of IgG and IgA in the blood. See *et al.*, (2002) in Sasmito *et al.*, (2006b) found an increase of NK cell activity and TNF- α in patients with end-stage cancer who consume genistein for 6 months.

The function of the immune system is affected by oxidative damage and hormonal changes. Phytochemicals found in plants have biological characteristics in improving the immune system. Genistein may increase resistance to B16F10 tumors in female rats, and enhance Tc and NK

cell activity. Genistein also increases the specific immune response that suppresses antigen and lower the levels of IgG1 through its competition during interaction with 17 β -estradiol estrogen receptor in rats BALB /c which were immunized with ovalbumin. In humans, the consumption of isoflavones contained in soy foods modulates cytokine production (Wang *et al.*, 2008).

Tempe contains antioxidant components such as isoflavones, vitamin E and β -carotene. Antioxidant compounds (isoflavones) in tempe may also contribute to gene expression (Rimbach *et al.*, 2008). Activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase was significantly increased by genistein (Rimbach *et al.*, 2008). Sierens *et al.*, (2001) stated the hypothesis that phytoestrogens in certain circumstances act as antioxidants and protect DNA from oxidative damage. They found that human lymphocytes that were incubated with genistein *in vitro* resistance to damage caused by H₂O₂. Nurrahman *et al.*, (2011) reported that rats fed with black soybean tempe diet for 35 days had higher T cell proliferation index than rats fed a standard diet. Based on the above explanation, it needs to do research on the role of black soybean tempe to the body's immune system. This study aims to determine the potential of black soybean tempe in modulating the activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and proliferation of human lymphocytes *in vivo*.

Materials and Methods

Materials

The materials used in this study are black soybean tempe of Mallika variety, tempe

inoculum, respondents blood, various chemicals for cell culture such as *Roswell Park Memorial Institute* (RPMI 1640, Sigma, USA), lipopolysaccharide (LPS, Sigma, USA), fithohemagglutinin (PHA, Sigma, USA), various chemicals for the isolation and analysis of lymphocyte proliferation, reagent kits for superoxide dismutase enzyme activity (BioVision, USA), reagent kit for catalase enzyme activity (BioVision, USA) and reagent kit of glutathione peroxidase enzyme activity (BioVision, USA).

Research Procedure

Tempe Extraction

Tempe that will be extracted first dried at a temperature of 40 - 45°C for 24 hours. The dried tempe then crushed to be a powder (60 mesh). Tempe flour then soaked in 70% ethanol (1:2) for 24 hours, and then filtered. Accommodated the obtained filtrate, add 70% ethanol to the residue and then filtered. The first filtrate is mixed with the second filtrate results. Evaporate the mixed filtrate to obtain a viscous fluid.

Tempe Extract Capsulation

Tempe extract is made capsules by adding tapioca to the extract with the ratio 4:1. The mixture is dried at a temperature of 40 - 45 ° C until dry. The dried material is inserted into the capsule.

Number of Subject Determination

Subjects that are included as respondents in this study are people who lived in the dorm. The number of respondents is 21 male aged between 19 to 24 years old, do not smoke, do not consume alcohol, no drugs, not take antioxidant supplements during the intervention, healthy (stated by

a doctor) and a normal nutritional status (normal BMI).

Respondents are divided into three groups. The first group of seven people did not consume tempe and tempe extract (placebo), the second group of seven people consume black soybean tempe extract capsules and the rest of the respondents consume black soybean tempe in the form of boiled processed tempe (seasoned with garlic and salt, then boiled until the water runs out). The interventions are conducted for 28 days in the respondents' residents. During the intervention all respondents only consume food provided by researcher. Every respondent in the treatment group consume tempe as much as 100 grams per day, while those who consume tempe extract consumed 3 capsules per day. Before the intervention, the respondents do not consume any soy foods for one week.

Blood Sampling

Blood sampling is performed at 0, 14 and 28 days for all subjects. It is performed by an expert medical analyst. Blood is taken 9 ml using sterile 10 ml *vacutainer* containing heparin. Then the blood was taken to the Laboratory of Immunology UGM to analyze the proliferation of T and B cell, the resistance of lymphocytes to hydrogen peroxide, to analyze the activity of SOD enzyme, catalase and glutathione peroxides. The measurement of SOD enzyme, catalase and glutathione peroxides activity is based on the method as stated in the kit.

