

CHEMICAL CHARACTERISTICS OF PUMPKIN SEED TEMPEH FROM SOYBEAN AND PUMPKIN SEEDS

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Abstract - The aim of this research was to find the effect of the combination of soybean and pumpkin seeds on the chemical characteristics of pumpkin seed tempeh. The pumpkin seed tempeh samples were analyzed for its water, ash, protein, fat, carbohydrate and crude fiber. Meanwhile, support data were isoflavone and zinc. Sensory hedonic was conducted by 25 untrained panelists for selecting the best formulation of soybean and pumpkin seeds in tempeh. The result shows the formulation of soybean and pumpkin seeds in various combinations (100:0, 85:15, 70:30, 55:45, and 40:60). It gives a significant effect on the water, protein, fat, carbohydrate, and crude fiber. Moreover, all acceptance on pumpkin seed tempeh is on the level of $\alpha = 0,01$ and $\alpha = 0,05$. The best combination of soybean and pumpkin seeds is 40:60, with chemical analysis results which are water (33,7%), ash (5,31%), protein (65,65%), fat (54,57%), carbohydrate (5,94%), crude fiber (14,06%) (db), zinc (30,05 ppm), and isoflavones (42,10 mg/100g). Chemical characteristics of the selective pumpkin seed tempeh have fulfilled SNI 3144-2015 regarding water, protein and fat.

Keywords: chemical, characteristics, soybean, pumpkin seeds, tempeh

I. INTRODUCTION

Tempeh is processed with fermented soybeans through the growth of *Rhizopus* sp which has high protein. In 2011, soybean production reached 851.286 tons. However, in 2012 and 2013, it decreased to 843.153 tons and 779.921 tons respectively (BPS, 2014). In Indonesia, the use of soybean is very high in producing tempeh which equals to 57%. Meanwhile, tofu manufacturing is about 38%, and the rest is in other preparations such as soy sauce, and other processed foods like tauco (Widowati *et al.*, 2010). Soybean demand has reached 2,54 million tons of dry seeds of soybean. Therefore, to meet the high needs of soybean, government imports the soybeans about 1,58 million tons annually (BPS, 2014). It makes the diversification of raw materials require tempeh in the increasing food security.

Then, tempeh has many benefits for the human body. It can lower cholesterol, flatulence, and diarrhea. Moreover, it can increase antioxidant enzyme like Superoxide Dismutase (SOD) and lower the risk of prostate and colon cancer (Astuti *et al.*, 2000).

Development of tempeh making has been done by using nuts or seeds such as jack bean, chickpea, lupin and kidney bean since the 1970s (Nout & Kiers, 2005). Similarly, Radiati and Sumarto (2015) said that tempeh had been done by using nuts such as bogor beans, green beans,

red beans, and peanuts. It had the protein of 18,3-28,5%. However, Kusnanto *et al.* (2013) stated that in the case of the seeds, tempeh containing 100% of rubber seeds had 8,6% of protein. Then, tempeh from durian seeds contained 3,4% protein as stated by Sawitri and Santoso (2017). Moreover, Andaka *et al.* (2017) and Harmoko *et al.* (2016) agreed that tempeh from jackfruit seeds investigated still had low protein (5,63%-5,96%). Qomariah and Utomo (2016) said that lamtoro gung seeds were used in tempeh production. The combination of soybean and lamtoro gung seeds was 90:10. Then, it had 23,43% of protein.

Tempeh mold grows and forms hyphae. It is white threads that cover the surface of soybean seeds and form the strands that bind mycelium soybean seeds with one another. Thus, it forms a structure with compact and dense texture.

Soybean (*Glycine max* L.) is a source of vegetable protein that is essential for humans as stated by Margono *et al.* (2000). Protein found in soy that is equal to 36,49 g per 100 g (USDA, 2016). Mapegau (2006) said that soybean had higher vegetable protein and relatively lower price compared to other protein sources. For example, it could be milk, meat, and fish. Thus, it made soybean as a food ingredient that could meet the needs of nutritious food for the general public.

