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Abstract: Dyslipidemia condition will effect on the occurrence of oxidative stress. one of the efforts to improve lipid profiles is by consuming food containing antioxidant which have the potential to improve lipid profiles (10). The aim of this study was to obtain a formula which contains the most nutrients and antioxidants from a combination of soybean tempe flour with bran. The method used in this study was an experiment. Black soybeans and yellow soybeans were processed into tempe and then molded, red bran and white bran were then heated using a Dreyer Cabinet with a temperature of 100oC for 20-30 minutes then sifted using an 80 mesh size sieve. Mix each (1) yellow soybean tempe flour + white bran flour, (2) black soybean tempe flour + white bran flour (BSF+ WBF) (3) yellow soybean tempe flour + red bran flour (YSF+ RBF), (4) black soybean tempe flour + red bran flour (BSF+ RBF), with a composition of 1: 1 and weighing 112 grams each, noncalorie sweetener 3 gram and 3 gram food flavoring. Results: The combination of YSF+ WBF with a composition of 1: 1 which had higher levels of protein, fat, vitamin E and low carbs compared to the combination of BSF+ WBF, YSF+ RBF or BSF+ RBF. Of the four best formulations received by the panelists, the combination of yellow soybean flour with white rice bran with a composition of 1: 1

Keywords: Combination of tempe and bran flour, nutrient content, anti hyperlipidemic and antioxidative stress

1. Introduction

Dyslipidemia is one of the risk factors for cardiovascular disease characterized by an increasing total cholesterol level (C-Total), low density-lipoprotein cholesterol (C-LDL), and a decrease in high density-lipoprotein cholesterol (C-HDL) and triglycerides, [1] due to complex interactions between genetic factors and lifestyle. [2,3,4] This condition can cause cholesterol build up on the arterial wall which will cause the atherosclerosis process.

Factors that cause dyslipidemia include the high energy intake foods, especially those derived from fat and carbohydrates, low fiber sustenance and lack of physical activity. Food intake correlates with the emergence of changes in body fat distribution, including an increase in total fat mass and central obesity

which triggers oxidative stress due to the prooxidant and antioxidant imbalances in the body. [5] Oxidative stress is an increasing in levels of free radicals in the body. This is related to the development of chronic and degenerative diseases, including cardiovascular disease which is a part of the aging process. [6]

The dyslipidemia prevalence continues to increase, especially in developing countries [7] based on data from the Basic Health Research in 2013 which increased to 50.7%. [8.9]

The main prevention of dyslipidemia is through efforts to control the level of C-Total, C-LDL, C-HDL and triglycerides to always be within normal limits, weight control, low cholesterol diet, regular exercise, and consuming food ingredients containing antioxidant compounds [10].

Antioxidants are compounds that can prevent the occurrence of free radicals by preventing oxidation reactions. The results of the research from Zubaidi et al. (2004); shows that the best exogenous antioxidant is vitamin E (Tocopherol) because it has the best effect when compared to vitamin A and vitamin C in reducing fat peroxidation. Addition of Tocopherol (vitamin E) to a diet can reduce the risk of coronary heart disease (CHD) by 34% .[11,12]

Other types of antioxidants besides vitamin E which have hypolipidemic features are isoflavones contained in soybeans.[13] The ability of isoflavones as antioxidants is to neutralize free radicals that cause cell damage similar to estrogen.[13,14] One of the soybeans products is tempe. The biological activity of tempe increases compared to soybeans, namely the increasing of isoflavone by 222.5% and the decreasing of phytic acid by 65% due to the reaction of phytate enzymes produced by *Rhizopus oligosporus*. Tempe is considered to be related to the activity of the superoxide dismutase (SOD) enzyme formed in the fermentation process after 24 to 60 hours which couldn't be found in soybean seeds prior so that it can increase the SOD levels in the body.[14] One alternative to extend the span life of tempe is to make it into tempe flour.[15]