The Measurement of Lymphocyte Proliferation

The measurement of lymphocyte proliferation is performed to determine the

in vitro activity of lymphocytes, which consists of lymphocytes isolation and lymphocyte proliferation.

Lymphocytes Isolation

The drawn blood centrifuge with the speed of 1514 g for 10 minutes to obtain plasma and lymphocyte layer. The heavier part of the blood (red blood cells) will be at the very bottom. Among the layers of red blood cells and blood plasma are *buffycoat* layer, where the layer containing lymphocytes. Dialiquot plasma then stored at -20°C, while the buffycoat layer is taken with Pasteur pipette slowly, and then put in 5 ml RPMI-1640 medium.

Further Lymphocyte cell separation with *Histopaque* use the principle of separation based on the density difference. The *Buffycoat* layer which mixed with synthetic medium of RPMI-1640 placed gently on the top of *histopaque* by 10 ml in 15 ml centrifuge tube, then perform 1430 g centrifugation for 30 minutes. The centrifuge result is a layer of white ring located between the synthetic medium RPMI-1640 and *Histopaque*. The white layer then aspirate by Pasteur pipette and put in 10 ml of synthetic medium RPMI-1640. The lymphocyte washing is done by a centrifuge cell suspension that was mixed with synthetic media for 10 minutes, 1228 g. The washing is done twice.

Proliferasi limfosit

Prior to culture, lymphocytes first calculated the amount obtained from each respondent. After that the number of lymphocytes from each of the respondents matched to a 2×10^6 /ml with standard medium. Cell suspension from one respondent is put into 12 wells (96 well

plate) of 100 µl each, then added 5 mg PHA mitogen, 1 µg lipopolisacride mitogen or 90 µmol/l hydrogen peroxide solution respectively in the three wells. For control, just add the medium. Then, they are cultured in an incubator with 5% CO₂ atmospheric humidity-95% air and 37° C. After 72 hours incubation, each well is added 10 µl solution of MTT 5 mg/ml. Then, it is incubated for next 4 hours. Living cells will react with MTT to form purple. The reaction with MTT is stopped by adding the stopper reagent which, is a 10% solution of SDS in 50 µl 0.01 N hydrochloric acid, in each well and allowed to stand overnight, and then the absorbance is measured with a microplate reader with a wave length of 550 nm.

Experimental Design

Experimental design used in this study is complete randomize design, where there are two independent variables, namely diet treatment (control, extracts tempe and black soybean tempe) and intervention length (0, 14 and 28 days). While the dependent variable is the proliferation index of T cells, B cells, lymphocytes resistance to hydrogen peroxide, the activity of superoxide dismutase enzyme, catalase and glutathione peroxidase. The data obtained are presented in tables and analyzed with factorial ANOVA.

Results and Discussion

T Cell Proliferation

Respondents' average stimulation index of T cell proliferation before intervention in the placebo, tempe extracts and tempe consumption group respectively are 2.005, 1.797 and 2.207. After 14 days intervention obtained average stimulation index of T cell proliferation respectively

are 1.950, 1.490 and 1.834. At the end of the intervention, the average stimulation indexes of each group are 2.775, 2.004 and 3.600. Table 1 shows the average stimulation index of T cell proliferation before and after the intervention of the three groups respondents.

Based on ANOVA statistical analysis it find that during the 14 days intervention, the average stimulation index value of T cell proliferation in the three groups of respondents are not significantly different. This means that in the 14 days interventions there is no shown a change in the immune system, particularly to the T cells. In the 28 days intervention increased stimulation index in all groups of respondents. However, the increase of stimulation index in the placebo and tempe extracts group were not significant, but is significantly increase ($p \leq 0.05$) for the tempe consumption group. Thus it can be said that eating tempe can enhance T cell proliferation.

