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Tempe Made from Green Beans (Vigna radiata L.) and Red Beans (Phaseolus vulgaris L.) as Functional Food

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ABSTRACT

Tempeh is a traditional Indonesian fermented food that is rich in protein and has various health benefits. This study aims to compare the nutritional content, bioactive compounds, and antioxidant activity in tempeh made from green beans (Vigna radiata L.) and red beans (Phaseolus vulgaris L.) as functional food. Tempeh is made by fermenting green beans and red beans using Rhizopus oligosporus inoculum. The levels of proteins, fats, carbohydrates, isoflavones, antioxidant activity and water were determined. The content of bioactive compounds such as isoflavones, total phenolic, and flavonoids was measured using the HPLC method. Antioxidant activity was measured by the DPPH method. The results showed that green bean tempeh has higher levels of antioxidants and carbohydrates compared to red bean tempeh. Red bean tempeh has higher levels of fat, protein, isoflavones and water. The content of isoflavones in green bean tempeh is much lower than that of red bean tempeh. The antioxidant activity of green bean tempeh is much higher than that of red bean tempeh.

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1. INTRODUCTION

Tempeh is one of the many traditional foods in Indonesia. In general, tempeh is made from fermented soybeans. The rise in soybean prices is very influential on the rise in price of tempeh, which resulted in a decline in the quality of tempeh producers to take advantage. Tempeh is one of the sources of protein for the people of Indonesia[9].According to the Nasional Standardization Agency (2012) Soybean-based tempeh has protein content protein (20,8 g/100g), Fat (8,8 g/100g), energy (201 kal/100g), calcium (155 g/100g), up vitamin B1 (0,19 mg/100g) [11]. As a result of significant price increases, there is a need for alternative raw materials for making tempeh, namely green beans and red beans. Both are included in the type of beans or cereals that are classified into functional food types.

Green beans (Vigna radiata L.) belong to the Family Leguminosae and sub family Phapilonaceae, genus Phaseolus, dan species radiatis (Putri, Sutjahjo and Jambo[10]. Green beans have a wide variety of benefits for the body and nutritional value is quite good. Green beans have a carbohydrate content (62.5%) which is composed of starch, sugar and coarse fiber. Mung bean starch contains 28.8% amylose and 71.2% amylopectin. In addition to carbohydrates, green beans also have other contents such as protein (22.2%), vitamin A 9 IU, vitamin B1 150-400 IU and various minerals such as sulfur, manganese, calcium and iron in it[2].

Red beans (Phaseolus vulgaris L.) belong to the family Leguminosae and sub family papilionoideae, genus Phaseolus, and species Phaseolus vulgaris L. (Wiyono, 2012). Red beans contain protein (23.1%) and carbohydrates (59.5%), both of which can be used as a source of nutrition. In addition to protein and carbohydrates, kidney beans also contain various minerals such as phosphorus, calcium and iron, vitamins A and B1, as well as bioactid components such as flavonoids and phytosterols [4].

With various contents contained in green beans and red beans, both have the potential to be processed into food with good nutritional value through various processes, such as fermentation. One of the fermented products of green beans and red beans is tempeh. The fermentation process carried out on green beans and soybeans can reduce antinutritional compounds, so that processed tempeh green beans and red beans will have better nutritional value for the body[6]Chemical changes that occur in the fermentation process of green beans and red beans into tempeh will make both beans more easily digested, so tempeh green beans and red beans are good for people who experience digestive disorders in order to get the nutritional content contained in green beans and red beans [10]

2. METHOD

2.1. Making Tempeh

Sorted green and red beans are washed thoroughly, then soaked in boiling water for about 12 hours. After the soaking process (red beans produce frothy soaking water) the beans are peeled and washed thoroughly. After the beans are declared clean, they are steamed for 30-60 minutes. Beans that have been steamed are allowed to dry at room temperature and then inoculated with yeast and fermented for 48 hours [3].