Tempe is included in the legumes products which when combined with grains can be a good formula to supplement essential amino acids because both types of food ingredients have limiting amino acids in addition to their antioxidant content. The limiting amino acids in tempe are the amino acids methionine and cystine, while in the grains are amino acids lysine.[16] One of the foods derived from grains is paddy that produces rice. The by-products of paddy mills are bran .[17] Rice bran has always been considered as waste, whereas rice bran has a fairly complete nutrient content.[18] Based on BPS data in 2015, 75.36 million tons of rice production in Indonesia would produce bran as much as 6 - 7.54 million tons from rice mills [19]. The types of rice bran depend on the rice varieties itself, there are white rice bran, red rice bran and black rice bran. Bran also contains vitamin E, B vitamin B complex s (B1, B2, B3, B5, and B6), essential fatty acids, fiber, amino acids, *g-oryzanol, polyphenol, and phytosterol*.[20] Components of bran which can reduce total cholesterol, triglycerides and LDL are *tocopherol, oryzanol*, and dissolves food fiber. The mechanism of tocopherol in reducing cholesterol levels is by suppressing lipid peroxidation through the catching of free radicals which include in peroxidation or through reaction with lipid peroxyl radicals. *α-tocopherol* is a powerful free radical chain antioxidant breaker and the most potential fat-soluble vitamin E isomer. [21]

Tempe and bran flour in addition to complementing amino acid types, they also have compounds in tempe flour in the form of isoflavones and compounds contained in bran in the form of tocopherol, as antioxidants chain breaker when combined will produce better strength to slow LDL oxidation. The results of the Nunes (2005) study in animals showed that the addition of isoflavones had no effect but when it is combined with synthetic vitamin E it was able to slow LDL oxidation by 45.6% compared to the atherogenic group ($p < 0.05$) [22]. Tempe flour and bran also contain food fiber. In small intestine, fiber can attach to cholesterol particles so that it prevents cholesterol from entering the bloodstream. Then, cholesterol with this fiber will come out of the body through feces. [23] The purpose of the study was to obtain a formula that contains the most optimum nutrients and antioxidants from a combination of yellow soybean flour with white rice bran.

2. Materials and Method

2.1. Materials

Materials: Yellow soybean tempe flour formula and white rice bran with a composition of 1: 1, 1: 2 and 2: 1, 3 grams of non-calorie sugar and 3 grams of food flavorings.
Material for chemical analysis:
The research materials used in the first year were: *Rice Bran*, soybean tempe and formula-making materials and reagents. Sequential chemical analysis materials are as follows: concentrated H2SO4, HgO, ZnSO4, 40% NaOH, 0.02 N NaOH, 0.02 N HCl, 10% K2SO4, standard α tocopherol, ascorbic acid, hexane, absolute ethanol, 60% KOH, Acetone , NaCl, Na2SO4 an hidrouse, Folin Ciocalteu, distilled water, PP and MR indicators, dye solution, 2% oxalic acid solution, and rice bran flour and soybean tempe flour.

2.2. Research methods

This type of laboratory experimental research is to find out the most optimum nutrient and antioxidant content from yellow and black soybean tempe flour formula with white and red bran. Formulation was done at UNIMUS Food Lab and Nutrition Analysis was carried out at PAU UGM Lab.

2.2.1. Making tempe flour: Yellow soybean tempe and black soybeans, blended by steaming for 15 minutes, cut into pieces with 1 cm thickness, dried, covered with flour and sifted using a 100 mesh sieve.

2.2.2. Making bran flour: The byproduct of white rice and red rice obtained from a rice mill, would then go through heating process using a cabinet dryer with a temperature of 100^{o}C for 20-30 minutes, then sifted with a size of 100 mesh sieves.

2.2.3. Making a variety of formula ingredients, mix each one (1) Combination of yellow soybean flour + white bran flour formula, (2) Combination of yellow soybean flour + white bran flour formula (3) Combination of yellow soybean flour + white bran flour formula, (4) Combination of yellow soybean flour + white bran flour formula, each with a composition of 1: 1 and weighing 112 grams each, adding 3 grams of non-calorie sweetener and 3 grams of food flavoring. After well mixed, each one is dissolved in cold water as much as 250 cc.

2.2.4. Chemical analysis
The soybean flour obtained was then sieved with a 60 mesh size filter. The analysis including analysis of protein levels (AOAC, 2005), fat content (AOAC, 2005), vitamin E (AOAC, 2005) 24 and isoflavone (daidzein and genistein) (Penalvo et al., 2004).

2.2.4.1. Protein Level Analysis
Samples were weighed 2 grams and put into a Kjedahl flask and then added 2 tablets of catalyst, 5 boiling stones, and 15 ml of concentrated H2SO4 and 3 ml 30% of H2O2. It would then be heated by muffle furnace in the fume hood with a temperature of 450^{o}C for 2 hours (until the sample is clear).