The increase of T cell stimulation index in respondents who consumed tempe is higher than in placebo and tempe extract group, so tempe consumption can increase T cell proliferation. Nurrahman *et al.*, (2011), which used rat as the object of study, found that the increased ability of T cells to proliferate in the group who consumed tempe was likely due to some components, such as isoflavones, unsaturated fatty acids, vitamin E, β-carotene and free amino acids. Those which contained in tempe had a role in improving the performance of T cells (Ramprasath *et al.*, 2005 and Rimbach *et al.*, 2008). Some amino acids which are contained in both soybean and tempe have a role to increase T cell proliferation, such as glutamine, which is an important energy source for lymphocytes to proliferate

(Schley and Field, 2002), sulfur amino acids such as methionine, cysteine, and cystine which are required by T cells in carrying out the functions of the immune system (Grimble and Grimbé, 1998) and arginine which is supplemented into rats feeding has shown an increase of cellular immune system, especially the increase of T cell response (Daly et al, 1990). Another possibility, there are components in tempe (isoflavones) that can interact with receptors on the surface of T cells thus activated to proliferate (Zhao *et al.*, 2005). According to Dixon and Ferreira (2002), genistein is one of the isoflavones contained in soybean and soy products (tempe) that is able to bind estrogen receptors.

Wang *et al.*, (2008) stated that consuming isoflavones that contained in soy foods can modulate cytokine production. The role of tempe components in lymphocytes proliferation is likely to stimulate the formation of lymphokine, especially interleukin-1 (IL-1) and interleukin-2 (IL-2). IL-1 is produced by macrophages, which affects to increase the proliferation and lymphocytes differentiation. IL-1 also plays a role to stimulate non-specific expression of various antigen receptors on the cell surface that indirectly increase the specific immune response.

In addition, IL-1 stimulates the production of lymphokines, including IL-2, B-cell growth factor, gamma interferon, and chemotactic factors. IL-2 is produced mainly by T cells that function to induce the proliferation of T cells, B cells and NK cells and activates macrophages (Pappa *et al.*, 2007).

However, this possibility has not been proved by research data that supported the role of tempe to the formation of IL-1 and

IL-2. Therefore, further research needs to examine the correlation between tempe and IL-1 production by macrophages and IL-2 by Th cells, so we can find out the mechanism of tempe role toward the increase of immune system. The increase of T cells proliferation activity leads to the improvement of the immune system. Thus, it can be concluded that the consumption of tempe and its extract may increase immune system, especially the cellular immune response.

B Cell Proliferation

The respondents' average stimulation index of B cell proliferation before the intervention in the placebo, tempe extracts and tempe consumption group respectively are 1.042, 1.062 and 1.065. After the 14 days intervention, the average T cell proliferation stimulation index are respectively 1.007, 1.003 and 1.002. At the end of the intervention, the average stimulation index become 1.100, 1.099 and 1.097. Table 2 shows the average stimulation index of B cell proliferation before and after the intervention of the three groups.

Based on ANOVA statistical analysis it find that after the 14 days intervention, the average stimulation index of B cell proliferation in group of tempe extract and tempe consumption is significantly different. This means that the intervention for 14 days show a change in the immune system.

At the 28 days intervention, there are an increase stimulation index in all groups of respondents. However, the increase index was not significant. Thus it can be said that eating tempe and its extracts cannot increase B cell proliferation.

Table.1 The Effect of Type of Consumption and length of intervention toward stimulation index of T cell proliferation

Type of Consumption	Length of intervention (day)		
	0	14	28
Placebo	2,005±0,477 ^a	1,950±0,369 ^a	2,775±0,300 ^a
Tempe Extract	1,797±0,469 ^a	1,490±0,239 ^a	2,004±0,533 ^a
Tempe	2,207±0,593 ^a	1,834±0,255 ^a	3,600±0,301 ^b

Note: different letters of each row and column indicated significantly different ($p \leq 0.05$)

Table.2 The effect of consumption type and intervention length toward B cell proliferation stimulation index

Type of consumption	Intervention Length		
	0	14	28
Placebo	1.042±0,091 ^a	1,007±0,010 ^{ab}	1,100±0,122 ^a
Tempe Extract	1.062±0,002 ^a	1,003±0,009 ^b	1,099±0,201 ^{ab}
Tempe	1,065±0,048 ^a	1,002±0,008 ^b	1,097±0,152 ^a

