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Sensory Profiles, Protein and Antioxidant Capacity of Tempeh with Sunflower and Sesame Seeds Substitution

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ABSTRACT

Background: Tempe is a traditional food from Indonesia that comes from fermentation process by several fungi including the mushrooms *Rhizopus oligosporus, Rhizopus oryzae or Rhizopus stolonifer*. In this study, soybeans were substituted with a mixture of sunflower seeds and sesame seeds. This study aimed to determine the effect of soybean substitution with a mixture of sunflower seeds and sesame seeds on the characteristics of tempeh. **Methods**: This type of research was experimental study with a randomized block design (RBD). The treatment given was 5 types of treatment with 3 repetitions. Data analysis used aim to determine the effect of treatment on organoleptic quality, protein content and antioxidant capacity using analysis of variance (ANOVA). **Results**: Substitution of soybeans with different mixtures of sunflower seeds and sesame seeds had a significant effect on organoleptic tests which included color, aroma, taste, overall acceptability, and differences had a significant effect on aroma quality, and protein content, and antioxidant capacity. Protein content ranges from 29.07% -35.76%. While the antioxidant capacity ranged from 51.06 mg/L GAEAC - 68.01 mg/L GAEAC. The best treatment was tempeh with a mixture of sunflower seeds and sesame seeds with the contain of 55% which was the most preferred by panelists with quality characteristics of white color, compact solid texture, no unpleasant odor, protein content of 35.76%, and antioxidant capacity of 68.01 mg/L GAEAC.

Keywords: Antioxidant, Protein, Sunflower, Sesame, Tempeh

INTRODUCTION

Tempeh is a worldwide source of plant-based protein from Indonesia. Tempeh is made from grains fermented by several fungi including *Rhizopus oligosporus, Rhizopus oryzae or Rhizopus stolonifer that form a white solid* [(1)]. Through this fermentation process, soybean seeds are broken down into simple compounds that are easily digested[(2)]. During fermentation, tempeh undergoes biochemical changes that provide health benefits. These changes make tempeh have many biofunctional properties such as angiotensin *I-converting enzyme (ACE)* inhibition, anticancer, antioxidant, antithrombotic, hypocholesterolemia, antidiabetic, and immunomodulatory activities [(3)]. Tempeh is a source of vegetable protein and is rich in other nutrients that are very beneficial for the human body [(4)].

Tempeh that is produced and consumed generally comes from soybeans. The availability of soybeans decreases every year because soybeans are not only intended for the basic ingredients of making tempeh but also for making tofu, tauco, oncom, soy sauce, and several other soy products [(5)]. The reduced availability of soybeans causes limitations on raw materials which have an impact on tempeh production which will decrease. Modification of tempeh raw materials is needed to overcome the decline in soybean availability. The modification is expected to add a variety of tempeh that can increase the acceptability of tempeh among young people by improving the sensory quality and nutritional value of tempeh..

Sunflower seeds can be utilized as raw material for tempeh products. In Indonesia, the availability of sunflower seeds is quite high[(6)], so that the utilization of sunflower seeds for making tempeh will produce a new innovation and will produce tempeh that is rich in vegetable protein and antioxidants due to the presence of phytochemical compounds in the raw material [(7)]. The benefits of sunflower seeds in accelerating the wound healing process come from the content of active substances including *β-sitosterol, flavonoids and linoleic acid* contained in the sunflower seeds. Flavonoids are able to limit the amount of free radicals, preventing excessive tissue damage to the wound area[(8)]. Apart from being an antioxidant, sunflower seeds also have potential as anti-inflammatory, anti-cancer, anti-hypertensive, analgesic, skin protectant, hypo cholesterol, antibacterial activity, sedative effect on nerves, muscles and blood vessels [(9)].