2.2. Antioxidant Test

Put 100 mM (1.5 mL) acetate buffer solution into the test tube, then added ethanol (2.805 mL) and DPPH mM (0.15) in methanol and then in vortex. Inserted (0.045 mL) sample in a test tube that has been divortex. Then the finished sample was measured using a spectrophotometer absorbance at λ=517 nm. The blank used is 0.045 mL of aquades as a substitute for the sample. The decrease in absorbance of the solution showed the presence of antioxidant activity[5].

$$\%inhibition\ DPPH = \frac{(abs_b - abs_s)}{abs_b}$$

2.3. Water Content Test

The sample is inserted into the crucible that has been weighed. The Crucible containing the sample is heated at a temperature of 105°C to ensure that the water evaporates completely. The Crucible is then cooled in a desiccator to room temperature. The cooled Crucible is weighed [1].

$$\%Water\ Content\ = \left(\frac{W_2 - W_3}{W_2 - W_3}\right) \times 100\%$$

2.4 Total Isoflavone Test

Prepared HPLC column and mobile phase (water/methanol 80:20). HPLC is calibrated by injecting standard solutions of isoflavones of different concentrations. A standard curve is made by plotting the standard concentration of isoflavones to the peak area of the chromatorgram. Samples prepared in methanol with appropriate concentrations were injected into HPLC, then recorded peak area of isoflavones [12].

$$isoflavone = \frac{peak\ area}{slope\ kurva} \times M$$

2.5. Carbohydrate Test

The sample moisture content test results are then calculated by the equation :

$$\%carbhhydrate = 100\% \ - \ \%water$$

2.6. Protein Test

The sample was put into kjedahl flask and added H_2SO_4 and K_2SO_4 (10 mL;1 gram). The kjedahl flask is then heated until a clear colored solution and white steam escape and then cooled. The heated solution is transferred into the distillation tube and added NaOH (20 mL). The distillation tube is connected to the distillation apparatus and the container tube. The distillation apparatus is heated until the ammonia is completely distilled and the distillate is accommodated in an Erlenmeyer containing the indicator H_3BO_3. The distillate is titrated using standard HCl until it turns pink[13].

$$\%protein = (\frac{(V1 \times N \times F \times 100)}{(W1 \times 0{,}014)})$$

2.7. Fat Test

Prepared Whatman filter paper and put the sample that has been weighed. Filter paper is inserted into the flask Soxhlet and poured non-polar organic solvent. Assembled tool Soxhlet. The Soxhlet is heated with *a heating mantle* for several hours until the sample is fully extracted. After heating, the non-polar organic solvent is transferred into the rotary evaporator to be evaporated until the remaining fat is left in the flask. Pumpkin dried in the oven to remove residual solvent. The dried pumpkin is weighed and the fat weight is calculated.

$$fat\ \% = \left(\frac{fat}{mass\ sample}\right) mass \times 100\%$$

3. RESULT AND DISCUSSION

Table 1. Test results on samples of Green bean and Red bean Tempeh

No	evidence for the	Sample	Methods	use of	Unit Test	Description
1.	Antioxidant	Green beans	DPPH	210.7372	mg/L	Maryam (2015)
		Red beans	DPPH	43.86	mg/L	Maryam (2015)
2.	Water content	Green beans	Gravimetric	56,19	%	Radiati et al (2016)
		Red beans	Gravimetric	61,79	%	Radiati et al (2016)
3.	Total isoflavon	Green beans	HPLC	59,00	mg/100g	Maryam (2016)
		Red beans	HPLC	126,33	mg/100g	Maryam (2016)
4.	Carbohydrates	Green beans	Gravimetric	62.9	g/100g	Ministry of Health (2017)
		Red Beans	Gravimetric	59.5	g / 100g	Ministry of Health (2017)
5.	Protein	Green beans	Kjedahl	22,2	g/100g	Ministry of Health (2017)
		Red beans	Kjedahl	23,1	g/100g	Ministry of Health (2017)
6.	Fat	Green Beans	Soxhlet	1,2	g/100g	Ministry of Health (2017)
		Red beans	Soxhlet	1,7	g/100g	Ministry of Health (2017)

3.1. Making Tempeh

In the production process of tempeh samples of green beans and red beans, done with the addition of traditional yeast containing *Rhizopus oligosporus* , *Rhizopus oryzae* and *Rhizopus stolonifer* contained in hibiscus leaves. *Rhizopus* contained in hibiscus leaves have the ability to degrade or hydrolyze several components consisting of a combination of monomers such as carbohydrates, fats and proteins contained in sample green beans and red beans into smaller molecules or referred to as monomers. In addition, secondary metabolites are produced through aerobic metabolism, resulting in vitamin E (alpha tocopherol), which acts as an antioxidant. Vitamin E can act as an antioxidant because there are double bonds in the structure of vitamin E as in Figure 1.