Note: same letters of each row and column is not indicated significantly different ($p \leq 0.05$)

Table.3 The Effect of consumption type and intervention length toward lymphocytes proliferation stimulation index

Consumption Type	Intervention Length (days)		
	0	14	28
Placebo	0,684±0,114 ^{ac}	0,515±0,082 ^a	0,689±0,112 ^{ac}
Tempe Extract	0,689±0,112 ^{ac}	0,427±0,066 ^b	0,693±0,132 ^{ac}
Tempe	0,736±0,035 ^c	0,551±0,059 ^a	0,907±0,092 ^d

Note: different letters of all row and column indicated significantly different ($p \leq 0.05$)

Table.4 The Effect of consumption type and intervention length toward SOD enzyme activity (% inhibition)

Consumption Type	Intervention Length (days)		
	0	14	28
Placebo	46,44±17,45 ^a	45,01±19,31 ^a	48,79±16,92 ^a
Tempe Extract	48,94±10,09 ^a	47,51±18,48 ^a	50,01±14,43 ^a
Tempe	50,01±17,68 ^a	51,79±19,88 ^a	59,65±12,29 ^a

Note: same letters of all row and column indicated insignificantly different

Lymphocytes Durability against Hydrogen Peroxide (H₂O₂)

The average of lymphocyte stimulation index before intervention in the placebo, tempe extracts and tempe consumption group are respectively 0.684, 0.689 and 0.736. After 14 days intervention, the average lymphocyte proliferation stimulation indexes respectively are 0.515, 0.427 and 0.551. At the end of the intervention the average stimulation index of each being are 0.689, 0.693 and 0.907. Table 3 shows the average stimulation index of lymphocyte proliferation before and after the intervention of the three groups of respondents. These data showed that the average of the three groups with intervention length 0, 14 and 28 days was lower than lymphocytes cultures which were not added hydrogen peroxide. This means that all lymphocyte cultures which were added hydrogen peroxide experienced an inhibited proliferation. Thus, hydrogen peroxide can suppress lymphocytes proliferation.

Based on statistical analysis with factorial ANOVA it find that there was significant effect of length and type of diet intervention toward lymphocyte proliferation stimulation index ($p \leq 0.05$). Before the intervention, the three groups showed insignificant difference of stimulation index. During the 14 days interventions, all three groups experienced a decrease stimulation index than before the intervention (0 day) significantly ($p \leq 0.05$), in which the group who consumed tempe experienced the smallest decrease than the other groups ($p \leq 0.05$). At the 28 days intervention, the stimulation index in all groups of respondents increase significantly ($p \leq 0.05$) compared to 14 days intervention. The average of stimulation index in group who consumed

tempe is higher than other groups at the 28 days intervention, while the stimulation index in the placebo group and tempe extract group was not significantly different. Based on the explanation above, it can be said that consuming tempe can increase lymphocytes resistance against hydrogen peroxide. According to Sierens *et al.*, (2001), human lymphocytes cells that are incubated with genistein *in vitro* were protected by H₂O₂.

The addition of hydrogen peroxide into the culture of lymphocytes can create an oxidative stress condition, which is the increase in the number of prooxidant (Panayiotidis *et al.*, (1999) and Sierens *et al.*, (2001)). In a state of equilibrium between prooxidant and antioxidant, prooxidant can be neutralized by enzyme superoxide dismutase, catalase, glutathione peroxidase and many other antioxidants (Langseth, 1995 and Yen *et al.*, 2004). SOD enzyme catalyzes the O₂⁻ dismutase to H₂O₂ (Yuan *et al.*, 2009). This enzyme inhibits the simultaneous presence of O₂⁻ and H₂O₂ derived from the formation of hydroxyl radicals (*OH). In human, SOD is in form of Mn-SOD mitochondrial and Cu-Zn SOD sitosolisik and extracellular (Nabet, 1996). Catalase enzyme is a human enzyme that was located in peroxisomes and hematis. Catalase degrades hydrogen peroxide into water (H₂O₂ ----→ H₂O + ½ O₂) (Nabet, 1996 and Yuan *et al.*, 2009). The enzyme glutathione peroxides is an enzyme that has selenium (Se) on the active side. This enzyme catalyzes the reduction of H₂O₂ and peroxides lipid (LOOH) using reduced glutathione (GSH) as a cofactor (Nabet, 1996). According to Meydani *et al.*, (1995), immune cells are very sensitive toward the changes in the balance of prooxidant and antioxidant.