Pumpkin seeds have also reduced the use of soybean as the main raw material of tempeh. Pumpkin seeds are considered as materials that are not used anymore. However, it can be developed into a material with high productivity values and nutritional value. Pumpkin seeds studied contain 37,8% to 45,5% oil and plant sterols that are useful to lower cholesterol and have a hypoglycemic effect (antidiabetic). It also serves as an antimicrobial and neuroprotectin (Hargono, 1999).

Pumpkin seeds contain 100 g of nutrient such as 265 mg of phytochemicals (phytosterols), 6 g of fiber, 20,9 g of polyunsaturated fatty acids (PUFAs), and antioxidants (1,9 mg of vitamin C, 2,18 mg of vitamin E, 9 g of beta carotene). It can reduce the effects of hypercholesterolemia. The total of isoflavone is 130-380 mg/100 g. The high content of soy isoflavones increases the antioxidant power as described by Koswara (2006). Meanwhile, high mineral content like 7,64 mg of zinc can reduce the risk of osteoporosis (USDA, 2016). It is recommended to consume pumpkin seeds per day by the US Food and Drug Administration (FDA) about 30-40 g/day.

According to BPS (2010), the production of pumpkins in Indonesia reached 369-846 tons. The availability and utilization of pumpkin in Indonesia are only used for the manufacture of seeds or traditional medicine. For diabetes, pumpkin seeds can be used as an ingredient substitution for soybean tempeh.

Thus, the food substitution program through a combination of soybean and pumpkin seeds in the tempeh can reduce the consumption of soybean as the main raw materials and improve the nutrition of tempeh. In this research, a combination of soybean with pumpkin seeds is expected to have a good chemical characteristic and can improve the nutritional values of tempeh.

II. METHODS

Raw materials used in this research are soybean, pumpkin seeds, and tempeh yeast. The soybean is obtained in Koperasi Produsen Tahu Tempe Indonesia (KOPTI – Indonesian Tofu and Tempeh Producer Cooperative) in Kalideres, West Java. The characteristics of soybean are old enough and have cream color and normal size. Meanwhile, the criteria of pumpkin seeds are dry enough and have light brown color and normal size. The pumpkin seeds are obtained in Toko Hasil Bumiku, Yogyakarta. Moreover, tempeh yeast used is specific brand of Raprima. Tempeh yeast is obtained from the Indonesian Institute of Sciences. The material for the analysis includes H_2SO_4 , HgO , K_2SO_4 , $NaOH$, $Na_2S_2O_3$, H_3BO_3 , HCl , hexane, petroleum ether, sodium phosphate, HNO_3 , and $HClO_4$.

Then, the tools used are oven, measuring instruments (scales and measuring cups), and supporting tools (containers, cutting boards, stoves, and pans). Moreover, the tools in the chemical analysis tools are porcelain, oven, desiccator, analytical balance, Kjeldahl digestion tube, Erlenmeyer flask, glass flask, glass beakers, pipettes, filter paper, pumpkin fat, furnaces, Soxhlet extractor, hot plate, and other analytical standard tests. The experiment design uses completely randomized design with five level treatments (100:0, 85:15, 70:30, 55:45, and 40:60) and three replications.

The preliminary research is conducted to determine the chemical content of raw materials, namely pumpkin seeds. It is to see the range of combination level of soybean and pumpkin seeds. Moreover, it is to determine the sensory hedonic and hedonic quality test. The combination of soybean and pumpkin seeds examined in the preliminary research is equal to four levels. There are 100:0, 70:30, 40:60, and 10:90. In the preliminary research, the testing is done through the sensory test in the form of hedonic, while the hedonic quality test is conducted by 20 untrained panelists. This research is conducted in several places. There are Laboratory of Food Technology, Universitas Sahid Jakarta, Food Chemical Laboratory in Bogor Agricultural University, and Saraswanti Indo Genetech Laboratory located in Bogor.