Apart from sunflower seeds, sesame seeds also have a high protein content and contain antioxidants, namely tocopherols and lignans (*sesamin, sesamol, and sesamolin*)[10)]. The color of the seed coat also varies depending on the variety: white, yellow, brown, gray, and black [(11)]. Sesame food products are beneficial to health because sesame contains compounds that can bind cholesterol in the blood, prevent hardening of the walls of blood vessels, maintain liver and kidney health, prevent cancer, and increase body fitness and vitality [(12)]. Sesame seeds that have been roasted release oil that is very fragrant and savory, besides that it also acts as an aroma binder and catalyst, so that the languorous odor in tempeh can be eliminated.

Based on the description above, research was conducted on the manufacture of tempeh substitute mixture of sunflower seeds and sesame seeds (namely Delijen). The processing of tempeh substituted with a mixture of

sunflower seeds and sesame seeds with the intention of increasing protein and antioxidant levels and reducing the use of soybeans, so as to optimize the use of other food ingredients that have nutritional value such as sunflower seeds and sesame seeds. The general objective of this study was to determine the effect of soybean substitution with sunflower seeds and sesame seeds on tempeh characteristics. The specific objectives of this study were to make tempeh with various treatments, determine organoleptic characteristics, determine the best treatment and analyze nutritional values including protein content and antioxidant capacity in tempeh.

METHODS

The material was imported soybeans that have been sorted and there are no defects in each seed, sunflower seeds that have been peeled off the skin, white sesame seeds that are intact and have no defects, tempeh starter from Raprima.

Tempeh Process

The process of making tempeh began with the preparation of the three basic raw materials. First, the preparation of sunflower seeds by sorting the sunflower seeds first, then washed in running water, boiling for 30 minutes, soaking for 24 hours, after that washed until clean from the charcoal skin, then steamed for 15 minutes and drained. Sesame seeds are first sorted, washed, drained, and then roasted. Then for soybeans, the first thing to do is sorting, washing in running water, boiling for 30 minutes, soaking for 24 hours, after soaking for a day then separated from the aromatic skin and split into 2 pieces, washed thoroughly, steamed for 15 minutes, and drained. For the flow of making tempeh can be seen in the following flow chart.

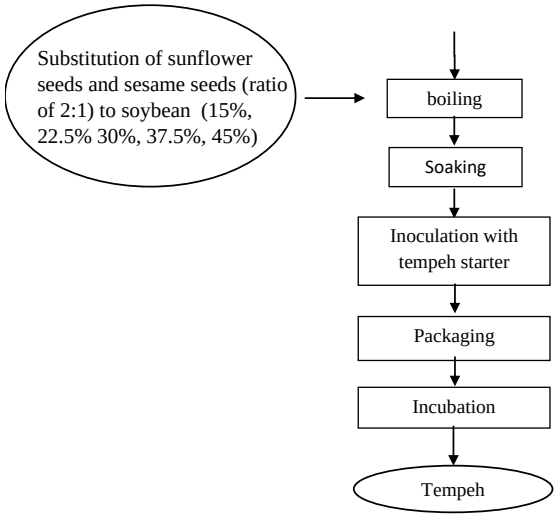

Fig 1. Production of Tempeh

RESULTS

Subjective analysis includes hedonic test of preference for color, texture, aroma, and overall acceptance in the test using raw tempeh while for the taste test using tempeh that has been fried. Hedonic quality tests include texture quality, color quality, and aroma quality tests using raw tempeh. The average value of the tempeh hedonic test can be seen in Table 2

TABLE 2. SCORE OF HEDONIC TEST OF TEMPEH

Treatments	Hedonic Test Average Value				
	Color	Texture	Scent	Taste	Overall Acceptability
P1	3,77±0,425^{d}	4,03±0,181^{a}	2,97±0,181^{c}	3,03±0,570^{d}	3,31±0,466^{c}
P2	3,83±0,375^{cd}	3,97±0,409^{a}	2,99±0,757^{c}	3,33±0,618^{c}	3,43±0,520^{c}
P3	3,97±0,316^{bc}	3,93±0,251^{a}	3,14±0,842^{c}	3,48±0,657^{c}	3,46±0,544^{c}
P4	4,03±0,608^{b}	3,90±0,302^{a}	3,77±0,750^{b}	3,93±0,650^{b}	4,07±0,596^{b}
P5	4,33±0,474^{a}	3,90±0,302^{a}	4,62±0,510^{a}	4,49±0,546^{a}	4,78±0,444^{a}

Notes: Different letters behind the mean indicate very significant differences based on LSD test for 1% level (P<0,01).