2.2.4.1.1. Distillation stage
Add 100 ml of distilled water to the flask containing the result of destruction, then put the flask into a distillation steam device. Take 25 ml of H3BO4 and put it in a 250 ml Erlenmeyer flask and add 2 drops of the methyl red indicator then the distillation device is paired.

2.2.4.1.2. Stage of titration
The distillate from the distillation result is titrated with a standard 0.2 N HCl solution to the endpoint of the titration which is marked by a change in color from yellow to pink. Protein Level Calculation:
(%): (S-B) x N HCl x 14,008 x 5.71 x 100% W x 100

Information :
W = sample weight (gram)
S = number of titration samples (ml)
B = Number of titrations of blank (ml)
V = Standard HCL volume in titration
14,008 = Nitrogen atomic weight
5.71 = Protein conversion factor

2.2.4.2. Measurement of fat content

Soxhlet flask is dried in an oven, cooled in a desiccator, and weighed. The mashed sample is weighed 5 gram (mashed) directly in filter paper and then covered with fat-free cotton. The filter paper containing the sample is placed in the Soxhlet extraction tool, then the condenser is mounted on top of it, and the fat flask is underneath it.

Diethyl ether solvents are poured into a fat flask. Refluxed for a minimum of 5 hours, until the solvent has come back down to fat flask changed clear colored. The solvent in the fat flask was distilled, the solvent was then collected. Furthermore, the fat flask containing the extracted fat was heated in an oven at 105^{o}C. After drying until the weight remains and cooled in a desiccator, the flask then weighed along with the fat.

2.2.4.3. Determination of Vitamin E (Alpha-Tocopherol) levels

The sample was weighed 10 g and then crushed with mortar, added 50 ml of 40% ethanol and 2.5 grams of ascorbic acid, refluxed until condensed, added 20 ml 60% of KOH and then it was refluxed for 5 minutes. Cooled with running water and filtered with a vacuum pump. The residue was extracted with acetone (twice) and was strained again. The filtrate which was produced, extracted with 30 ml of hexane (twice), using a separating flask. The organic phase was washed with 25 ml of saturated NaCl (twice) and filtered with Na2SO4 anhydrous. 10 ml was taken then was steamed with a rotary evaporator at 40^{o}C for 1 hour. Working solution: dissolved 10 mg of standard tocopherol into 100 ml of absolute ethanol. Standard solution: made a series of standard solutions by diluting the working solution using absolute ethanol with the following composition:

Table 1. Standard solutions

Material	Unit	Sample	Blanko 1	Blanko 2	Blanko 3
Concentration	Mg/l	5	10	15	20
Working Solution	ml	0.5	1	1.5	2
Absolute Ethanol	ml	9.5	9	8.5	8

Procedure: 200 µl of both sample and standard was taken, 200 µl 20% ascorbic acid was added and then vortexed for 30 seconds. 1 ml of 95% ethanol was added, the vortexed again for 30 seconds, hexane was added and then vortexed again for 30 seconds. Left for few seconds, next, take the upper phase. Centrifuge at 2000 rpm for 10 minutes. We measured by using a spectrophotometer at an excitation wavelength of 295 nm and emission wavelength of 340 nm.

2.2.4.4. Analysis of Isoflavone Profiles

A sample of 100 gram was crushed, then dried at 40°C and crushed again. Sample powder 1 - 2 grams was extracted with 5 ml 1M HCl in 80% ethanol and incubated for 1 hour at 800°C. Then, it was put in the shaker for 2 minutes and centrifuged 2140 times for 2 minutes. The supernatant was filtered, while the residue was added 2.5 ml 80% ethanol and then put in the shaker and re-centrifuged. Netxt, it was filtered and the supernatant obtained was combined with the first supernatant (Penalvo et al., 2004). The condition of HPLC: HPLC Simadzu, isocratic, sample volume of 20 µl, column: C 18, eluent: methanol and acetonitrile (97: 3), detector: SPD 10A, flow rate: 1ml / min, temperature: 25 - 27°C, wavelength 260 nm and LC10AD pumps.