The analysis results show that the resistance of lymphocytes to the addition of hydrogen peroxide from rat of treated group is higher than the control group, although the average of all groups experienced inhibition. This happens because tempe contains components that function in improving the antioxidant status of lymphocytes such as isoflavones, vitamin E, β -carotene, amino acids and minerals Cu, Mn and Zn. So, possibly lymphocytes from the group who consumed tempe and the extract are first activated in the body by tempe components (isoflavones). Another possibility is hydrogen peroxide which cannot penetrate lymphocytes or can penetrate but can be neutralized by cells' antioxidant system.

Activity of Superoxide Dismutase Enzyme

The average of SOD enzyme activity of respondents' plasma before the intervention in the placebo, tempe extracts and tempe consumption group respectively are 46.44, 48.94 and 50.01 percent inhibition. After 14 days intervention, the average value of SOD enzyme activity respectively are 45.01, 47.51 and 51.79 percent inhibition. At the end of the intervention, the average of SOD enzyme activity each become 48.79, 50.01 and 59.65 percent inhibition. Table 4 shows the average value of SOD enzyme activity before and after the intervention of the three groups.

Based on statistical analysis by ANOVA it find that at the 14 days intervention, the average value of SOD enzyme activity in the three groups of respondents are not significantly different. This means that interventions during 14 days have not shown any changes in SOD enzyme

activity. While at 28 days intervention, SOD enzyme activity in the placebo, tempe extracts consuming and tempe consuming group increase. There are a tendency that respondents who consumed tempe experience higher increase than other respondents. However, the increase in SOD enzyme activity is not significant.

Activity of Catalase Enzyme

The average values of catalase enzyme activity of respondents' plasma before the intervention in the placebo, tempe extracts consumption and tempe consumption group are respectively 54.66, 54.73 and 54.54 nmol/min/ml. After the intervention for 14 days, the average values are respectively 53.90, 53.24 and 53.67 nmol/min/ml. Then, at the end of the intervention the average values are respectively 54.54, 54.34 and 55.37 nmol/min/ml. Table 5 shows the average value of catalase enzyme activity before and after the intervention of the three groups of respondents.

Based on statistical analysis by ANOVA it find that during the 14 days intervention, the average values of catalase enzyme activity in the three groups of respondents are not significantly different. This means that interventions during the 14 days have not shown any changes in the activity of catalase enzyme. While in the 28 days intervention, the activity of catalase enzyme increase in the group of respondents who consumed tempe, but it decrease in the placebo and tempe extracts group. However, the increase and decrease of catalase enzyme activity are not significant.

Glutathione Peroxide Enzyme Activity

The average values of glutathione peroxides enzyme activity of respondents'

Table.5 The Effect of consumption type and intervention length toward catalase enzyme activity (nmol/min/ml)

Type of consumption	Intervention Length (days)		
	0	14	28
Placebo	54,66±3,83 ^a	53,90±4,99 ^a	54,55±4,37 ^a
Tempe Extract	54,73±2,87 ^a	53,24±3,57 ^a	53,67±2,72 ^a
Tempe	54,54±4,49 ^a	54,43±5,15 ^a	55,37±1,56 ^a

Note: same letters of all row and column indicated insignificantly different

Table.6 The Effect of consumption type and intervention length toward activity of glutathione peroxides enzyme (nmol/min/ml)

Consumption Type	Intervention Length		
	0	14	28
Placebo	233,14±16,83 ^a	234,71±24,63 ^a	233,40±14,59 ^a
Tempe Extract	248,10±21,00 ^a	248,41±23,32 ^a	248,12±18,00 ^a
Tempe	257,00±24,78 ^a	257,11±16,65 ^a	258,98±12,34 ^a

Note: same letters of all row and column indicated insignificantly different

plasma before the intervention in the placebo, tempe extracts and tempe consumption group are respectively 261.71, 238.10 and 257.00 nmol/min/ml. After the intervention for 14 days, the average values are respectively 250.43, 228.41 and 255.69 nmol/min/ml. At the end of the intervention, the average are respectively to be 256.40, 236.83 and 258.98 nmol/min/ml. Table 6 shows the average activity of glutathione peroxidase enzyme before and after the intervention

of the three groups of respondents.