Hedonic quality test of tempeh includes quality test of texture, color, and aroma. The average value of hedonic quality test can be seen in Table 3.

TABLE 3. SCORE OF HEDONIC QUALITY TEST OF TEMPEH

Average Value of Hedonic Quality Test			
Treatment	Color Quality	Texture quality	Fragrance quality
P1	2,83±0,461^{a}	2,87±0,342^{a}	1,81±0,652^{c}
P2	2,80±0,474^{a}	2,87±0,342^{a}	1,89±0,726^{c}
P3	2,77±0,503^{a}	2,83±0,375^{a}	1,98±0,734^{c}
P4	2,73±0,503^{a}	2,80±0,402^{a}	2,34±0,584^{b}
P5	2,73±0,498^{a}	2,80±0,402^{a}	2,93±0,251^{a}

Notes: Different letters behind the mean indicate very significant differences based on LSD test for 1% level (P<0,01).

The mean value of hedonic test on the color of tempeh was in the range of 3.77 - 4.33. The highest mean score of 4.33 (liked) was in the mixture of sunflower seeds and sesame seeds 45% with soybeans 55% (P5) and the lowest mean score of 3.77 (neutral) in the mixture of sunflower seeds and sesame seeds 15% with soybeans 85% (P1). For the color quality of Tempe, the average value is in the range of 2.73 - 2.83. The highest average value of 2.83 (white) was in the mixture of 45% sunflower seeds and sesame seeds with 55% soybean (P5) and the lowest average value of 2.73 (white) in the mixture of 15% sunflower seeds and sesame seeds with 85% soybean (P1).

The mean value of hedonic test on the texture of tempeh is in the range of 3.90 - 4.03. The highest mean score was 4.03 (liked) in the mixture of sunflower seeds and sesame seeds 15% with soybean 85% (P1) and the lowest mean score was 3.90 (liked) in the mixture of sunflower seeds and sesame seeds 15% with soybean 85% (P1). For the texture quality of tempeh Delijen, the average value is in the range of 2.87 - 2.80. The highest average value of 2.87 (compact) was in the mixture of sunflower seeds and sesame seeds 15% with soybean 85% (P1) and the lowest average value of 2.80 (compact) in the mixture of sunflower seeds and sesame seeds 15% with soybean 85% (P1).

The mean value of hedonic test on the aroma of tempeh is in the range of 2.97 - 4.62. The highest average value of 4.62 (liked) was in the mixture of sunflower seeds and sesame seeds 45% with soybeans 55% (P5) and the lowest average value of 2.97 (neutral) in the mixture of sunflower seeds and sesame seeds 15% with soybeans 85% (P1). For the aroma quality of Tempe Delijen, the average value is in the range of 1.81 - 2.93. The highest average value of 2.93 (no smell) was in the mixture of sunflower seeds and sesame seeds 45% with soybeans 55% (P5) and the lowest average value of 1.81 (slightly smell) in the mixture of sunflower seeds and sesame seeds 15% with soybeans 85% (P1).