The process of making tempeh using pumpkin seeds is done by mixing all the ingredients with suitable combinations. The soybeans are previously boiled for 4 hours with ground water and a temperature of $100^\circ C$. Then, it is soaked for 12 hours. The husk is discarded, and it is steamed for an hour. Moreover, for pumpkin seeds, it is dried for 12 hours with the sun. Then, the husk is separated from the seed. After that, it is soaked for 30 minutes and steamed for 1 hour. Both materials are chilled for 1 hour and mixed with 0,2% of Raprima yeast or 100 g total weight of each combination. Once it is mixed with yeast, the inoculation and printing with banana leaves can be done. Moreover, fermentation is done for 32 hours with the temperature of $25-31^\circ C$. The combination of soybean, pumpkin seeds, and yeast in tempeh can be seen in Table 1.

Table 1 Combination of Soybean, Pumpkin Seeds, and Yeast in Tempeh

Raw material	Treatment				
	100:0	85:15	70:30	55:45	40:60
Soybean (g)	100	85	70	55	60
Pumpkin seeds (g)	0	15	30	45	40
Yeast (%)	0,2	0,2	0,2	0,2	0,2

The chemist characteristics and sensory test (hedonic) is done in primary research. Sensory hedonic is conducted by 25 untrained panelists to select the best combination of soybean and pumpkin seeds in tempeh. Then, the chemical characteristics observed in pumpkin seed tempeh include water, ash, protein, fat, carbohydrate, and crude fiber. The supporting characteristics are the isoflavone and zinc. For comparison of quality, it uses SNI 3144-2015 regarding the quality of soybean tempeh (BSN, 2015).

III. RESULTS AND DISCUSSIONS

The results of the analysis of raw materials of pumpkin seeds in the preliminary research according to USDA (2016) can be seen in Table 2. Table 2 shows that the results of water in pumpkin seeds which are 5,81% tends to be higher than soybean which is 8,54%. Then, the ash in pumpkin seeds is 4,10%. The pumpkin seeds' protein is 30,23% which are lower than soybean. Soybean is 36,49%. Similarly, the fat in pumpkin seeds about 31,76% is higher than soybean which is 19,94%. Moreover, the carbohydrate in pumpkin seeds is 12,54%. It is than carbohydrate in soybean which is 30,16%. Meanwhile, the crude fiber in pumpkin seeds which are 6% tends to be lower than the soybean which is 9,3%. Then, the zinc in pumpkin seeds is 27,20 ppm.

Table 2 The Results of the Analysis of Raw Materials by Comparing Pumpkin Seeds and Soybeans

Parameter	Pumpkin	Seeds	Soybean
Water (%)	5,81	5,23**	8,54**
Ash (%)	4,10	-	-
Protein (%)	30,23	30,23**	36,49**
Fat (%)	31,76	49,05**	19,94**
Carbohydrates (%)	12,54	10,71**	30,16**
Crude fiber (%)	15,56	6,00**	9,30**
Zinc (ppm)	27,20	-	-

** USDA (2016)

Then the data analysis of pumpkin seeds is used in the discussion of the results of the chemical characteristics in tempeh on primary research. The average percentage of the hedonic test by panelists who likes tempeh with the combination of soybean and pumpkin seeds can be seen in Table 3.

Table 3 The Average Percentage of the Hedonic Test by Panelists

Sensory	Combination (Soybean: Pumpkin Seeds)			
	100:0	70:30	40:60	10:90
Color (%)	67	72	61	28
Aroma (%)	28	72	83	44
Texture (%)	72	95	67	61
Overall (%)	61	78	78	39
Average (%)	57	79,3	72,3	43

Based on the hedonic test results in Table 3, it shows that the average percentage of panelists who the combination of soybean and pumpkin seeds on pumpkin seed tempeh is 43-79,25%. The combination of soybean and pumpkin seeds are selected on the primary research which has a percentage of $\geq 50\%$ of like and very like. Therefore, the combination of soybean and pumpkin seeds which are 10:90 is not selected because it has a percentage 43% of like and very like or it is $< 50\%$. From the results of the preliminary research, it is found that the range of the primary research is the combination of soybean and pumpkin seeds of 100:0, 70:30, and 40:60. Moreover, the result of hedonic quality in pumpkin seed tempeh is in the preliminary research which can be seen in Table 4.