2.2.4.5. Experimental Design.
The experimental design in this study was a completely randomized design (CRD) of one factor. Analysis of nutrient content data (protein levels, fat, carbohydrate, vitamin E and isoflavone profile) using the one-way ANOVA (Analysis of Variance) statistical method. Furthermore, a further Multiple Comparison test (posthoc test) was conducted with the Tukey test to find out the real differences between treatments. Based on the data obtained, the average value was taken and displayed in table form and then compared between treatments

3. Results

Table 2. Average Nutrient Content Formula Combination of Tempe Flour with Bran Per 100 gr

No	Composition	Unit	TREATMENT			
			YSF +WBF	BSF+WBF	YSF+RBF	BSF+RBF
1	Protein Level	g	43,30 ± 0,28^{d}	30,03 ± 0,34^{b}	29,47 ± 0,56^{a}	34,25 ± 0,49^{c}
2	Fat Content	g	21,70 ± 0,44^{d}	20,78 ± 0,43^{c}	17,05 ± 0,31^{b}	15,37 ± 0,62^{a}
3	Carbohydrate Level	g	36,72 ± 0,72^{a}	36,72 ± 0,88^{a}	37,05 ± 0,31^{b}	38,84 ± 0,28^{b}
4	Vitamin E level	mg	600,51± 7,07^{d}	362,79± 1,37^{a}	550,35± 2,76^{c}	415,43± 2,29^{b}
5	Isoflavones level	mg	77,28 ± 0,76^{b}	70,82 ± 0,55^{a}	90,80 ± 1,20^{d}	86,88± 0,66^{c}

Information:
Signs of different letters on the same line show significant differences ($p \leq 0,05$)

1. YSF = Yellow Soybean Flour TKK
2. WBF = White Bran Flour TBP
3. BSF = Black Soybean Flour TKH
4. RBF = Red Bran Flour TBM

The ANOVA test results showed that there was a significant effect between yellow soybean flour, white rice bran flour, black soybean flour and red rice bran flour towards protein level, fat level, carbohydrate level, vitamin e levels, and isoflavones level of formula flour. This was indicated by the value of the p-value of each parameter was ≤ 0.05. Further testing using the LSD method with a significance level of 95% showed that the highest protein level was found in the YSF + WBF formula reaching 43.30 ± 0.28 grams, then the BSF + RBF formula (34.25 ± 0.49 grams), BSF + WBF formula (30.03 ± 0.34 grams), and the lowest protein level was found in the YSF + RBF formula as much as 29.47 ± 0.34 grams. Statistically there are differences in each treatment.

The LSD test results at the 95% significance level in the fat level showed the highest fat level to the lowest respectively is YSF + RBF (15.37 ± 0.62 grams), YSF + RBF (17.05 ± 0.31 grams). BSF + WBF (20.78 ± 0.43 grams), and YSF + WBF (21.70 ± 0.44 grams) which are statistically different for each treatment. While the LSD advanced test at the 95% level of significance for the highest carbohydrate parameters was the BSF + RBF formula (38.84 ± 0.28 grams), not significantly different from BSF +

WBF (36.72 ± 0.72 grams) and YSF + WBF (36.72 ± 0.88 grams), but significantly different was the YSF + RBF formula (17.05 ± 0.31 grams).

The average results of vitamin E level of the further test using the LSD test with a significance level of 95% showed that there were significant differences in each formula. The highest levels of vitamin E was found in the YSF + WBF formula (600.51 ± 7.07 mg / 100 grams), then YSF + RBF (550.35 ± 2.76 mg / 100 grams), BSF + RBF (415.43 ± 2.29 mg / 100 grams), and the lowest is the BSF + WBF formula (362.79 ± 1.37 mg / 100 grams). While the results of the LSD further test was at the 95% level of significance in the isoflavones data showed a significant difference in each treatment whereas the highest isoflavone levels were found in the YSF + RBF formula which was 90.80 ± 1.20 mg / 100 grams, then the 86 BSF + RBF formula , 88 ± 0.66 (mg / 100 grams), next the YSF + WBF formula (77.28 ± 0.76 mg / 100 grams), and the lowest is the BSF + WBF formula of 70.82 ± 0.55 mg / 100 grams.

4. Discussion

The combination formula of tempe flour and rice bran flour which was evaluated including the composition of nutrients and antioxidant compounds from four combinations of formulation treatments namely (1) combination formula of yellow soybean tempe flour + white bran flour, (2) Black soybean tempe flour + white bran flour, (3) Yellow soybean flour tempe + red bran flour, (4) Black soybean tempe flour + red bran flour. The parameters used to assess the composition of nutrients and antioxidant compounds including proximate, fatty acids, vitamin E, isoflavones and fiber content.