The mean value of hedonic test on the flavor of tempeh is in the range of 3.03 - 4.49. The highest average value of 4.49 (liked) was in the mixture of sunflower seeds and sesame seeds 45% with soybeans 55% (P5) and the lowest average value of 3.03 (neutral) in the

mixture of sunflower seeds and sesame seeds 15% with soybeans 85% (P1). For the overall acceptance of Tempe, the average value is in the range of 3.31 - 4.78. The highest average value of 4.78 (liked) was in the mixture of sunflower seeds and sesame seeds 45% with soybeans 55% (P5) and the lowest average value of 3.31 (neutral) in the mixture of sunflower seeds and sesame seeds 15% with soybeans 85% (P1). Objective analysis of tempeh was carried out with the aim of knowing the chemical characteristics which include analysis of proximate content and antioxidant capacity with the help of laboratory tests. The average value of the objective analysis can be seen in Table 4.

Table 4. Protein and antioxidant capacity of tempeh

Treatment	Protein Content (%)	Antioxidant Capacity (mg/L GAEAC)
P1	29,07±2,79^{c}	51,1±1,08^{d}
P2	29,30±3,28^{c}	58,1±3,34^{c}
P3	31,79±0,75^{bc}	63,2±3,28^{b}
P4	33,60±1,23^{ab}	65,2±2,58^{ab}
P5	35,76±2,36^{a}	68,01±2,02^{a}

Notes: Different letters behind the mean indicate significant differences based on BNT test for 5% level (P<0.05).

Based on the results of laboratory analysis, the average value of tempeh protein content is in the range of 29.07% - 35.76%. The highest protein content was found in the mixture of 45% sunflower seeds and sesame seeds with 55% soybean (P5) and the lowest in the mixture of 15% sunflower seeds and sesame seeds with 85% soybean (P1). The average value of antioxidant capacity of Delijen tempeh is in the range of 51.1 mg/L - 68 mg/L. The highest antioxidant capacity was found in the mixture of 45% sunflower seeds and sesame seeds with 55% soybean (P5) and the lowest in the mixture of 15% sunflower seeds and sesame seeds with 85% soybean (P1). Determination of the best treatment of Delijen tempeh is obtained based on the average value of subjective analysis. The

best treatment assessment can be seen in Table 4.

TABLE 5. BEST TREATMENT BASED ON SUBJECTIVE ANALYSIS

Subjective Analysis	Hedonic				
	P1	P2	P3	P4	P5
Color	3,77^{d}	3,83^{cd}	3,97^{bc}	3,74^{b}	4,33^{a}
Texture	4,03^{a}	3,97^{a}	3,93^{a}	3,90^{a}	3,90^{a}
Aroma	2,97^{c}	2,99^{c}	3,14^{c}	3,77^{b}	4,62^{a}
Taste	3,03^{d}	3,33^{c}	3,48^{c}	3,93^{b}	4,49^{a}
Overall Acceptance	3,31^{c}	3,43^{c}	3,46^{c}	4,07^{b}	4,78^{a}
	Hedonic Quality				
Color Quality	2,73^{a}	2,73^{a}	2,77^{a}	2,80^{a}	2,83^{a}
Texture Quality	2,87^{a}	2,87^{a}	2,83^{a}	2,80^{a}	2,80^{a}
Aroma Quality	1,81^{c}	1,89^{c}	1,98^{c}	2,34^{b}	2,93^{a}
Total Notation a	3	3	3	3	8

Notes: Different letters behind the mean indicate highly significant differences (P<0.01).

Based on the results of the assessment, the best treatment is a mixture of sunflower seeds and sesame seeds 45% with soybeans 55% (P5) because it has the highest level of preference for texture, aroma, taste, overall acceptance, color quality, texture quality and aroma quality. Delijen tempeh with the best treatment has a protein content of 35.76% and antioxidant capacity of 68.01 mg/L GAEAC.