Table 4 Hedonic Quality Result of Pumpkin Seed Tempeh in the Preliminary Research

Quality Parameter	Combination (Soybean: Pumpkin seeds)		
	100:0	70:30	40:60
Color	2,8	3,7	4,1
Aroma	2,7	3,8	3,7
Texture	3,4	4,2	3,8

Description:

Color: 1= Cream, 2= Yellowish, 3= Yellow, 4= Rather greenish yellow, 5= Greenish yellow

Aroma: 1= Strongly unique aroma and acidic of tempeh, 2= No unique aroma and acidic of tempeh, 3= Rather unique aroma and acidic of tempeh, 4= Unique aroma and no acidic of tempeh

Texture: 1= Strongly not compact, 2= Not compact, 3= Rather compact, 4= Compact, 5= Very compact

Based on the hedonic test results in Table 4, it shows that tempeh with a combination of soybean and pumpkin seeds 100:0, 70:30 and 40:60 have a quality value from 2,8 to 4,1. It has the characteristics of the color which are yellow to rather greenish yellow. Meanwhile, the quality value to the aroma is 2,7 to 3,7. It has rather unique aroma and acidic of tempeh. For texture, the quality score ranges from 3,4 to 3,8. It shows that the texture of tempeh is rather compact and compact. Based on the results of the hedonic test in the preliminary study, the researchers investigate the combination of soybean and pumpkin seeds from 100:0 to 40:60 in the main study. Therefore, the level of a combination of soybean and pumpkin seeds used in the primary research is 100:0, 85:15, 70:30, 55:45, and 40:60. Those are with characteristics that have yellow to rather greenish yellow, rather unique aroma and acidic of tempeh, and rather compact to compact texture of tempeh. Moreover, the average value of the chemical characteristics of tempeh with a combination of soybean and pumpkin seeds is in Table 5.

In Table 5, there is decrease in the soybean and increase in pumpkin seeds, there is a decrease in the water content in tempeh ($\alpha = 0,01$). For example, there is a decrease in water in pumpkin seed tempeh ($\alpha = 0,01$). This is because the water holding capacity in soybean is higher than the pumpkin seeds. Lower water level causes the texture to be denser and more compact as described by Pabesak *et al.* (2013). Meanwhile, soybean contains relatively higher protein and carbohydrate that is easy to bind water than pumpkin seeds. This is based on the protein and carbohydrate whose polar and ionizing compounds can bind a lot of water. It is stated by Soekarto & Adawiyah, (2012).

When this is compared to the quality requirements of SNI 3144-2015 regarding the numbers of soybean tempeh, water in pumpkin seed tempeh is 33,27-50,95%. It is in conformity with a maximum of 65%.

Based on observations in Table 5, ash in tempeh is not different from one to another. The lowest average value is 40:60 and the highest combination is 70:30. The ash in pumpkin seed tempeh is about 5,31 to 6,51%. There is no standard determination of the ash in soybean in SNI 3144-2015 regarding quality requirements of soybean tempeh.

In Table 5, by decreasing soybeans and increasing pumpkin seeds, there is a decrease in the protein in tempeh ($\alpha = 0,05$). This is seen from the preliminary test results on the protein. Protein in pumpkin seeds which are 30,23% is lower than soybean which is 36,49% (USDA, 2016). Thus, the tempeh with a combination with more pumpkin seeds has lower protein than the combination of more soybeans.