Based on Table 2, it can be seen that the four formulations of yellow tempe flour, black soybean tempe with white bran and red bran have different level of protein, fat, carbohydrate and fiber. The formula with a combination of yellow soybean tempe flour with white rice bran flour has a higher protein, fat, vitamin E nutrient content compared to the other three formulations. Protein was the highest compared to other combination formulas. Both of these ingredients are food ingredients that contain protein. The combination of the two food ingredients contributed protein to the combination formula of yellow soybean tempe flour and white bran up to 43.30% ± 0.28 and provided a complementary effect of essential amino acids.

Soybeans during the fermentation process become tempe, *Rhizopus* and bacteria produce protease enzymes so that the protein was then decomposed into free amino acids. The amount of amino acid released increased and reached its peak after 24 hours to 72 hours of fermentation as much as 7.3% - 30%. The increasing of these amino acids release would improve the nutritional value of tempe, the *digestibility protein corrected amino acid score* (PDCAAS) which reaches 0.8 - 0.9 or 80-90% of animal protein[26]. Amino acids found in soybean tempe are arginine, glycine and alanine, while amino acids found in bran are leucine, valine and vinyl alanine so that they complement each other[27]. The results of Utari's research (2011) proved that the highest amino acid in tempe is arginine[28].

Consumption of soy protein was ≥25 grams per day has a hypocholesterolemic effect caused by the synergistic role of several components in soybeans[29]. According to Erdman (2000) stated that soybean protein has been proven can stimulate an increasing activity in LDL receptor so that it can reduce the levels of LDL[30]. The results of the study of Baum et al. (1998) showed that the increasing activity in LDL receptor was higher in the group which was given intervention in soy protein than casein protein[31]. Arginine found in soybeans is a metabolic precursor that has the potential to increase production[32]. Arginine is a substrate that is ready to be synthesized to produce

Nitric Oxide (NO) with the help of NO Synthases (NOSs) [33]). The arginine mechanism against lipid profiles is through the mechanism of decreased lipogenesis by Nitric Oxide. Nitric Oxide can slow the glucose synthesis, glycogen, and fat in the liver and adipose [34]). While the dominant amino acids found in bran was leucine. Leucine is one of the essential amino acids that play a role in the stability of blood sugar, through the metabolism of the insulin hormone so that it can reduce the insulin secretion and glucagon, and can slow lipogenesis because it plays a role in lipid and glucose homeostasis [30])

The combination formula of yellow soybean flour with white rice bran flour also contains the highest fat among the three other formulas, up to 21.70 ± 0.44. The type of fat content in the formula includes

unsaturated fatty acids. The results of Agrof's study, (1997) stated that the fatty acid content in tempe is oleic acid, linoleic and linolenic acids[35], meanwhile the fat content in bran according to Orthoefer (2005), there are about 20% of rice bran oil (RBO) with unsaturated fatty acids oleic and acidic acids linoleic (70-90%), [36].

Oleic acid is the second largest free fatty acid in tempe and bran after linoleic acid. Oleic acid is beneficial for the body as a substitute for saturated fat (SAFA) which can reduce blood cholesterol by increasing cholesterol (C) -HDL so that it can reduce the risk of cardiovascular disease.[37] Linoleic acid can increase C-HDL and decrease C-LDL, this is different from the role of other fatty acids that tend to increase blood fat levels, linolenic acid is more effective in reducing blood triglycerides than linoleic acid. Another role of linoleic and linolenic acid is to strengthen transplanting and prevent damage to skin tissue and help transport and metabolize cholesterol so that it can reduce cholesterol levels in the blood, in addition to the combination formula of yellow tempe with white bran contains the highest vitamin E among the three other formulations as much as 600.51 ± 7.07 mg.

Vitamin E is a chain breaker antioxidant and fat soluble which is very important because it can improve lipid profile by removing free radicals and lipid peroxidation. Research results of Zubaidi et al (2004); Bourassa et al. (2006) showed that the best exogenous antioxidant is vitamin E (Tocopherol) because it has the best effect compared to vitamin A and vitamin C in reducing fat peroxidation. ([11]). Gaziano, (2004) reported that the addition of Tocopherol (vitamin E) to the diet can reduce the risk of coronary heart disease (CHD) by 34%. ([12]). ([38]).

5. Conclusion

Of the four best formulations received by the panelists, the combination of yellow soybean flour with white rice bran with a composition of 1: 1

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