Based on statistical analysis by ANOVA it find that during the 14 days intervention, the average value of glutathione peroxides enzyme activity in three different groups by increasing T cell proliferation and the resistance of lymphocytes to hydrogen peroxide. Consumption of black soybean tempe tends to enhance B cell proliferation, SOD, catalase and glutathione peroxides enzyme activity. Thus it can be said that consuming tempe

of respondents are not significantly different. This means that interventions during the 14 days have not shown any changes in the activity of glutathione peroxides enzyme. At the 28 days intervention, the activity of glutathione peroxides enzyme increase in the group of respondents who consumed tempe, whereas it decrease in the placebo and tempe extracts group. However, the increase and decrease of the glutathione peroxides enzyme activity are not significant. Thus it can be said that eating tempe and its extracts cannot increase and decrease the activity of glutathione peroxides enzyme.

Black soybean tempe consumption in humans can enhance the immune system can enhance the immune system, especially the cellular immune system

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References

- Daly, J.M., J. Reynolds, R.K. Sigal, J. Shou and M.D. Liberman. 1990. Effect of dietary protein and amino acids on immune function. *Cri. Care Med.*, 18:86 – 93.
- Dixon, R.A. and D.Ferreira. 2002. Genistein. *Phytochem.*, 60: 205 – 211.
- Grimble, R.F. and G.K. Grimble. 1998. Immunonutrition: role of sulfur amino acids, related amino acids, and polyamines. *Nutr.*, 14:605 – 670.
- Hermana, M. Karmini dan D. Karyadi. 1996. Komposisi dan nilai gizi tempe serta manfaatnya dalam peningkatan mutu gizi makanan. *Dalam. Sapuan dan N. Soetrisno*, eds. 1996. *Bunga Rampai Tempe Indonesia, Jakarta*, hal. 61-67. Yayasan Tempe Indonesia, Jakarta.
- Meydani, S.N., Wu D., Santos, M.S. and Hayek, M.G. 1995. Antioxidant and respon in aged person: overview of present evidence. *Am.J.Clin.Nutr.*, 62:1462-1476.
- Nabet, F.B. 1996. Zat gizi antioksidan penangkal senyawa radikal pangan dalam system biologis. *Dalam. Zakaria et al.*, (eds.). *Proseding Seminar Senyawa Radikal dan Sistem pangan: Reaksi Biomolekuler, Dampak terhadap Kesehatan dan Penangkalan*, hal. 8-15. Kerjasama PAU IPB dengan Kedutaan Perancis, Jakarta.
- Nurrahman, F.R. Zakaria, D. Sayuti dan Sanjaya. 1999. Pengaruh konsumsi sari jahe terhadap perlindungan limfosit dari stres oksidatif pada mahasiswa Pesantren Ulil Al-Baab Bogor. *Dalam. Proseding Seminar Nasional Teknologi Pangan*, hal. 707-715. PATPI, Jakarta.
- Nurrahman, M. Astuti, Suparmo dan M.H.N.E. Soesatyo. 2011. The effect of black soybeans tempe and it's ethanol extract on lymphocyte proliferation and IgA secretion in Salmonella typhimurium induced rat. *Afri. J. Food Scie.*, 5(14): 775 – 779.
- Panayiotidis, M., O. Tsolas and D. Galaris. 1999. Glucose oxidase-produced H₂O₂ induces Ca⁺-dependent DNA damage in human peripheral blood lymphocytes. *Free Rad. Biol & Med.*, 26(5/6): 548 – 556.
- Pappa, C., S. Miyakis, G. Tsirakis, A. Sfiridaki, A.Alegakis, M. Kafousi, E.N. Stathopoulos, and M.G. Alexandrakis. 2007. Serum levels of interleukin-15 and interleukin-10 and their correlation with proliferating cell nuclear antigen in multiple myeloma. *Cyt.*, 37: 171 – 175.
- Ramprasath, V.R., P.Shanthi and P.Sachdanandam. 2005. Evaluation antioxidant effect of *Samecarpus anacardium* Linn. Extract on the components of immune system in adjuvant arthritis. *Vas. Pharmacol.*, 42:179 – 186.
- Rimbach, G., C.B. Saadatmandi, J. Frank, D. Fuchs, U. Wenzel, H. Daniel, W.L. Hall and P.D. Weinberg. 2008. Dietary isoflavones in the prevention of cardiovascular disease-A molecular prespective. *Food and Chem. Toxicol.*, 46:1308-1319.
- Sasmito, E., Rumiya, S. Rahayu W., E. Andriyani dan R. Istikharah. 2006a. Pengaruh pemberian susu kuda terfermentasi terhadap imunitas vaksin hepatitis A pada tikus Balb/c. *Maj. Farma. Ind.* 17(1): 13-18.
- Sasmito, E., S. Mulyaningsih, E.K. Untari, dan R. Widyaningrum. 2006b. Aktivitas imunostimulan susu kedelai