Table 5 The Average Value of the Chemical Characteristics of Tempeh (db)

Parameter (%)	Combination of Soybean: Pumpkin Seeds				
	100:0	85:15	70:30	55:45	40:60
Water **	50,95±3,44 ^a	43,71±1,07 ^b	39,33±5,03 ^{bc}	37,78±1,26 ^{cd}	33,27±1,44 ^d
Ash	5,78±2,08	6,47±0,23	6,51±0,76	5,41±1,83	5,31±0,74
Protein *	91,04±8,61 ^a	66,28±13,70 ^b	66,17±10,55 ^b	73,00±0,72 ^{ab}	65,65±0,91 ^b
Fat level*	41,21±1,89 ^a	53,12±6,84 ^b	51,68±1,91 ^b	54,15±5,05 ^b	54,57±5,31 ^b
Carbohydrate*	25,00±5,70 ^a	18,43±10,16 ^{ab}	13,26±5,53 ^{ab}	4,96±3,68 ^b	5,94±5,29 ^b
Crude fiber *	30,79±5,21 ^a	26,64±2,32 ^{ab}	22,34±4,51 ^{bc}	18,32±0,46 ^{cd}	14,06±1,85 ^d

Description:

Different levels between letters show highly significant or significant effect between treatment

(**Significance $< \alpha = 0,01$, *Significance $< \alpha = 0,05$)

Proteolytic activity of *Rhizopus oryzae* and *Rhizopus oligosporus* is important for the improvement of protein during tempeh fermentation process. Mold use amino acids (albumin and globulin) and soluble base for its growth (Handoyo & Morita, 2006). Compared to the quality requirements of SNI 3144-2015, the protein of pumpkin seed tempeh is 65,65-91,04%. It is suitable with the minimal of 15%.

In Table 5, decreasing soybeans and increasing pumpkin seeds affect the increase in the fat in tempeh ($\alpha = 0,05$). This is because of the influence of Polyunsaturated Fatty Acids (PUFA) of 20,9 g (USDA, 2016). Moreover, it is because pumpkin seeds are rich in linoleic fatty acid (43,56%), oleic acid (24,38%) and linolenic acid as said by Hargono (1999). The fatty acid increases the levels of fat in soybean. In the preliminary test result on the fat in pumpkin seeds which are 31,76% is higher than soybean which equals to 19,94%. When it is compared with the levels of fat in the quality requirements of SNI 3144-2015 regarding soybean tempeh, fat in pumpkin seed tempeh is 41,21-54,57%. It is appropriate with the minimal of 7%.

Based on observations in Table 5, decreasing soybeans and increasing pumpkin seeds affect the decrease in carbohydrate in tempeh ($\alpha = 0,01$). From the preliminary test results, the carbohydrate in pumpkin seeds is 12,54% which are lower than the carbohydrate in soybean which is 30,16%. This results in tempeh combination with more pumpkin seeds so that the carbohydrate levels is lower. Carbohydrate levels in pumpkin seed tempeh are 4,96-25%. There is no standard determination of carbohydrates in tempeh according to SNI 3144-2015.

In Table 5, the decrease in the number of soybeans and the increase in a number of pumpkin seeds influence the decline in crude fiber in tempeh ($\alpha = 0,05$). The crude fiber in pumpkin seeds is 6% which are lower than soybean which is 9,3%. Therefore, tempeh combination with more pumpkin seeds makes crude fiber levels lower. It is similar to carbohydrate in soybean tempeh and pumpkin seeds. The increasing number of pumpkin seeds decrease crude fiber and carbohydrates in tempeh. Winarno (2008) stated that the building blocks of crude fiber were composed of the coarse polyblock saccharide such as hemicellulose and cellulose which are parts of carbohydrates.

Compared to the fiber in SNI 3144-2015, fiber in pumpkin seeds which are 4,06-26,64% is not appropriate. The maximum is 2,5%. This means that the fiber in pumpkin seed tempeh is higher than in SNI. Moreover, higher coarse fiber should be better. The high crude fiber in pumpkin seeds can limit the absorption of carbohydrates so the intake of glucose in the body can decrease and control blood sugar levels. In addition, Cahyadi (2007) described that the consumption of high fiber could aid the digestive tract and prevent obesity. The hedonic value with a combination of soybean and pumpkin seeds in tempeh can be seen in Table 6.