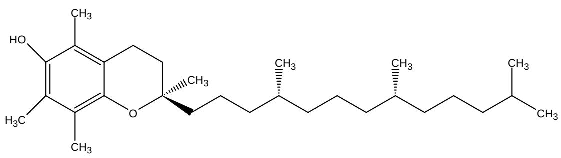

Figure 1. Structure of Vitamin E

Double bonds contained in the structure of vitamin E cause electron delocalization, so it will inhibit free radicals produced or formed from oxidative stress processes.

3.2. Antioxidant Test

Antioxidants have an ability to inhibit free radicals consisting of various kinds. The types of free radicals that can be inhibited depend on their antioxidant activity. When an antioxidant activity has a large value, it is directly proportional to the inhibition of free radicals will be higher[8]. Based on the results of Table 3.1 obtained in mung bean samples showed antioxidant activity of 210.7372 mg/L, while for red bean samples showed a value of 43.86 mg / L. Where both of these results show that the samples of green bean-based tempeh has a higher antioxidant activity than the antioxidant activity of red bean-based tempeh samples, so that green bean tempeh can reduce free radicals better than red bean tempeh.

3.3. Water Content Test

In tempeh samples made from green beans and red beans based on data obtained in Table 3.1 shows the presence of water contained in the sample of 56.19% for green beans and 61.79% for red beans. The high water content in the sample will cause tempeh to be more easily damaged, this is because spoilage microorganisms such as bacteria, fungi and mold in addition *to Rhiszopus* will be easier to grow in humid conditions, so tempeh will easily decay. The occurrence of decay in tempeh is characterized by the texture of tempeh that becomes mushy and sticky, the aroma is less pleasant and the distinctive taste of tempeh that changes. Therefore, in the long storage process, tempeh with green bean base material will be more durable because the water content contained is smaller than red bean-based tempeh. This is also because the texture of red beans is also more watery than green beans, so the water content of red bean-based tempeh has a higher water content[5].

3.3 Total Isoflavone Test

In the total isoflavone test based on the data in Table 3.1 showed a value of 59.00 mg/100g for mung bean samples and 126.33 mg / 100g. According to Maryam's research [7] during the processing of tempeh making, the bioconversion of isoflavone compounds has occurred in both samples This bioconversion reaction through the process of hydrolysis of isoflavone compounds that bind to carbohydrates resulting in free isoflavone compounds known as aglycones which have higher activity than isoflavones that still bind to carbohydrates. During the fermentation process, the hydrolysis reaction is caused due to the growth of fungi that produce the enzyme β glycosidase, resulting in the release of aglican isoflavone compounds from sugar compounds. This aglican isoflavone compound will undergo further transformation to form compounds that have higher biological activity. In both samples showed results for red bean-based tempeh has a higher content of isoflavones compared to green bean-based tempeh samples.

3.5 carbohydrate, Protein and fat test

Other ingredients contained in both samples shown in Table 3.1 such as protein, carbohydrate, and fat, do not have significant differences, these contents are produced from samples of basic ingredients tempeh. The small amount of this content value is based on the initial content value contained in the sample. As for other factors that affect these contents occur in the fermentation process, where these three compounds are degraded into monomers. Based on the data, green bean-based tempeh has an advantage in the amount of carbohydrates of 62.9 g/100g compared to red bean-based tempeh which only contains carbohydrates of 59.5 g/100g. However, red bean-based tempeh had a higher protein and fat content compared to green bean-based samples of (23.1;1.7 g/100g), while green beans only contained (22.2;1.2 g/100g) protein and fat.

4. CONCLUSION

The results of the analysis of the content contained in the samples of green beans and red beans obtained green bean-based tempeh has a higher antioxidant and carbohydrate content compared to red bean-based tempeh, and has a longer shelf life than red bean tempeh because it has a lower water content. Meanwhile, red bean-based tempeh has the advantage of isoflavones, protein and fat content, however, its storage power is lower than green bean-based tempeh because it has a higher water content.

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