- terhadap imunoglobulin (IgG, IgA) dan proliferasi sel limfosit pada tikus Balb/c yang diinduksi hepatitis A. *Maj. Farma. Ind.*, 17(3): 156-161.
- Schley, P.D. and C.J.Field. 2002. The immune-enhancing effect of dietary fibers and prebiotics. *Br. J. Nutr.*, 87: 221 – 230.
- Sierens, J., J.A. Hartley, M.J. Campbell, A.J.C. Leathem and J.V. Woodside. 2001. Effect of phytoestrogen and antioxidant supplementation on oxidative DNA damage assessed using the comet assay. *Mut. Res.*, 485:169-176.
- Sudigbia, P. 1996. Tempe dalam penatalaksanaan diare anak. *Dalam. Sapan dan N. Soetrisno (eds.). 1996. Bunga Rampai Tempe Indonesia, Jakarta*, hal. 71-82. Yayasan Tempe Indonesia, Jakarta.
- Suparmo and P. Markakis, 1987. Tempeh Prepared from Germinated Soybeans. *J. Food Sci.*, 52(6):1736–1737.
- Tejasari. 2007. Evaluation of ginger (*Zingiber officinale* Roscoe) bioactive compounds in increasing the ratio of T-cell surface molecul of CD3+CD4+:CD3+CD8+ in vitro. *Mal. J. Nutr.*, 13(2): 161-170.
- Wang, J., Q. Zhang, S. Jin, D. He, S. Zhao and S. Liu. 2008. Genistein modulate imune responses in collagen-induced rheumatoid arthristis model. *Mat.*, 59: 405-412.
- Xu, B.J. and S.K.S. Chang. 2007. A Comparative study on phenolic profils and antioxidant of legums as affected by extraction solvents. *J. Food Sci.* 72(2):159-166.
- Yen, G.C., P.D. Duh, and H.J. Su. 2004. Antioxidant properties of lotus seed and its beffect n DNA damage in human lymphocytes. *Food Chem.*, 89: 379 – 385.
- Yuan, C., X.Huang, L. Cheng, Y. Bu, and G.Liu, F. Yi, Z. Yang and F. Song. 2009. Evaluation of antioxidant and immune activity of *Phellinus ribis* glucan in mice. *Food Chem.*, 115: 581 – 584.
- Zakaria-Rungkat, F. Nurrahman, Prangdimurti,E. Tejasari.2003. Antioxidant and Immunoenhancement Activities of Ginger (*Zingiber officinale* Roscoe) Extract and Compounds in *In Vitro* and *In Vivo* Mouse and Human Systems. *Nutr. and Food*, 8: 96-104
- Zhao, J.H., S.J. Sun, H. Horiguchi, Y. Arao, N. Kanamori, A. Kikuchi, E. Oguma and F. Kayama. 2005. A soy diet accelerates renal damage in autoimune MRL/Mp-lpr/lpr mice. *Int. Immunopharmacol.*, 5: 1601-1610.