Table 6 The Average Value of the Hedonic in Tempeh

Parameter	Combination Soybean: Pumpkin				
	100:0	85:15	70:30	55:45	40:60
Overall	3,3 ^a	3,6 ^{bc}	3,5 ^b	3,6 ^{bc}	3,8 ^c

Description: 1 = strongly dislike, 2 = dislike, 3 = rather like, 4 = like, 5 = very like

Table 6 shows that decrease in the number of soybeans and the increase in a number of pumpkin seed affect the increase in average value of the hedonic in tempeh ($\alpha = 0,01$). The average value of hedonic in tempeh with a combination of soybean and pumpkin seeds ranges from 3,5-3,8 (rather like to like). The hedonic value in tempeh with a combination of soybean and pumpkin seeds is the combination of 40:60, 85:15, and 55:45. The combination of soybean and pumpkin seeds selected is 40:60 because it has the greatest combination of pumpkin seeds. However, from the observation, increasing the use of pumpkin seeds makes tempeh have rather greenish yellow, unique aroma and non acidic tempeh, and denser and compact texture. This is because the water in pumpkin seeds is smaller than soybean. Lower water levels cause the texture to be more dense and compact as stated by Pabesak *et al.* (2013).

Then, the supporting data regarding the tempeh with the combination of soybean and pumpkin seeds (40:60) is the most preferred combination. Supporting test is conducted by isoflavone and zinc. The result of isoflavones and zinc can be seen in Table 7.

Table 7 Test Results of Isoflavone and Zinc

Nutritional Content	Soybean Tempeh	Pumpkin Seed Tempeh Soybean: Pumpkin Seeds (40:60)
Isoflavones	43,05 mg/ 100 g	42,10 mg/100 g
Zinc	10,05 ppm	30,05 ppm

Table 7 shows that the isoflavone in soybean tempeh is 43,05 mg/100 g, while pumpkin seed tempeh has 42,10 mg/100 g. According to Istiani (2010), the differences in the total amount of isoflavones in making tempeh are influenced by the characteristics of an isoflavone compound. It is reactive and easily oxidized so that it can bind other compounds into new compounds. The other characteristics can be growing conditions, soybean varieties, climatic conditions, and the time of planting soybeans. This occurs during the process of ripening and fermenting soybeans. According to Hidayat *et al.* (2010), fermentation increases the number of isoflavones in soybean tempeh.

Moreover, zinc in pumpkin seed tempeh is 30,05 ppm. It is higher than soybean tempeh which is 10,05 ppm. It is influenced by the levels of zinc pumpkin seeds which is 27,20 ppm. It tends to be higher compared with soybean. Zinc is very useful to improve the performance of Superoxide Dismutase (SOD) contained in tempeh (Utari *et al.*, 2010). SOD is needed for the body against free radicals. Zinc contained in pure soybean tempeh is almost equivalent to chicken meat. In this research, the zinc in pumpkins seeds tempeh is higher compared to soybean. The other benefit of zinc is to help the process of antioxidants in the body. There are 70 enzymes to more than 200 enzymes requiring zinc for the activity in the body. Then, zinc is also very beneficial to the body's immunity as explained by Mann *et al.* (2007).

IV. CONCLUSIONS

There is a difference in the quality of the chemical water ($\alpha = 0,01$), protein, fat, carbohydrate, fiber, and the average hedonic value of the overall in tempeh ($\alpha = 0,05$).

The selected combination of soybean and pumpkin seeds is 40:60. It has the characteristics of water (33,27%), ash (5,31%), protein (65,65%), fat (54,57%), carbohydrates (5,94%), crude fiber (14,06%), isoflavone (42,10 mg/100g), and zinc (30,05 ppm). Chemical characteristics of the best pumpkin seed tempeh are in accordance with SNI 3144-2009 on water, protein, and fat. There is no standard determination of ash and carbohydrates in tempeh in the SNI 3144-2015 regarding quality requirements of soybean tempeh.

However, the characteristics that are behind the target is crude fiber. It is still higher than the requirement in SNI 3144-2015. From the results, the combination of soybean and pumpkin seeds affect the levels of protein and fat. Therefore, the further research can be done is how to determine the effect of amino acids and free fatty acids.

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