DISCUSSION

Based on the results of subjective data analysis with ANOVA analysis, it shows that different treatments on tempeh products have a very significant effect on color, taste, aroma, overall acceptance, but there is no effect on the texture of tempeh. The hedonic test results on the color of Delijen tempeh with a mixture of sunflower seeds and sesame seeds 45% with soybeans 55% obtained the highest average value with white hedonic quality. The white color produced in tempeh is the growth of mold

mycelium on the surface of the seeds[13]. In the tempeh fermentation process, the fine hyphae will cover the entire surface of the soybeans which results in tempeh having a white and compact color[14]. The Least Significant Difference Test on the color of Delijen tempeh was not significantly different due to the composition of yeast, type of yeast, and the same manufacturing procedure in each treatment. Although it has a color quality that is not much different, judging from the panelists' preference for the color of Delijen tempeh, it is influenced by the amount of use of sunflower seeds and sesame seeds. The higher the composition of sunflower seeds and sesame seeds, the higher the panelists liking value of the tempeh products produced.

The hedonic test results on the texture of Delijen tempeh with a mixture of sunflower seeds and sesame seeds 15% with soybeans 85% obtained the highest average value with the hedonic quality of compact solid texture. The texture of tempeh is influenced by mold mycelium and incubation conditions. The quality of the mold mycelium is determined by the type of mold (species or variant of Rhizopus spp.) used. Incubation conditions consist of aeration, namely the availability of oxygen for mold growth, as well as incubation temperature in accordance with the optimum temperature for mold growth[15]. The Least Significant Difference Test on the color of Delijen tempeh was not significantly different due to the composition of yeast, type of yeast, and the same manufacturing procedure in each treatment. Although it has the same texture quality, judging from the panelists' preference for the texture of Delijen tempeh, it is influenced by the amount of sunflower seeds and sesame seeds used. Tempeh with the smallest sesame seed addition obtained the highest favorability score in the sensory test. The increased use of sesame seeds causes the growth of mold in tempeh to be incompletely bound, which results in a slightly brittle texture[1].

Aroma is the smell of food products, odor itself is a response when volatile compounds from a food enter the nasal cavity and are perceived by the olfactory system[16]. Hedonic test results on the aroma of tempeh Delijen with a mixture of sunflower seeds and sesame seeds 45% with soybeans 55% obtained the highest average value with the hedonic quality of the aroma does not smell bad. The lower the composition of the use of a mixture of sunflower seeds and sesame seeds, the aroma of tempeh produced tends to smell languorous. The odor of tempeh is caused by enzymatic activity that

breaks down various macromolecules of tempeh raw materials, such as proteases that break down proteins and lipases that break down fats so that they are simpler in size and produce volatile compounds[17]. Soy raw materials can also cause languorous odor in tempeh because soybeans contain the enzyme lipoxygenase[18]. The use of sesame seeds in tempeh can help break down the languorous odor in tempeh because it has aromatics that can reduce the languorous odor in tempeh and the nature of sesame seeds that can bind the aroma[1]. The aromatic components found in sesame seeds are *oleic acid, stearic acid, and palmitic acid*. This aromatic component will be stronger and more volatile when subjected to the heating process[23]. Sunflower seeds also have a distinctive aroma and can reduce the odor of tempeh.

A flavor is a compound or mixture of chemical compounds that can affect the body's senses, such as the tongue as a sense of taste. The hedonic test results on the taste of tempeh delijen with a mixture of sunflower seeds and sesame seeds 45% with soybeans 55% obtained the highest average value. Tempeh flavor can arise due to the formation of peptides or amino acids because R. microsporus produces enzymes during the fermentation process. The size of the peptide and the type of amino acid determine the bitter, savory or sour taste of tempeh. Where microorganisms that produce enzymes, such as *Bacillus spp*. that produce protease enzymes are found in abundance during tempeh fermentation. Different types of *Rhizopus* can also produce different flavors[13]. The higher the substitution of the mixture of sunflower seeds and sesame seeds, the more favorable the taste of tempeh produced. This is because after going through the frying process, sunflower seeds have a distinctive flavor that causes tempeh to have a new taste, besides the savory sesame flavor also causes the taste of tempeh to be preferred over soybean tempeh in general. Overall acceptance includes an assessment of the taste, texture, color and aroma of the delijen tempeh. The hedonic test results on the overall acceptance of delijen tempeh with a mixture of sunflower seeds and sesame seeds 45% with soybeans 55% obtained the highest average value. The treatment was preferred by panelists because the tempeh did not have a strong aroma, was bright white in color, had a compact texture, and had a savory and distinctive taste from sunflower seeds, and was in accordance with the standardization of tempeh.

There are two types of protein, animal protein and vegetable protein. One example of

vegetable protein that has a high protein content is from nuts[19]. The average value of protein content shows that the highest protein content is found in a mixture of 45% sunflower seeds and sesame seeds with 55% soybeans and the lowest is found in a mixture of 15% sunflower seeds and sesame seeds with 85% soybeans. The increase in protein content in tempeh is caused by differences in the concentration of substitution of a mixture of sunflower seeds and sesame seeds, the more substitution of a mixture of sunflower seeds and sesame seeds used, the greater the protein content in the resulting tempeh. The high protein content in delijen tempeh is due to the protein content of the raw material, namely for sunflower seeds, which is 30.6 grams and sesame seeds 19.3 grams /100 grams of material[4]. The increase in protein content is also due to the growth of microbial cell biomass, especially by *Rhizopus* fungi from the tempeh yeast inoculum used[20]. The proteolytic activity of *R. oligosporus* and *R. oryzae* is instrumental in increasing protein during the tempeh fermentation process[15]. The higher the protein content of a food ingredient, the higher the quality of the food ingredient. In 25 grams of Delijen tempeh, which is equivalent to 1 medium piece, it contains 9 grams of protein.

Antioxidant capacity describes the ability of an antioxidant compound to inhibit the rate of free radical formation reactions[21]. The average value of antioxidant capacity showed that the highest antioxidant capacity was found in the mixture of 45% sunflower seeds and sesame seeds with 55% soybeans and the lowest was found in the mixture of 15% sunflower seeds and sesame seeds with 85% soybeans. Antioxidant capacity varies due to different types and amounts of raw materials, different tempeh processing, and different types of molds during the fermentation process[13]. The antioxidant capacity of tempeh is determined by the amount of antioxidants present in the tempeh. The more the amount of antioxidants, the more its capacity will increase and will have better potential as a functional food[22]. The utilization of sunflower seeds as raw material for tempeh has a higher antioxidant capacity compared to regular soybean tempeh[7]. Sesame seeds contain *Sesamin, sesamolin, sesaminol glucoside*, and *vitamin E* which are the main antioxidant compounds in sesame seeds. [10].

CONCLUSION

Delijen tempeh with different substitutions of sunflower seeds and sesame seeds has a very significant effect on

organoleptic tests which include color, aroma, taste, overall acceptance, aroma quality, protein content and antioxidant capacity. Tempeh Delijen with the most organoleptically acceptable characteristics is P5 with 55% soybean composition and 45% sunflower seed and sesame seed mixture. Obtained a hedonic value of color 4.33 (liked), texture 3.90 (liked), aroma 4.62 (liked), taste 4.49 (liked), and overall acceptance 4.78 (liked). Tempe Delijen P5 which is the best treatment has characteristics in accordance with SNI 3144: 2015 which is the standardization of tempeh which is evenly white in color, has a compact solid texture, and does not smell bad. Laboratory results showed that increased substitution of sunflower seeds and sesame seeds affected the increase in protein content and antioxidant capacity in each treatment, with an average value of protein content (29.07 - 35.79%) and an average value of antioxidant capacity (51.06 - 68.01 mg/L GAEAC).

Suggestions that can be given based on this research are that further research is expected to conduct further analysis of macronutrients (fat and carbohydrates), especially on unsaturated fatty acid levels because sesame seeds and sunflower seeds have high unsaturated fat content values and other micronutrients (vitamins and minerals). As well as for tempeh producers, it is hoped that they will be able to develop innovations in making tempeh with sunflower seeds and sesame seeds.

Conflict of Interest

The author(s) declare that they have no conflict of interest.

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